Slightly lower incidence of ectopic pregnancies in frozen embryo transfer cycles versus fresh in vitro fertilization-embryo transfer cycles: a retrospective cohort study

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Objective: To analyze the incidence of ectopic pregnancies (EPs) in fresh and frozen/thawed cycles.
Design: A retrospective cohort study on the incidence of EPs in all fresh IVF cycles from January 2002 until December 2012. This was compared with the incidence of tubal pregnancies in patients undergoing transfer of frozen/thawed embryos during the same time period.
Setting: The IVF program at Fertility Center, AZ Jan Palfijn, Gent, Belgium.
Patient(s): A total of 11,831 patients undergoing IVF (i.e., the entire population of the IVF Center) were retrospectively analyzed.
Intervention(s): The IVF cycles, fresh IVF-ET, frozen/thawed ET. Laparoscopy for treatment of EP.
Main Outcome Measure(s): Primary end point: incidence of EPs in both groups. Secondary end points: clinical pregnancy rate (PR), rate of EPs per clinical pregnancy.
Result(s): In the fresh IVF cycle group, 10,046 patients underwent oocyte retrieval; 9,174 of them had an ET; 2,243 of these patients had a clinical pregnancy. Of these, 43 (0.47%) appeared to have an ectopic localization of their pregnancy. In the group of the patients undergoing frozen/thawed ET (1,785 patients) there were 467 pregnancies and 6 ectopic implants (0.34%). The incidence of the EPs per established clinical pregnancy was 1.92% for the fresh vs. 1.28% for the frozen/thawed cycles.
Conclusion(s): No significant difference could be demonstrated on the incidence of EP in fresh IVF cycles vs. frozen/thawed cycles in a large cohort of patients. (Fertil Steril® 2014;101:162–5. ©2014 by American Society for Reproductive Medicine.)
Key Words: Ectopic pregnancy, IVF, frozen/thawed cycle, relative risk

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Infertility patients have always been associated with a higher risk of tubal pregnancy (1). Tubal surgery, including microsurgical operations and laparoscopic interventions such as salpingectomy or tubal reanastomosis, led to an astonishing number (15%–20%) of ectopic pregnancies (EPs) (2). Even after IVF where the tubal passage was bypassed and the embryos were transferred directly in the uterine cavity, still a significant number of ectopic localizations of the gravidity were found (3). The reasons are numerous, such as tubal disease (4), increased uterine contractions due to ovarian stimulation (5), dysfunction of the uterine musculature due to high P levels (6), and side effects of the medication (7).

Recently it has been reported (8) that the EP rate is significantly reduced after the replacement of frozen/thawed embryos. To corroborate a retrospective analysis of the data of the IVF Center of the Hospital Jan Palfijn in Gent (Belgium) was performed from January 1, 2002 to December 31, 2012.

MATERIALS AND METHODS

All patients who underwent a fresh IVF treatment and had an ET between

Received April 24, 2013; revised September 16, 2013; accepted October 3, 2013; published online November 12, 2013.
W.D. has nothing to disclose. K.O. has nothing to disclose. G.M. has nothing to disclose. P.D. has nothing to disclose.
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Fertility and Sterility® Vol. 101, No. 1, January 2014 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2013.10.002

January 1, 2002 and December 31, 2012, were included (group A). They were compared with all patients who had a transfer with a frozen/thawed cycle during the same period of time (11 years) (group B). The treatment procedures and the comparison of data were approved by the local ethical committee as being part of the standard quality control measurements.

Group A patients were stimulated with FSH (recombinant FSH; Puregon, MSD and Gonal F, Serono), sometimes in combination with urinary extracted FSH (hMG; Menopur, Ferring). The stimulation was performed with pituitary suppression and the administration of GnRH antagonists (Cetrotide 0.25 mg, Serono or Orgalutran 0.25 mg/0.5 mL, MSD) from day 6 onward until triggering. Final oocyte maturation was achieved by administration of 5,000–10,000 IU of hCG (Pregnyl, MSD) as soon as three follicles reached 18 mm in diameter at the time of ultrasound evaluation of the stimulation. Historically, 10,000 IU hCG was administrated until 2008 to trigger ovulation. From 2009 onward the dose of hCG was reduced to 5,000 IU only to minimize the risk for ovarian hyperstimulation syndrome (OHSS). In less than 10% of patients—selected indications such as endometriosis or poor embryo quality in a previous stimulation cycle—down regulation of the cycle with GnRH agonists before ovarian stimulation (long protocol stimulation) was chosen for controlled stimulation. In these patients the triggering was performed by the administration of hCG. The oocyte retrieval took place by an ultrasound-guided transvaginal approach 36 hours after triggering. Fertilization of the oocytes took place either by IVF or intracytoplasmic sperm injection (ICSI), according to the sperm quality. An indication for ICSI was found if the total number of fast progressive motility sperm was $\leq 1.10^6$ and/or normal morphology was $\leq 3\%$ (Krüger criteria) or if a poor fertilization was seen in previous cycles. The embryos were cultured in either G1 Plus (Vitrolife) or ISM1 (Origio). Embryo transfer was generally performed on day 3 after fertilization. Only in case of repeated failure of implantation and if sufficient embryos of good quality were available, blastocyst culture was chosen with ET on day 5 after pick-up. If only one or two embryos were available for transfer, it took place on the second day after fertilization (<5%). The number of transferred embryos varied from one to three, according to the Belgian legislation (Table 1). Supernumerary embryos of good quality (9) A or B type, less than 10% fragmentation, seven to eight-cell stage on day 3, or blastocyst stage on day 5 were frozen (slow freezing with cryogenesis).

