

Comparable implantation rates with fresh vs frozen embryo transfer suggests that controlled ovarian hyperstimulation has an adverse effect on conception outcome

J. H. Check, D. Katsoff, D. Brittingham, D. Summers-Chase, C. Wilson

*The University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School at Camden
Cooper Hospital/University Medical Center, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology & Infertility,
Camden, New Jersey (USA)*

Summary

Purpose: A retrospective comparison of fresh vs frozen embryo pregnancy rates.

Methods: All frozen embryos transferred used in the analysis including deselected embryos from the oocyte retrieval cycle, and twice-frozen embryos.

Results: Pregnancy and implantation rates following fresh or frozen embryo transfers were similar.

Conclusion: The similar outcomes despite the obvious disadvantages for the frozen-thawed embryo suggests that some other factor reduces the chance of embryo implantation on oocyte-retrieval cycles. An adverse affect of controlled ovarian hyperstimulation on the uterine environment is a strong possibility.

Key Words: Frozen Embryo Transfer; Deselection; Ovarian hyperstimulation.

Introduction

There are data suggesting that controlled ovarian hyperstimulation (COH) with the stimulation of multiple mature follicles for oocyte retrieval may exert an adverse influence on subsequent implantation. One study matched in vitro fertilization (IVF) patient characteristics to those of oocyte donors with regard to age and previous conception [1]. Despite the transfer of similar numbers of embryos and findings of no difference in embryo morphology for standard IVF patients and recipients, the clinical and ongoing pregnancy rates (PRs) and implantation rates were significantly higher in recipients [1]. Another study, using shared oocytes for donors and recipients, found twice the PR in recipients versus donors despite the recipient group averaging ten years older [2]. The same group subsequently repeated this study to be sure that the previous one was not influenced by the presence of hydrosalpinges in a much higher percentage of donors than recipients, but found, once again, significantly higher implantation rates in the recipients [3]. Thus these two latter studies support the one by Paulson *et al.* that COH may adversely affect subsequent PRs and implantation rates [1].

Though transferring frozen-thawed embryos would escape the potential adverse effect of COH, the consequences of freeze-thawing may negatively affect implantation rates. However, if the adverse affect of freeze-thawing was less than the affects of COH, it may be better to freeze all embryos and defer fresh ET. Before embarking on a prospective randomized trial to determine if it may be advisable to defer transfer on a stimu-

lated cycle, we retrospectively compared PRs and implantation rates in two age groups following fresh or frozen ET.

Materials and Methods

A retrospective comparison of fresh versus frozen embryo PRs and implantation rates according to age (<39 vs >40) for 1997 and 1998 were made. Normally, the intention was to transfer embryos in a cycle with COH. Sometimes, because of a risk of ovarian hyperstimulation syndrome, in the opinion of the clinicians, or because of an inadequate endometrial thickness (<8 mm), or a homogeneous hyperechogenic pattern by sonography, all embryos were cryopreserved [4-10].

Embryo selection was used when transferring either fresh or frozen embryos by allowing twice as many embryos as intended to transfer to cleave to the 72 hour stage, and the best half, based on number of blastomeres and fragmentation, were transferred; those remaining were either frozen or refrozen (or discarded if very poor quality). Fragmentation scores were based on assigning 1 to those with no fragmentation; 2 for those with 25% or less (but not zero); 3 for 26-50%; and 4 for those with >50% fragmentation. The extra embryos not in the group used for the selection of the embryos for fresh transfer were cryopreserved at the 2 pronuclear (2PN) stage.

Whenever 2PN cryopreserved embryos were available they were used for thawing for frozen ET because their quality was unknown as opposed to the multi-cell embryos where they were frequently of lesser quality because of the deselection process. If there were insufficient 2PN embryos to transfer, they were mixed with multi-cellular embryos. There were many retrieval cycles where all embryos were allowed to go to multi-cell stage because the number of embryos formed were not so large, and thus only frozen multi-cell deselected embryos were available for transfer.

Thus frozen ETs could exist of only those cryopreserved at the 2PN stage, or exclusively those at the multi-cell stage or could be mixtures of both. Multi-cell embryos used for transfer

Revised manuscript accepted for publication September 25, 2000

could be once-frozen or twice-frozen. Refrozen embryos were the last ones used [11].

All ETs occurred three days after oocyte retrieval. Assisted hatching using acidic Tyrode's solution was performed on all frozen-thawed embryos prior to transfer [12]. Hatching was performed also on fresh embryos especially in older patients or those younger ones with thickened zona pellucida [13].

