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

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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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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.

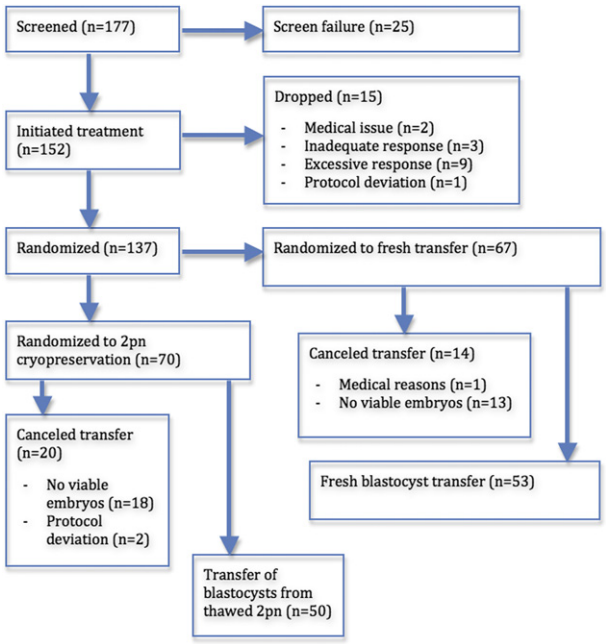
Quinn's Advantage Protein Plus Cleavage Media and Quinn's Advantage Protein Plus Blastocyst Media (Sage) were used to culture all embryos. The morphologically best one or two blastocysts were transferred on the first day on which at least one good expanded blastocyst appeared. Supernumerary expanded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



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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.

TABLE 1

Comparison of potential confounders in the fresh group and cryopreservation group.

Variable	Fresh	Cryopreservation	*P* value
Blastocyst transfers	53	50	
Age at stimulation (y)	32.9 ± 3.7	33.0 ± 3.8	.8302
Patients ≥ 35 y old	19 (35.8)	18 (36.0)	1.0000
Anovulatory	5 (19.2)	1 (24.5)	.2061
Tubal factor	11 (20.8)	15 (30.0)	.3649
Endometriosis	2 (3.8)	2 (4.0)	1.0000
Male factor	25 (47.2)	20 (40.0)	.5520
Multiple factors	13	20	.2141
Unexplained infertility	3 (5.7)	5 (10.0)	.4798
Body mass index (kg/m^2)	24.2 ± 4.4	25.4 ± 5.8	.4038
Cycle day 3 FSH (IU/L)	7.1 ± 1.3	6.8 ± 1.5	.3349
Antral follicles	11.8 ± 2.0	12.4 ± 1.9	.0951
Days of stimulation	10.5 ± 1.4	10.4 ± 1.7	.6458
FSH + hMG dosage (IU)	3,000 ± 735	2,948 ± 825	.8018
E_2, day of trigger (pg/mL)	3,418 ± 1,501	3,076 ± 1,438	.2336
P, day of trigger (ng/mL)	1.13 ± 0.55	1.12 ± 0.61	.7209
Follicles, day of trigger[a]	18.2 ± 6.5	16.9 ± 6.7	.2489
hCG dosage (IU)	4,206 ± 3,000	4,635 ± 2,742	.3418
Oocytes retrieved	14.1 ± 6.4	12.9 ± 4.7	.4772
MII oocytes	11.2 ± 5.1	9.9 ± 4.0	.2712
Bipronuclear oocytes	10.0 ± 4.8	8.1 ± 3.6	.0275
No. thawed	N/A	8.1 ± 3.6	
No. survived	N/A	7.2 ± 3.3	
Survival (%)	N/A	90.5 ± 12.8	
Blastocysts[b]	3.6 ± 2.3	2.5 ± 1.6	.0064
Blastulation rate (%)[c]	37.2 ± 17.1	32.8 ± 16.1	.1590
Supernumerary blastocysts	1.8 ± 2.3	0.7 ± 1.4	.0034
Cycles with supernumerary blastocysts	30	14	.0051
Transferred blastocysts	1.79 ± 0.41	1.78 ± 0.42	.8781
Day-5 blastocyst transfer	37/53	18/50	.0008
Day-6 blastocyst transfer	16/53	32/50	.0008
Endometrial thickness (mm)	10.1 ± 1.8	10.0 ± 2.7	.4272

Note: Values are number (percentage) or mean ± SD.