In group B patients the frozen/thawed embryos were transferred after optimalization of the cycle with clomiphene citrate (CC) (100 mg/d; Clomid, Sanofi-Aventis) from day 5 until day 9 of the cycle, and induction of ovulation with 5,000 IU of hCG as soon as a follicle of 20 mm was visualized by ultrasound examination. For the patients without ovulation, E_2 valerate (Progynova, Bayer) was given vaginally in increasing doses (from 2 mg/d on day 1 to 6 mg/d on day 9). Progesterone (600 mg) (Utrogestan, Piette-Besins) was administered to mimic the luteal phase as soon as a 9-mm thick endometrium was visualized. Embryo transfer took place 5–7 days after hCG administration. In the anovulatory group the embryo replacement was performed 4–6 days after the initiation of micronized P (Utrogestan).

Human chorionic gonadotropin and P levels were measured 13 days (day 3 of transfer) after transfer, except for the blastocyst transfers where a pregnancy diagnosis already took place 11 days after transfer. For the patients who had a transfer on day 2 (4-cell stage), hCG level was measured on day 14 after transfer. In all patients with positive hCG measurement, the administration of micronized P vaginally was continued and a confirmation of the clinical pregnancy (echographic visualization of gestational sac) was realized by vaginal ultrasound examination approximately 10 days later. Subsequent follow-up of the pregnancy development, the evolution of the stimulated ovaries, and the presence of fetal heart activity 5 weeks after transfer were established.

The diagnosis of EP was made either by direct extrauterine visualization of the gestational sac or by finding an empty uterine cavity with increasing hCG levels more than 500 ng/mL. In all patients suspect for an ectopic localization of the pregnancy, the final confirmation was made by direct laparoscopic visualization. Laparoscopy was performed in all patients with hCG levels >1,000 ng/mL and with an empty cavity on ultrasound examination. Laparascopic longitudinal incision of the tube, or salpingectomy in badly damaged tubes, was performed in all of those patients. This intervention was, without exception, performed laparoscopically. Especially in the group of tube-conserving surgery an adequate follow-up of the decreasing hCG levels postoperatively was established.

The percentages EP per ET obtained in patients undergoing fresh autologous ETs and those undergoing transfer with frozen/thawed embryos were compared. The same was done for the percentage of EPs per clinical pregnancy. The Fisher's exact test was performed one-sided at the 5% significance level. In doing so it is tested whether it is more likely to have an EP when receiving fresh ET (compared with frozen/thawed ET).

TABLE 1

Belgian legislation limits for number of transferred embryos.

Age cycleNo.	< 36 y-1 d	36 y to 40 y-1 d	40 y to 43 y-1 d	43 y to 45-1 d
1	1	2	No max	No max
2	1 or 2	2	No max	No max
3	2	3	No max	No max
4	2	3	No max	No max
5	2	3	No max	No max
6	2	3	No max	No max
Cryopreserved cycle	2	2	2	2

Decleer. Relative risk of EUG in frozen IVF cycle. Fertil Steril 2014.

RESULTS

From January 1, 2002 to December 31, 2012, 9,174 fresh autologous ETs were performed. They were the result of 10,046 oocyte retrievals from the same period. This number resulted in 2,243 pregnancies. The overall clinical pregnancy

TABLE 2

Results 2002-2012.

	Fresh	Frozen	Total	Significant (*P* value)
Retrieval	10,046		10,046	–
Retrieval with ET	9,174		9,174	–
Cryopreservation		1,785	1,785	–
Clinical pregnancy (n)	2,243 (24.45%)	467 (26.16%)	2,710	–
Ectopic pregnancy (n)	43	6	49	–
Ectopic pregnancy per ET	0.47%	0.34%	0.45%	NS 0.29 (>.05)
Ectopic pregnancy per clinical pregnancy	1.92%	1.28%	1.81%	NS 0.23 (>.05)

Note: NS = not significant.

