ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Frozen-thawed day 5 blastocyst transfer is associated with a lower risk of ectopic pregnancy than day 3 transfer and fresh transfer

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Objective: To determine the ectopic pregnancy rate with fresh versus frozen-thawed embryo transfers, and factors associated with ectopic pregnancy in patients undergoing IVF-ET.
Design: Retrospective analysis.
Setting: Institutional IVF center.
Patient(s): A total of 3,183 patients who received 3,340 blastocysts transfers: 1,994 fresh transfers and 1,346 frozen-thawed transfers.
Intervention(s): Patients received fresh day 3 embryos (F-D3 group), fresh day 5 blastocysts (F-D5 group), frozen-thawed day 3 embryos (T-D3 group), or frozen-thawed day 5 or 6 blastocysts (T-D5 and T-D6 groups).
Main Outcome Measure(s): Ectopic pregnancy rate.
Result(s): The ectopic pregnant rates were 2.4% in the F-D3 group, 1.7% in F-D5, 1.9% in T-D3, 0.3% in T-D5, and 0.5% in T-D6. The ectopic pregnant rate of the T-D3 group was significantly greater than that of the T-D5 and T-D6 groups (1.9% vs. 0.3% and 0.5%). The ectopic pregnancy rate of the F-D5 group was significantly greater than that of the T-D5 group (1.7% vs. 0.3%).
Conclusion(s): Frozen-thawed day 5 blastocyst transfer is associated with a lower ectopic pregnancy rate than frozen-thawed day 3 transfer and fresh transfer in patients undergoing IVF-ET. (Fertil Steril® 2014;■:■–■. ©2014 by American Society for Reproductive Medicine.)
Key Words: Blastocyst, ectopic pregnancy, embryo, IVF, ovarian stimulation

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The incidence of ectopic pregnancy is ~1%–2%, and ~98% of ectopic pregnancies occur in the fallopian tube (1). The primary causes of tubal pregnancy include tubal inflammation, tubal obstruction, and tubal dysplasia or functional abnormalities, which affect the embryo movement from the fallopian tube to the uterine cavity and thereby result in an ectopic pregnancy (2). During IVF-ET, the embryo is directly transferred into the uterine cavity; therefore, in theory the incidence of ectopic pregnancy should be low. However, among patients who have become pregnant after IVF-ET the incidence of tubal pregnancy is 2%–5% (3, 4), which is 2%–3% higher than the incidence of ectopic pregnancy in women who conceive naturally (2). Studies have suggested that the primary reason for this increased rate is various tubal pathologic changes (5–9). For a tubal pregnancy to occur in an IVF cycle, however, the embryo must move from the uterine cavity into the fallopian tube and some authors have suggested that increased uterine contractility in stimulated cycles is responsible for pushing the embryo into the tube (10). Other authors have suggested there is a negative effect on the endometrium due to ovarian stimulation (11) or stronger signals from the tubal epithelia than from the uterine epithelia for implantation (12). Most embryos transferred into the uterine cavity during IVF-ET are in the cleavage stage. During natural conception,

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embryos of this stage are still located in the fallopian tube and enter the uterine cavity only after they develop into blastocysts. Therefore, day of transplantation is a variable that has been examined with respect to the ectopic pregnancy rate, and study has suggested that there is no difference in the ectopic pregnancy rate between day 3 and day 5 transfers (13). Other variables to be considered are type of embryo (fresh vs. frozen-thawed) and type of cycle (natural vs. stimulated). A number of studies have suggested that the ectopic pregnancy rate is lower after frozen-thawed embryo transfer than after fresh transfer (11, 14), and others have suggested no difference (15–18).

The purpose of the present study was to determine if there is a difference in the ectopic pregnancy rate with fresh versus frozen-thawed transfers in patients undergoing IVF-ET. We also sought to determine factors associated with ectopic pregnancy and if there is an influence of transfer day.

MATERIALS AND METHODS

Patients

This study was approved by the Institutional Review Board of the Sixth Affiliated Hospital, Sun Yat-Sen University, and owing to the retrospective nature the requirement of informed consent was waived. This was a retrospective case-control trial that included patients who became pregnant (intrauterine and ectopic pregnancies) after receiving IVF-ET at our reproductive center from June 2010 to November 2013. Patients with combined intrauterine and ectopic pregnancies were excluded. For analysis, patients were grouped as follows: patients receiving fresh day 3 embryos (F-D3 group), fresh day 5 blastocysts (F-D5 group); frozen-thawed day 3 embryos (T-D3 group); frozen-thawed day 5 blastocysts (T-D5 group); and frozen-thawed day 6 blastocysts (T-D6 group). Each group was then divided into an intrauterine pregnancy group and an ectopic pregnancy group.