[a] Follicles at least 10 mm in mean diameter.

[b] Blastocysts transferred or cryopreserved.

[c] Blastocysts per (thawed or fresh) 2PN oocyte.

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Patients with extreme high response received GnRH agonist trigger alone to avoid ovarian hyperstimulation syndrome, their embryos were cryopreserved, and they were dropped from this study. Those with response high enough to incur limited ovarian hyperstimulation syndrome risk were given GnRH agonist with concomitant low-dose hCG. There were 19 subjects in the fresh group and 10 in the cryopreservation group (*P*=.0838) receiving this "dual trigger." In the fresh group, the ongoing pregnancy rates were 63.2% (12 of 19) with the dual trigger and 44.1% (15 of 34) with hCG alone (*P*=.2542). In the cryopreservation group, the ongoing pregnancy rates were 80% (8 of 10) with the dual trigger and 77.5% (31 of 40) with hCG alone (*P*=1.0000).

Among the 74 subjects who received hCG alone, there was a significantly greater ongoing pregnancy rate per transfer in the cryopreservation group than in the fresh group (*P*=.0041). Among the 29 subjects who received the dual trigger, the ongoing pregnancy rates did not differ significantly between the cryopreservation group and the fresh group (*P*=.4311). Among the 74 patients who received only hCG trigger, the clinical pregnancy rates were 50.0% (17/34) in fresh transfers and 82.5% (33/40) in the cryopreservation group (relative risk of implantation failure = 2.86, 95% CI, 1.35 to 6.06, *P*=.0055). Among the 29 that received both hCG and the agonist, the clinical pregnancy rates were 63.2% (12/19) in fresh transfers and 90.0% (9/10) in the cryopreservation group (relative risk of implantation failure = 3.68, 95% CI, 0.52 to 25.90, *P*=.2008).

The overall relative risk of implantation failure (lack of clinical pregnancy) was 2.83 (95% confidence interval [CI], 1.40–5.70) in the fresh group relative to the cryopreservation group. The attributable risk percentage of implantation failure due to COS exposure was 64.7% (95% CI, 28.6%–82.5%). The relative risk of a transferred fresh blastocyst failing to implant was 2.09 (95% CI, 1.46–3.00) when compared with a blastocyst derived from PTEC.

In cycles with frozen supernumerary blastocysts, the ongoing pregnancy rates were 100% (14 of 14) in the cryopreservation group and 56.7% (17 of 30) in the fresh group (*P*=.0034). The implantation rates were 89.3% (25 of 28) and 41.8% (23 of 55), respectively (*P*<.0001).

The clinical pregnancy rates per retrieval were 60.0% (42 of 70) in the cryopreservation group and 43.3% (29 of 67) in the fresh group (*P*=.0607).

TABLE 2

Outcomes in the fresh group and cryopreservation group.

Variable	Fresh	Cryopreservation	*P* value
Retrievals	67	70	
Canceled transfers (rate, %)	14 (20.9)	20 (28.6)	.3280
Blastocyst transfers	53	50	
Pregnancies (rate, %)	36 (67.9)	45 (90.0)	.0079
Clinical pregnancies (rate, %)	29 (54.7)	42 (84.0)	.0013
Ongoing pregnancies (rate, %)	27 (50.9)	39 (78.0)	.0072
Early pregnancy losses (rate, %)	7 (19.4)	6 (13.3)	.5481
Transferred blastocysts	95	89	
Fetal hearts (implantation rate, %)	37 (38.9)	63 (70.8)	<.0001

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DISCUSSION

The cryopreservation group was associated with significantly greater rates of clinical pregnancy per transfer, ongoing pregnancy per transfer, and implantation when compared with fresh controls. Because the bulk of potential confounding variables did not have significant differences between the two groups, and those that were significant favored the fresh group, we conclude that the difference in outcomes was probably not due to confounding variables but more likely due to superior endometrial receptivity in the cryopreservation group. The *P* value for the comparison of clinical pregnancy rate per transfer was less than the specified significance level (.03) for the interim test point; this study was halted per the study protocol.

