Assisted Hatching Does Not Enhance IVF Success in Good-Prognosis Patients¹

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Purpose: The role of assisted hatching in good-prognosis IVF patients was evaluated in a prospective, randomized, controlled pilot study, which was followed by a retrospective observational series.

Methods: After assisted hatching was proved successful in a mouse embryo study, 20 good-prognosis IVF patients were randomly assigned to either assisted hatching (13) or no assisted hatching (7; the controls). Following this series, 27 good-prognosis IVF patients were retrospectively evaluated to determine the outcome with assisted hatching.

Results: In the prospective study, clinical pregnancies resulted from 3 (23%) of 13 patients in the hatching group, compared to 3 (43%) of 7 in the control group. Implantation rates were similar: 9.6% in the hatching group and 10.7% in the controls. In the retrospective series, the 11.1% implantation rate with assisted hatching was significantly less than the 42.9% implantation rate seen with traditional IVF.

Conclusions: Implantation and pregnancy rates are high in young women undergoing traditional IVF. Assisted hatching is not beneficial in these patients.

KEY WORDS: in vitro fertilization; micromanipulation; assisted hatching; implantation.

INTRODUCTION

Assisted hatching may improve success in poor-prognosis in vitro fertilization (IVF) patients, although its

role in those with a good prognosis is not clear (1). Assisted hatching adds to the cost and complexity of IVF, and there is a potential for damage to the embryo with this procedure. To determine if there is a benefit with assisted hatching in young, good-prognosis IVF patients, we initiated a series of studies. In this report, we describe the outcome of our initial laboratory quality control study, pilot prospective randomized series, and retrospective observational data with and without assisted hatching.

MATERIALS AND METHODS

An initial series of quality control (QC) studies was performed using frozen/thawed two-cell mouse embryos. Acid Tyrode's was used to create a gap in the zona, which resulted in a hatching rate of 23 of 23 on day 5. The hatching rate on day 5 for the nonmanipulated controls was 1 of 23.

In the prospective randomized study, 20 patients requiring IVF were recruited and computer randomized, 13 to the assisted hatching group, and 7 to the control group (no assisted hatching). Because funding was limited, additional patients could not be enrolled. Patients with no prior IVF experience were eligible if they were age 30 or younger, had a basal follicle stimulating hormone (FSH) level of less than 10 mIU/ml (conversion to SI units, 1.0), a normal endometrial cavity, and normal semen parameters including strict morphology (2). Patients 35 years of age or younger with prior IVF experience were eligible if at least six embryos resulted from the prior IVF attempt, the overall fertilization rate was at least 50%, and the uterine cavity was normal. Institutional review board consent was obtained for all patients.

Ovulation induction with FSH, 225 IU/day, was initiated after luteal-phase down-regulation with leuprolide acetate was achieved. Patients were monitored with

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serial ultrasound and estradiol levels. Human chorionic gonadotropin (hCG) was given when at least two follicles reached a mean diameter of at least 18 mm. Ultrasound-directed transvaginal oocyte retrieval was performed 35–36 hr later.

Oocytes were inseminated approximately 4–6 hr after retrieval and cultured in human tubal fluid (HTF; Irvine Scientific, Santa Ana, CA) supplemented with 7.5% synthetic serum substitute (SSS; Irvine Scientific), in microdroplets under oil. Pronuclear embryos were transferred to HTF with 15% SSS microdroplets under oil and cultured for 48 hr. Seventy-two hours after oocyte retrieval, the most advanced, morphologically normal-appearing embryos were selected for transfer (3).

Embryos from patients assigned to the hatching group were placed in 100- μ l microdroplets of HTF with Hepes and 15% SSS overlaid with oil. Acid Tyrode's introduced with a Narishige micromanipulator (Tokyo) was used to erode the zona (4). When the zona was eroded, expulsion of acid was stopped and the embryo was returned to the culture medium, rinsed thoroughly, and returned to the incubator until transfer.

Embryo transfer was performed 3 days after retrieval, and luteal support with hCG and progesterone was provided. Transvaginal ultrasound was performed to confirm an intrauterine pregnancy 4 weeks after transfer.

Patients in the retrospective analysis were limited to those age 30 years or younger who had a basal FSH level of 10 mIU/mL or less, a normal uterine cavity, and normal semen parameters. Assisted hatching was performed at the discretion of the embryologist, as described. Transfers in which no embryos underwent assisted hatching were compared to those in which at least 50% of the transferred embryos had assisted hatching. For this follow-up period, transfers were performed 2 days after oocyte retrieval.

