



## **PREGNANCY/IMPLANTATION RATES AS RELATED TO AGE FOLLOWING TRANSFER OF FROZEN EMBRYOS PRODUCED BY ICSI**

M. L. CHECK  
J. H. CHECK  
D. SUMMERS-CHASE  
K. SWENSON  
W. YUAN

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

A study has suggested that one drawback of ICSI is that if these embryos are cryopreserved they have lower implantation rates after thawing and transfer as compared to other frozen embryos derived from conventional oocyte insemination. Other studies have not shown such adverse effects on pregnancy rates following frozen embryo transfer (ET) of embryos formed by ICSI. The study presented here evaluated the largest number of frozen ET cycles of embryos following ICSI, which were compared to couples having frozen ET with embryos formed by conventional insemination. In women age 39 and younger, the clinical, viable, pregnancy rates and implantation rates were very similar. Similar rates were reached for the older group. These data convincingly demonstrate that fertilization by ICSI does not adversely effect the implanting capacity of frozen-thawed embryos.

**Keywords** cryopreservation, embryo, ICSI, implantation, pregnancy

There are conflicting opinions as to whether fertilization of oocytes by ICSI produces embryos that are less likely to result in pregnancy after cryopreservation and subsequent embryo transfer after thawing. Van Steirteghem et al. [13] found a delivery rate of only 5% after transfer of these thawed embryos. However, Al-Hasani et al. [1] and Hoover et al. [8] found comparable delivery rates after transfer of frozen-thawed embryos fertilized by ICSI versus conventional insemination.

The present retrospective review is the largest study comparing pregnancy and implantation rates following frozen ET according to whether the oocyte was fertilized by ICSI or by conventional insemination. The study was designed to evaluate whether advanced age influences either positively or negatively the ability to result in a successful pregnancy.

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Address correspondence to Jerome H. Check, MD, PhD, 7447 Old York Road, Melrose Park, PA 19027, USA.

## MATERIALS AND METHODS

### General Design

A retrospective review of the outcome from transfer of all frozen embryos performed in the years 1997 to 1999 was made. Excluded were frozen embryo transfers of donor egg recipients and frozen donor embryos. Also evaluated were the outcomes following fresh embryo transfer in which embryos were fertilized by ICSI. The study included frozen embryo transfers from women who had controlled ovarian hyperstimulation despite elevated early follicular phase serum FSH levels or women who had been poor responders in previous cycles. All fresh transfer cycles involving ICSI were from 1997 to 1999, as were all the frozen ETs. The time of fertilization of the embryos evaluated for frozen ET could have occurred prior to 1997. The results of frozen embryo transfers were stratified according to whether the oocytes were fertilized by ICSI or conventional insemination and by age ( $\leq 39$  or  $>40$  years).

### Controlled Ovarian Hyperstimulation Regimens

One controlled ovarian hyperstimulation regimen used leuprolide acetate beginning in the midluteal phase for 10 days by itself then gonadotropins using various combinations of recombinant FSH by itself or hMG by itself or mixtures of both in dosages ranging from 150 IU to 600 IU per day. The leuprolide acetate could be used for 10 days only in dosages ranging from 0.5 to 0.020 mg daily or could be continued when the gonadotropins were started. Another typical COH regimen used leuprolide acetate beginning in the early follicular phase in dosages ranging from 0.05 to 0.75 mg daily along with various combinations of gonadotropins.

### ICSI Procedure

If needed, the ICSI technique was performed on all mature oocytes [10]. For each oocyte, a motile sperm was immobilized with an injection pipette in a drop of polyvinylpyrrolidone (Scandinavian IVF Science AB, Goteberg, Sweden) and then injected into the ooplasm. The injected oocytes were placed in human tubal fluid (HTF; Irvine Scientific, Irvine, CA) and 10% synthetic serum substitute (Irvine Scientific) and incubated for  $\geq 16$  h before evaluation for signs of fertilization (two pronuclei).

### Oocyte Insemination Procedure

For standard insemination semen was prepared using the Percoll gradient technique. Oocytes were inseminated with 25,000 sperm per oocyte and incubated for  $\geq 16$  h before evaluation for signs of fertilization.

### Cryopreservation Procedure

Pronuclear embryos were cryopreserved using a one-step addition of cryoprotectant. The embryos were equilibrated in 1.5 M 1,2-propanediol and frozen in 0.25-mL straws in an alcohol bath controlled rate freezer. The straws were preloaded with 0.12 mL of 1 M sucrose followed by 1 cm of air and column of 1,2-propanediol containing the embryos. Seeding was performed at  $-6^{\circ}\text{C}$ . The straws were cooled at  $-0.4^{\circ}\text{C}$  per minute down to  $-40^{\circ}\text{C}$  and then plunged into liquid nitrogen [3].

### Thawing Technique

Thawing the embryos involved a one-step dilution of the cryoprotectant [3]. The straws were warmed to room temperature in air for 2 min and the columns were shaken down. The

straws were then inverted in 37°C water bath for 3 min. The straws were inverted again at room temperature for 1 min before the contents were expelled into a petri dish. The thawed embryos were placed in a dish of phosphate-buffered saline for 10 min before being rinsed and placed in an organ culture dish containing 1 mL of warmed gassed HTF + 10% synthetic serum substitute covered with mineral oil. The embryos were allowed to cleave for another 48 h before transfer. Assisted embryo hatching was performed prior to transfer of the 72-h-old embryos [5].

