ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Revisiting the management of recurrent implantation failure through freeze-all policy

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Objective: To determine whether a freeze-all policy for in vitro human blastocysts improves the ongoing pregnancy rate in patients with recurrent implantation failure (RIF).
Design: Prospective cohort study.
Setting: Single private center.
Patient(s): A total of 171 women with RIF divided into two groups: freeze-all policy group (n = 81) and fresh embryo transfer (ET) group (n = 90).
Intervention(s): Freeze-all policy.
Main Outcome Measure(s): Ongoing pregnancy rate.
Result(s): The clinical pregnancy rate (52% vs. 28%; odds ratio [OR] 1.86; 95% confidence interval [CI], 1.29–2.68) and ongoing pregnancy rate (44% vs. 20%; OR 2.2; 95% CI, 1.04–3.45) were statistically significantly higher in the freeze-all group than the fresh ET group, respectively. The implantation rate was also statistically significant (freeze-all group 44.2% vs. fresh ET group 15.8%; OR 2.80; 95% CI, 2.00–3.92).
Conclusion(s): The freeze-all policy statistically significantly improved the ongoing pregnancy and implantation rates. Thus, a freeze-all policy is likely to be the new key to helping open the black box of RIF. These findings also are useful for further investigating the adverse effect of controlled ovarian stimulation on in vitro fertilization outcomes. (Fertil Steril® 2017;■:■–■. ©2017 by American Society for Reproductive Medicine.)
Key Words: Freeze all policy, recurrent implantation failure

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Recurrent implantation failure (RIF), a distressing clinical problem that affects about 10% of intracytoplasmic sperm injection (ICSI) cycles (1), refers to the failure in a woman younger than 40 years to achieve a clinical pregnancy within three or more consecutive embryo transfer (ET) cycles in which four good-quality embryos are transferred (2). Recurrent implantation failure may be attributed to multiple or interlinked causes, such as defective embryonic quality, inadequate endometrial receptivity, or both (3).

Various approaches have been used to improve ICSI outcomes in cases of RIF, such as attempts to improve the quality of embryos, the receptivity of the endometrium, or the interaction between embryos and endometrium (4, 5). Randomized, controlled studies have indicated that blastocyst transfer and salpingectomy may improve clinical outcomes in couples with RIF (2). Hysteroscopy in the preceding cycle has been reported to improve pregnancy outcomes in couples with three or more failed ET cycles (6), but this evidence has recently been contradicted by a multicenter, randomized controlled trial that showed hysteroscopy to be of no value in RIF (7). Furthermore, other techniques such as assisted hatching are still considered controversial in RIF patients (8), and the same debates

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continue with zygote intrafallopian transfer, cocultures, and preimplantation genetic screening (2, 3, 9).

Embryo implantation, a highly regulated process influenced by ovarian hormones, requires a receptive endometrium, a functional blastocyst, and reciprocal blastocyst–endometrium interaction (10). Supraphysiologic hormone levels during the follicular phase (11) of controlled ovarian stimulation (COS) may result in reduced endometrial receptivity and an impaired uterine environment, which in turn lowers the implantation rate in ICSI cycles and decreases the probability of pregnancy (12). In attempts to provide a more physiologic environment for ET and improve implantation, many studies have suggested a freeze-all policy in which the entire cohort of embryos is electively cryopreserved for transfer in a consecutive frozen-thawed cycle. Freeze-all may improve implantation as well as pregnancy when compared with fresh ET after ICSI (13–15).

We are not aware of any published studies to date that have evaluated whether a freeze-all policy can improve implantation and pregnancy in patients with RIF. Therefore, in hopes of establishing a new approach for RIF management, we investigated whether a freeze-all policy improves ongoing pregnancy rates in women with RIF.

MATERIALS AND METHODS

Patient Selection and Study Design

This prospective cohort study was conducted at a specialized fertility and gynecology center from April 2014 to October 2016. The research ethics committee of the center in which the study was conducted approved the protocol. On the day of oocyte retrieval and before the retrieval procedure, all couples were asked to sign informed consent forms with all of the details of the study written out and explained.