Comparisons between the groups were made by *t* test, with the Mann-Whitney rank sum test performed when the test for normality failed. Implantation and pregnancy rates were compared by chi-square and Fischer exact tests.

RESULTS

In the prospective, randomized study, there was no difference in age, number of embryos transferred, number of blastomeres, or embryo grade when the hatching group was compared to the controls (Table I). The implantation rate per embryo transferred was 5 (9.6%)

Table I. Comparison of Assisted Hatching and Control Groups: Prospective Study

	Hatching	Control
Number	13	7
Age ^a	30 $\pm$ 0.9	30 $\pm$ 0.8
Prior pregnancy	7	5
Prior IVF	4	2
Diagnosis		
Tubal	8	5
Endometriosis	3	2
Unexplained	2	0
Transferred embryos ^a	4.0 $\pm$ 0	4.0 $\pm$ 0
No. blastomeres ^a	8.1 $\pm$ 0.4	7.3 $\pm$ 0.5
Embryo grade	2.1 $\pm$ 0.1	2.1 $\pm$ 0.2
Implantation ^b	5/52 (9.6%)	3/28 (10.7%)
Clinical pregnancy	3/13 (23%)	3/7 (42%)
Delivered	2/13 (15%)	3/7 (42%)

^a Mean $\pm$ SE.

^b Number of gestational sacs per number of embryos transferred.

of 52 with hatching and 3 (10.7%) of 28 in the control group. Clinical pregnancies resulted in 3 of 13 patients in the hatching group, compared to 3 of 37 of the controls.

In the retrospective series, the implantation rate was 36 (42.9%) of 84, significantly higher than the 3 (11.1%) of 27 implantation rate seen in those patients who had assisted hatching performed for at least 50% of the transferred embryos ($P < 0.05$). The delivery rate was 16 (76%) of 21 in the control group, compared to 1 (17%) of 6 in the hatching group. This difference did not reach statistical significance. Of note, the two groups were not equal, because more rapidly dividing, morphologically normal embryos were transferred in the traditional IVF group (Table II).

When the two clinical studies are combined, the difference in implantation remains significant: thirty-nine (35%) of 112 embryos implanted with traditional

Table II. Comparison of Assisted Hatching and Traditional IVF: Retrospective Study

	Hatching	Controls	P
Number	6	21	
Age ^a	28.2 $\pm$ 1.0	25.3 $\pm$ 0.9	NS
Oocytes ^a	17.2 $\pm$ 4.3	19.3 $\pm$ 1.9	NS
Fertilized ^a	9.1 $\pm$ 1.0	13.5 $\pm$ 1.4	NS
Transferred embryos ^a	4.5 $\pm$ 0.2	4.0 $\pm$ 0.1	NS
Blastomeres ^a	3.4 $\pm$ 4.3	4.3 $\pm$ 0.1	0.007
Grade ^a	2.1 $\pm$ 0.2	1.4 $\pm$ 0.1	0.0001
Implantation ^b	3/27 (11.1%)	36/84 (42.9%)	0.045
Clinical pregnancy	1/6 (17%)	17/21 (81%)	NS
Deliveries	1/6 (17%)	16/21 (76%)	NS

^a Mean $\pm$ SE.

^b Number of gestational sacs per number of embryos transferred.

IVF compared to 8 (10%) of 79 with assisted hatching ($P = 0.003$). The difference in delivery rates, 9 (68%) of 28 in the traditional IVF group versus the 3 (16%) of 19 in the hatching cohort, approached significance ($P = 0.05$).

DISCUSSION

We report very high implantation and pregnancy rates in young women with low basal FSH levels undergoing traditional IVF. Assisted hatching clearly was not beneficial in our study. Indeed, even though the implantation rates were similar in the prospective series, delivery rates tended to be higher with traditional IVF. When we added our retrospective series, traditional IVF was clearly superior to assisted hatching. The embryo quality was better in the traditional IVF group, compared to those who had assisted hatching performed for at least 50% of all transferred embryos. However, it is not likely that this small discrepancy between embryo quality was responsible for the large difference seen in the implantation and pregnancy rates between the two groups.

Assisted hatching has been shown to enhance implantation and pregnancy rates in poor-prognosis IVF patients (1). There is a potential to cause damage to

the embryo with exposure to the acid Tyrode's solution used for the procedure, and that may have occurred in our series. However, with the high implantation pregnancy rate seen in young women with normal basal levels, it is unlikely that the added cost and complexity required to perform assisted hatching are beneficial in this good-prognosis group.

In conclusion, assisted hatching is not beneficial and traditional IVF provides superior implantation and pregnancy rates in good-prognosis IVF patients. Selective use of assisted hatching would appear to be prudent.

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