Decleer. Relative risk of EUG in frozen IVF cycle. Fertil Steril 2014.

rate (PR) per oocyte retrieval for this 11-year period was 22.3% and the PR per transfer was 24.4%. The implantation site in 43 patients (0.47%) was an ectopic location of the trophoblast by ultrasound confirmation. In terms of number of ectopics per realized pregnancy the relative risk was 1.92%.

Concerning the group of patients who underwent transfer with frozen/thawed embryos, the number of treated patients was 1,785 during the period from beginning in January 2002 until the end of December 2012 and yielded 467 pregnancies. Only 6 of them were noted to be extrauterine gravidities. This was equal to a relative risk of 0.34 for a patient who started with a frozen ET cycle to end up finally with an EP. In terms of the risk of the achieved pregnancy being localized outside of the womb, the risk of the patient with frozen/thawed ET was 1.28%.

In two patients (one in the frozen group and one in the fresh cycle group) an heterotopic pregnancy was found with a gestational sac both in the uterine cavity and outside of the uterus (Table 2).

DISCUSSION

Uterine motility, and more specific fundic contractions, are important in the implantation phase as shown by recent studies about the negative connotation of enhanced uterine contractility, the implantation rates, and hence the final PRs (10, 11). For this reason, during the past years attempts have been made to reduce the contractility of the womb by administration of medical drugs, such as oxytocin antagonist-like Atosiban (Ferring) to enhance the implantation rates, especially in woman with repeated failure of implantation and good embryo quality (12, 13). It was obvious that the stimulation itself and the peritoneal irritation that was caused by the ovarian enlargement secondary to medical stimulation of the ovaries by gonadotropins, followed by the surgical aggression with pick-up were among the most important factors influencing the enhanced contractility of the womb demonstrated in patients undergoing IVF (14). This also could explain the difference of ectopic implantation rate between embryos transferred in a stimulated cycle vs. embryos transferred in an optimized cycle, or a cycle with endometrium preparation mimicking the natural endometrial growth. In those cases there was no ovarian hyperstimulation, no peritoneal irritation, no transvaginal needle aspiration, and no surgical intervention.

Even in terms of the respective rates of EPs per obtained PR, the number of ectopic localizations of the pregnancy was lower in the group that achieved a pregnancy after a transfer of frozen and subsequently thawed embryos, although the PR per transfer in our center was lower in this group of patients than in the fresh autologous ET group. It is to be expected that by the introduction of the new technique of freezing embryos by vitrification, the implantation rates will easily equal the implantation rates of fresh embryos (15). This would directly imply that the relative risk of ectopic implantation of a transferred embryo would decrease further in terms of risk per obtained pregnancy. The decreased risk is one of the most important and dangerous complications of IVF (and fertility treatment in general), by freezing embryos in the stimulated cycle and transferring them in a natural cycle or an artificial cycle mimicking the natural, would be a further argument to freeze all embryos. Especially in those patients who have other disadvantages and potential risks in the original stimulated cycle, as in patients with polycystic ovary (PCO) with enhanced risk for OHSS, the decision to freeze all obtained embryos could reduce the risk for OHSS and extra uterine gravidity, without losing chances in the realization of an intact intrauterine pregnancy.

A very interesting feature was the realization that half of the EPs after frozen ET (3 of 6) occurred in the past year. All of these patients had no spontaneous cycle and had endometrium preparation with E_2 valerate (4–6 mg daily). This coincidence might be due to a change of cilia movement in the fallopian tubes induced by the administration of exogenous E_2.

In general, differences between the number of ectopic locations are much less than those illustrated in the article by Shapiro et al. (8). Maybe these investigators were dealing with patients with prolonged embryo culture and blastocyst transfers.

In conclusion, although the study is a retrospective analysis, the cohort of analyzed patients is sufficient to clearly diminish the relative risk of ectopic implantation in patients after transferring a frozen/thawed ET. It is to be expected that this advantage will be empowered with the higher implantation rates with the new embryo conservation techniques (like vitrification) being introduced at present. Additional, preferably prospective, cohort studies will be needed. This to evaluate and measure the differences in terms of implantation rates both in the uterus or in the fallopian tubes.

Acknowledgments: The authors acknowledge the study nurses Lieve Declercq and Caroline Van de Steene and the staff of the IVF Center AZ Jan Palfijn Gent for their invaluable help in the retrospective research of the data of more than 10,000 patients. We also thank Ir. Katrien Verschueren (Living Statistics) for the statistical analysis.

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