Women were eligible for the study if they were younger than 38 years, had no uterine abnormalities as assessed by a transvaginal ultrasound, had no post-ICSI pregnancies after three fresh ET cycles with ≥ 4 high-quality embryos transferred, had unexplained implantation failure, and agreed to participate and signed an informed consent form. High-quality embryos were defined as [1] cleaved—seven to eight cells on day 3, with symmetrical blastomeres and no or less than 10% fragmentation by volume according to Istanbul consensus (16); or [2] blastocyst stage—AA, AB, and BA embryos that reached stage 3, 4, or 5 of blastocoelic expansion on day 5 of culture according to the Gardner and Schoolcraft grading system (17). The study design also included midluteal pituitary down-regulation COS cycles, only fresh semen samples, and women with ≥ 8 mm endometrial thickness at the day of the human chorionic gonadotropin trigger shot. All ICSI cycles that did not result in day-5 blastocysts were excluded.

A total of 171 women with RIF were randomly assigned on the day of oocyte retrieval by use of a computer-based Microsoft Excel spreadsheet into two groups: the freeze-all group, which included 81 women who underwent the freeze-all protocol on day 5 after ICSI followed by a consecutive frozen-thawed embryo transfer (FET); and the fresh ET group (comparison group), which included 90 women who underwent a conventional ICSI followed by day-5 fresh ET. Assigning the participants into the freeze-all or fresh ET protocols was performed through an Excel table, where the first woman was assigned to the freeze-all group and the second woman assigned to the fresh ET; this sequence continued consecutively throughout the study. This study limited the treatment to only one cycle per participant. No hysteroscopies or saline sonograms were performed during in the treatment cycles for the enrolled patients.

COS and Oocyte Retrieval

Pituitary down-regulation was achieved by starting gonadotropin-releasing hormone agonists (Lucrin; Abbot) on day 21 of the preceding cycle, and it was continued throughout the treatment cycle. On treatment cycle day 2, recombinant follicle-stimulating hormone (FSH, Gonal-F; Serono) and/or human menopausal gonadotropin (Menogon; Ferring) were initiated after laboratory and ultrasound confirmation of down-regulation. The dosage of FSH and/or human menopausal gonadotropin was individualized at 150–450 IU daily, according to patient age, body mass index, antral follicle count, and response to ovarian stimulation. When three or more follicles were ≥ 18 mm and the mean diameter of the main cohort was 14–15 mm, ovulation was induced by injection of 250 μg/0.5 mL of recombinant human chorionic gonadotropin (Ovitrelle; Serono). Oocyte retrieval was performed 36 hours after the recombinant human chorionic gonadotropin trigger under transvaginal ultrasound guidance.

Semen Sample Processing, Oocyte Denudation, and ICSI

In all patients, ICSI was performed using fresh sperm ejaculates. The semen samples were collected by masturbation after an abstinence period of 3 to 5 days. Semen samples were prepared using a discontinuous sperm gradient centrifugation (ISolate; Irvine Scientific).

Oocytes were denuded 4 hours after retrieval using 40 μL of hyaluronidase (Irvine Scientific) for 30–60 seconds with mechanical aid pipetting. The metaphase-II oocytes were inseminated by ICSI at $\times 400$ original magnification. Groups of three injected oocytes per droplet were cultured in oil-covered (Irvine Scientific) droplets of 50 μL of Single Step Medium (Irvine Scientific) with 10% Serum Substitute Supplement protein (Irvine Scientific). The injected oocytes were then cultured in a gas phase of 6% CO_2, 5% O_2, 89% N_2 inside an incubator chamber at 37°C, which was continued throughout the 5 days of culture (same gas phase and temperature).

Zygote and Blastocyst Morphology Assessment

The fertilization check was performed 17 ± 1 hours after ICSI, and oocytes with two pronuclei were considered normally fertilized. The morphologic features of the blastocysts were assessed according to the Gardner and Schoolcraft criteria (17). Three different parameters were used for grading blastocysts: expansion and hatching stage, and grades of inner cell mass and trophectoderm. Grade A blastocysts included expansion and hatching of 4–6 and inner cell mass/trophectoderm of AA, AB, and BA. Grade B blastocysts included