The embryos were frozen using a simplified method in which a slow cooling program is started at the seeding temperature of -6°C in an alcohol-bath controlled-rate freezer. 1,2 propanediol was used as the cryoprotectant [14]. A one-step fast thawing procedure at room temperature was used and the cryoprotectant was removed from the embryos in one step with a sucrose solution [14].

Various COH regimens were used including leuprolide acetate (LA) started in mid-luteal phase and continued even when gonadotropins (usually 300 IU daily) were started 11 days later (preferred regimen for younger patients); or LA was started in the early follicular phase and continued even when 300 IU gonadotropins were started usually around day five (regimen used by many women in the late 30's whose baseline FSH was normal). Patients age 40 or over or those with elevated gonadotropins on day three were frequently treated in mid-luteal phase with a reduced amount of LA (0.5 mg) for ten days only with gonadotropins started at 450 IU daily, or a microdose protocol where they received only 0.1mg LA from early follicular phase with 450 IU gonadotropins started on day three and this was usually preceded by one cycle of oral contraceptives.

Most frozen ET cycles used oral estradiol started on day two at 2 mg with graduating doses after five days up to 4 mg x four days, then 6 mg for five days, or sometimes 8 mg if the endometrial lining was insufficient. If premature luteinization occurred, then LA was given at 1 mg x ten days beginning in mid-luteal phase and oral estradiol was started after ten days of LA.

All patients having ET in 1997 and 1998 were included without exception. Though there have been some studies suggesting that embryos fertilized by intracytoplasmic sperm injection do not fare well with freezing [15], that has not been our experience [16] and these patients were not excluded.

Results

The clinical and viable PRs and implantation rates following fresh versus frozen ET in women age ≤ 39 and those ≥ 40 are shown in Table 1. No significant differences were seen when comparing any of these parameters between fresh versus frozen transfers according to age group in either 1997 or 1998.

Table 1. — Pregnancy (clinical and viable) and implantation rates - fresh vs frozen transfer.

	Fresh (≤ 39)	Frozen (≤ 39)	Fresh (≥ 40)	Frozen (≥ 40)
<i>1997</i>				
No. Transfers	292	212	93	43
Clinical PR	46.9%	47.6%	22.6%	27.9%
Delivered PR	42.8%	41.0%	19.4%	23.2%
Implantation rates	21.9%	20.6%	8.9%	11.6%
Avg. cell stage transferred	6.3	5.1	5.9	5.3
Avg. fragmentation score	2.0	2.1	2.0	2.1
<i>1998</i>				
No. Transfers	296	208	94	54
Clinical PR	47.9%	42.8%	31.4%	29.6%
Delivered PR	44.3%	34.6%	17.4%	18.5%
Implantation rates	21.6%	20.2%	11.5%	13.5%
Avg. cell stage transferred	6.4	5.6	6	5.1
Avg. fragmentation score	2.1	2.1	2.1	2.0

Discussion

There are data suggesting that increasing the number of blastomeres in the embryos transferred correlates positively with outcome [17]. The frozen embryos in both age groups and in both years studied had a lower number of blastomeres compared to fresh embryos.

The fact that no differences were found between PRs or implantation rates following fresh or frozen ETs despite the trauma of freeze-thaw (sometimes even twice) and the adverse advantage of embryo deselection, supports previous data that the COH regimen adversely affects implantation [1-3]. The possibility thus exists that by deferring fresh ET with cryopreservation of all embryos, the PRs and implantation rates of first ETs may increase by allowing the best quality embryos to be transferred after freeze-thaw.

This retrospective comparative study, and one case study [18] has provided us, and hopefully will provide other centers, the encouragement to perform a prospective randomized study comparing PRs and implantation rates following first transfers in which one group will receive fresh embryos with a selection procedure (the same as is presently being conducted), and the other group will have all embryos cryopreserved at the 2PN stage, and transfer with embryo selection will occur two months later after thawing the frozen embryos on artificial estrogen and progesterone replacement. We plan on initiating the study first in the 40 year and older group.

If an IVF center is not interested in deferring all fresh ETs in favor of frozen ETs on non-hyperstimulated cycles, they should certainly give some consideration to this approach if the patient has failed to conceive following two or three fresh transfers.