### Source of Embryos

The majority of embryos used for frozen ET were the supernumerary ones left over following fresh ET. However, some resulted from cycles where fresh ET was deferred and all embryos frozen related to risk of ovarian hyperstimulation syndrome or inadequate endometrial sonographic characteristics at the time of the injection of human chorionic gonadotropin.

### RESULTS

For women age 39 or younger (based on age at time of transfer), there were 336 frozen transfers of embryos formed by conventional insemination of oocytes and 301 ETs from embryos derived from oocytes fertilized by ICSI. The respective clinical and viable pregnancy rates and implantation rates of 43, 38, and 19% in women having conventional insemination of oocytes were very similar to the results from the group of comparable aged women having frozen ETs resulting from ICSI (42, 35, 18%) (Table 1). There were no significant differences found when comparing similar categories for frozen ET, ICSI vs. non-ICSI according to age at time of retrieval.

The results from the 77 frozen ETs (conventional insemination) vs. the 50 frozen ETs (ICSI) in women age 40–43 years were also very similar (Table 1) (35, 29, 15% vs. 28, 24, 9%). There were no significant differences found when comparing similar categories for frozen

**Table 1.** Pregnancy and implantation rates according to the age at time of transfer in fresh vs. frozen ICSI cycles vs. frozen cycles with conventional insemination

	Fresh ICSI cycle		Frozen ICSI cycles		Frozen non-ICSI cycles	
	Age ≤39	Age 40–43	Age ≤39	Age 40–43	Age ≤39	Age 40–43
Transfer frozen ET ( <i>n</i> )	451	110	301	50	336	77
Average age			33.0	41.4	33.5	41.1
Pregnancies ( <i>n</i> )	215	39	145	15	165	33
Pregnant/transfer (%)	48	36	48	30	49	43
Clinical ( <i>n</i> )	193	34	126	14	145	27
Clinical/transfer (%)	43	31	42	28	43	35
Viable ( <i>n</i> )	175	26	106	12	126	22
Viable/transfer (%)	39	24	35	24	38	29
SAB/clinical pregnancy ( <i>n</i> )	14	27	20	21	19	22
ET ( <i>n</i> )	1512	421	1059	202	1190	293
Average ( <i>n</i> ) ET	3.2	3.7	3.5	3.7	3.4	3.7
Total sacs implanted ( <i>n</i> )	296	50	192	18	224	43
Implantation rate	20	12	18	9	19	15

ET, ICSI vs. non-ICSI according to age at time of retrieval. The Society for Assisted Reproductive Technology (SART) requires reporting statistics for frozen ET according to the age at time of transfer. Our data for frozen ET was also evaluated according to the age at time of retrieval (Table 2). These data were very similar when comparisons were made at age of transfer. The average ages in Tables 1 and 2 were similar in those having ICSI vs. conventional insemination and thus age did not act as a confounding variable.

There were no statistical differences found in either age group for clinical and viable pregnancy rates and implantation rates when comparing fresh vs. frozen ETs of embryos fertilized by ICSI (Table 1). The pregnancy rate for fresh ETs for the younger group was 43, 39, and 20% vs. 42, 36, and 18%, respectively. In the older group these rates were 31, 24, and 12% for fresh transfers vs. 30, 25, and 12% for frozen transfers.

## DISCUSSION

The study presented here is the largest one to date evaluating the effects of ICSI on subsequent pregnancy rates following transfer of frozen-thawed embryos. They show that for women age 39 years or younger, ICSI does not deleteriously affect success following frozen ET. Similarly, for women aged 40 years or greater there were no significant differences in clinical or viable pregnancy rates. None of the patients used in this study overlapped with our previously reported small series [8]. In fact, in the previous study only patients having fresh transfer deferred in favor of frozen ET were included, whereas in the present study, all frozen embryo transfers were included even if the female patient had had a previous fresh embryo transfer.

The technique of freezing that we employed [3] differed from the one used by Van Steirteghem et al. [13]. It would appear that when using the simplified freezing technique with one-step removal of cryoprotectant, fertilization of oocytes by ICSI does not adversely affect subsequent

**Table 2.** Comparison of clinical and viable pregnancy rates and implantation rates according to the age at time of retrieval in frozen embryo transfers of embryos formed from conventional insemination vs. ICSI

	Frozen ICSI cycles		Frozen non-ICSI cycles	
	Age ≤39	Age 40-43	Age ≤39	Age 40-43
Transfer frozen ET ( <i>n</i> )	309	44	369	51
Average age	33	41	33	41
Pregnancies ( <i>n</i> )	148	12	180	21
Pregnant/transfer (%)	48	27	49	41
Clinical ( <i>n</i> )	128	12	158	17
Clinical/transfer (%)	41	27	43	33
Viable ( <i>n</i> )	108	10	137	14
Viable/transfer (%)	35	23	37	28
SAB/clinical pregnancy ( <i>n</i> )	20	25	18	24
ET ( <i>n</i> )	1091	176	1304	204
Average ( <i>n</i> ) ET	3.5	3.7	3.4	3.9
Total sacs implanted ( <i>n</i> )	194	16	245	28
Implantation rate	18	9	19	14

chances of conception following frozen ET. The one caveat may be a slight tendency for lower implantation rates following frozen ET in the older reproductive population.

Several studies have supported the conclusion that ICSI does not impair pregnancy rates or survival rates of frozen-thawed embryos [2, 4, 6, 7, 9, 11, 12]. This study is the first to evaluate the advanced age group of 40 or older and found that these embryos are not adversely effected by ICSI when frozen and thawed.

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