Polycystic ovary syndrome (PCOS) was defined as the presence of ≥ 2 of the after 3 criteria after excluding other diseases such as congenital adrenal hyperplasia, Cushing syndrome, and androgen secreting tumors: 1) sparse ovulation or anovulation; 2) clinical manifestations of androgen excess and/or hyperandrogenism; 3) ultrasonography showing polycystic ovary (unilateral ovary or bilateral ovaries have more than 12 ovarian follicles 2–9 mm in diameter and/or ovarian volume >10 mL) (19).

Male-factor infertility was defined as oligoasthenoteratozoospermia or an azoospermia (sperm concentration $<15 \times 10^6$/mL, vitality $<40\%$, motility $<32\%$, normal morphology $<4\%$ [20]).

IVF-ET

Controlled ovarian stimulation protocol. A conventional long protocol of 1.3 mg long-acting GnRH agonist (Ipsen Pharmaceuticals), injected at the midluteal phase of the previous menstrual cycle, was used at our center. Fourteen days later, 150–300 IU recombinant FSH (Puregon, NV Organon; or Gonal-F, Serono) was administrated and continued daily. When there were more than two follicles ≥ 18 mm in diameter as determined by transvaginal ultrasound, 10,000 IU hCG (Livzon Pharmaceutical Group) was administrated and oocyte retrieval performed 36 hours later.

IVF and embryo culture. IVF was carried out 39–40 hours after hCG injection. Intracytoplasmic sperm injection (ICSI) was performed if the concentration of motile sperm was $<1 \times 10^6$/mL after sperm preparation, otherwise a conventional IVF method was used. The result was observed 16–18 hours after fertilization, and appearance of two pronuclei was considered to indicate normal fertilization. The embryo was then placed in Quinn Advantage Cleavage Medium (Sage) and cultured until 72 hours after ovulation (37°C; 6% CO_2, 5% O_2, 89% N_2). The embryo was then placed in Quinn Advantage Blastocyst Medium (Sage) and cultured until 5–6 days after ovulation with the same incubator conditions.

Observation of cleavage-stage embryos. Embryo morphology was observed 48 hours (day 2) and 72 hours (day 3) after oocyte retrieval. The grading criteria for the embryos were as follows: grade 1: the size of the blastomeres was uniform, with no fragmentation; grade 2: the blastomere size was slightly uneven with fragmentation $<20\%$; grade 3: the blastomere size was heterogeneous or with fragmentation 20%–50%; and grade 4; fragmentation $>50\%$. Available embryos were defined as 72 hours after oocyte retrieval with more than five cells and grade 1–2. Both transferred and frozen embryos met the standard of available embryos.

Culture and observation of blastocysts. Blastocysts were observed on the 5th and 6th days after oocyte retrieval, and each blastocyst was evaluated based on the Gardner grading system, which grades according to the degree of cyst expansion (21). Blastocysts meeting the following criteria were transferred or frozen: grade 2 at D5 or than grade $>$3BB (both B grades in the evaluations of ICM and TE) at D6.

In the F-D3 group, one to three available embryos were transferred on the 3rd day after oocyte retrieval. In the F-D5 group, one to two blastocysts of grade $>$3BB were transferred on the 5th day after oocyte retrieval; grade 2 blastocysts were transferred if the blastocysts did not reach grade 3 on the 5th day after oocyte retrieval.

Embryo freezing and thawing. Vitrification was performed for embryo freezing with the use of a Cryotop (Kitazato Corp.) device. Solution A was a HEPES solution (Sage) containing 12 mg/mL human serum albumin (Sage). Solution B was solution A with the addition of 7.5% dimethyl sulfoxide (Sage) and 7.5% ethylene glycol (EG; Sage). Solution C was solution A with the addition of 15% dimercaptosuccinic acid, 15% EG, and 1 mol/L sucrose (Sage). Briefly, the embryo was balanced with the use of solution A for 1 minute, moved into solution B for 3–5 minutes, and then placed in solution C. The sample was placed in a tube within 60 seconds and then stored on liquid nitrogen.

For thawing, the sample was taken out of the liquid nitrogen and rapidly placed in a 1.0 mol/L sucrose solution for 1 minute, 0.5 mol/L sucrose solution for 1 minute, 0.33 mol/L sucrose solution for 2 minutes, 0.2 mol/L sucrose solution for 3 minutes, and then placed in HEPES solution for 5 minutes.