References

- Paulson R. J., Sauer M. V., Lobo R. A.: "Embryo implantation after human in vitro fertilization: importance of endometrial receptivity". *Fertil. Steril.*, 1990, 53, 870.
- Check J. H., O'Shaughnessy A., Lurie D., Fisher C., Adelson H. G.: "Evaluation of the mechanism for higher pregnancy rates in donor oocyte recipients by comparison of fresh with frozen embryo transfer pregnancy rates in a shared oocyte programme". *Hum. Reprod.*, 1995, 10, 3022.
- Check J. H., Choe J. K., Katsoff D., Summers-Chase D., Wilson C.: "Controlled ovarian hyperstimulation adversely affects implantation following in vitro fertilization-embryo transfer". *J. Assist. Reprod. Genet.*, 1999, 16, 416.
- Check J. H., Nowroozi K., Choe J., Dietterich C.: "Influence of endometrial thickness and echo patterns on pregnancy rates during in vitro fertilization". *Fertil. Steril.*, 1991, 56, 1173.
- Check J. H., Lurie D., Dietterich C., Callan C., Baker A.: "Adverse effect of a homogeneous hyperechogenic endometrial sonographic pattern, despite adequate endometrial thickness on pregnancy rates following in vitro fertilization". *Hum. Reprod.*, 1993, 8, 1293.
- Dickey R. P., Olar T. T., Cruole D. N., Taylor S. N., Rye P. H.: "Endometrial pattern and thickness associated with pregnancy outcome after assisted reproduction technologies". *Hum. Reprod.*, 1992, 7, 418.
- Gonen Y., Casper R. F.: "Prediction of implantation by sonographic appearance of endometrium during controlled ovarian stimulation for in vitro fertilization (IVF)". *J. In Vitro Fertil. Embryo Transfer*, 1990, 7, 146.

- [8] Grunfeld L., Walker B., Bergh P. A., Sandler B., Hofmann G., Navot D: "High-resolution endovaginal ultrasonography of the endometrium: A noninvasive test for endometrial adequacy". *Obstet. Gynecol.*, 1991, 78, 200.
- [9] Sher G., Herbert C., Maassarani G., Jacobs M. H.: "Assessment of the late proliferative phase endometrium by ultrasonography in patients undergoing in-vitro fertilization and embryo transfer (IVF/ET)". *Hum. Reprod.*, 1991, 6, 232.
- [10] Smith B., Porter R., Ahuja K., Craft I.: "Ultrasonic assessment of endometrial changes in stimulated cycles in an in vitro fertilization and embryo transfer program". *J. In Vitro Fertil. Embryo Transfer*, 1984, 1, 233.
- [11] Baker A., Check J. H., Lurie D., Hourani C., Hoover L. M.: "Pregnancy achieved with pronuclear-stage embryos that were cryopreserved and thawed twice: a case report". *J. Assist. Reprod. Genet.*, 1996, 13, 713.
- [12] Check J. H., Hoover L., Nazari A., O'Shaughnessy A., Summers D.: "The effect of assisted hatching on pregnancy rates after frozen embryo transfer". *Fertil. Steril.*, 1996, 65, 254.
- [13] Cohen J., Elsner C., Kort H., Malter H., Massey J., Mayer M. P. et al.: "Impairment of the hatching process following IVF in the human and improvement of implantation by assisted hatching using micromanipulation". *Hum. Reprod.*, 1990, 5, 7.
- [14] Baker A. F., Check J. H., Hourani C. L.: "Survival and pregnancy rates of pronuclear stage human embryos cryopreserved and thawed using a single step addition and removal of cryoprotectants". *Hum. Reprod. Update*, 1997, 2 (CD-ROM).
- [15] Van Steirteghem A. C., Van der Elst J., Van den Abbeel E., Joris H., Camus M., Devroey P.: "Cryopreservation of supernumerary multicellular human embryos obtained after intracytoplasmic sperm injection". *Fertil. Steril.*, 1994, 62, 775.
- [16] Hoover L., Baker A., Check J. H., Lurie D., Summers D.: "Clinical outcome of cryopreserved human pronuclear stage embryos resulting from intracytoplasmic sperm injection". *Fertil. Steril.*, 1997, 67, 621.
- [17] Jones G. M., Trounson A. O., Lolatgis N., Wood C.: "Factors affecting the success of human blastocyst development and pregnancy following in vitro fertilization and embryo transfer". *Fertil. Steril.*, 1998, 70, 1022.
- [18] Check J. H., Choe J. K., Nazari A., Summers-Chase D.: "Ovarian hyperstimulation can reduce uterine receptivity. A case report". *Clin. Exp. Obst. Gyn.*, 2000, 27, 89.

Address reprint requests to:
JEROME H. CHECK, M.D., Ph.D.
7447 Old York Road
Melrose Park, PA 19027 (USA)