The impact of COS on endometrial receptivity was reflected in a clinical pregnancy rate in the fresh group of approximately two thirds of the rate in the cryopreservation group. Implantation failure (absence of clinical pregnancy) occurred in 16% of transfers in the cryopreservation group and 45.3% of transfers in the fresh group. The difference in those two rates, 29.3%, estimates the proportion of fresh transfers with implantation failure that may be attributed to endometrial impairment after COS and represents the majority (64.7%, the attributable risk) of all implantation failures in the fresh group. This suggests that the majority of implantation failures after conventional COS in this population are due to endometrial impairment from COS.

The observed clinical pregnancy rate of 84.0% and the implantation rate of 70.8% in the cryopreservation group are encouraging and suggest that PTEC may offer a competitive alternative to the use of fresh embryos or thawed embryos transferred after only a brief survival assessment. However, this study was not designed to evaluate PTEC as an overall primary therapy. The observed difference between the two study groups in clinical pregnancy rates per retrieval was not statistically significant, although the *P* value of .0607 was suggestive of a trend that might have been revealed with a greater sample size.

The presence of frozen supernumerary blastocysts is an indicator of good morphologic quality in the transferred sibling blastocysts. All 14 of the PTEC cycles that had supernumerary blastocysts achieved ongoing pregnancy in their PTEC cycle, with 89% of their transferred blastocysts implanting. Only morphologic techniques were used to select embryos for transfer in this study, suggesting that morphology alone may be sufficient for selecting viable blastocysts for transfer in the presence of a receptive endometrium. In the absence of a receptive endometrium, embryo selection for fresh transfer may be futile, and cryopreservation may be the better option.

There was no evidence that the "dual trigger" impaired success in either study group. In both study groups the ongoing pregnancy rates were greatest in patients receiving the dual trigger, although that difference was not statistically significant. It is plausible that the use of the dual trigger in some cycles reduced the observed difference between the cryopreservation group and the fresh group, because the difference in ongoing pregnancy rate between the two study groups was greatest among those who received hCG only. However, there were insufficient dual triggers in this study to support such a conclusion.

Levi et al. (21) compared fresh oocyte donation cycles and fresh autologous cycles and found no evidence of impaired endometrial receptivity after ovarian stimulation, whereas the present study found evidence of substantially impaired endometrial receptivity. The prior study (21) focused on young (age <33 years), good responders subjected to relatively brief ovarian stimulation (mean 20.0 retrieved oocytes after only 8.4 days of stimulation), perhaps limiting the degree of endometrial impact. The present study used normal responders (8 to 15 antral follicles) over a broad age range (age <41 years) who underwent a mean of 10.5 days of ovarian stimulation, and may be more representative of typical IVF cycles. The duration of ovarian stimulation has been associated with implantation potential (22). It is possible that the combination of young high responders and brief stimulation tends to produce an ample number of rapidly developing embryos while also reducing the frequency of precocious *P* elevation, resulting in excellent embryo–endometrium synchrony (9).

Despite the prospective randomized design of this study, there are potential weaknesses because endometrial receptivity could not be directly observed, but only inferred. We cannot rule out the possibility of embryonic factors affecting the difference in outcomes between the two study groups. It is possible that the fewer 2PN oocytes that were available in the cryopreservation group and the freeze–thaw of those 2PN oocytes resulted in inferior blastocyst cohorts available for transfer, and if true, this would result in underestimation of the endometrial impact of COS in this study. Another possibility is that the freeze–thaw procedure preferentially culled out flawed embryos, potentially resulting in a greater proportion of viable blastocysts being transferred in the cryopreservation group and therefore possibly exaggerating the estimated endometrial impact of COS. There is also the possibility that cryopreservation and thaw actually improve some proportion of embryos, and if true, this would cause the effect attributed to COS to be exaggerated.

The present study used an antagonist protocol in the cycles of ovarian stimulation, and this may have affected the results, given that lower success rates have been reported in antagonist protocols (23). However, a previous randomized controlled trial using an agonist protocol also found superior success rates in frozen–thawed embryo transfers when compared with fresh transfer (13).

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