



A RANDOMIZED STUDY COMPARING THE EFFECT OF STANDARD VERSUS SHORT INCUBATION OF SPERM AND OOCYTE ON SUBSEQUENT PREGNANCY AND IMPLANTATION RATES FOLLOWING IN VITRO FERTILIZATION EMBRYO TRANSFER

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

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
and Infertility, Camden, New Jersey, USA

Some data suggest that some sperm are associated with a toxic product that lowers embryo implantation rates when these sperm come in contact with the zona pellucida. The possibility exists that shortening the incubation time could improve pregnancy rates. A randomized prospective study was initiated to evaluate pregnancy and implantation rates with transfer of embryos formed by conventional overnight vs short (2-h) co-incubation of sperm and oocytes. The clinical and viable pregnancy rates were 41 and 29% for conventional co-incubation versus 30 and 30% for short co-incubation. Implantation rates were also similar (17.9 vs 17.8%). Short incubation does not seem to impact any advantage over conventional co-incubation techniques in couples undergoing in vitro fertilization where the male factor is normal.

Keywords implantation, overnight insemination, short co-incubation

Certain sperm abnormalities, e.g., a subnormal hypoosmotic swelling (HOS) test [3] or an abnormal stress test [2], may be associated with subfertility not related to poor fertilization but to defective implantation despite apparent normal cleavage to 3-day-old embryos. The problem may be related to a factor attached to the sperm (and thus impairing the functional integrity of the sperm membrane) that gets transferred to the zona pellucida (ZP) by the supernumerary sperm that are attached [6]. A proteinaceous nature to this toxic factor was suggested by improvement of HOS scores and subsequent pregnancies following intrauterine insemination (IUI) after treatment of the sperm with a protein digestive enzyme, chymotrypsin [6]. Further

Address correspondence to Jerome H. Check, MD, PhD, 7447 Old York Road, Melrose Park, PA 10927, USA.

support to this hypothesis was provided by demonstrating normal pregnancy rates (PRs) despite subnormal HOS scores following in vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI) [6].

Subnormal HOS test scores are not very common, but a much higher percentage of patients undergoing IVF have subnormal stress tests [2]. Intracytoplasmic sperm injection is much more complicated than conventional co-incubation techniques, requiring far greater skill by the embryologist, and thus is not available in all IVF centers and adds a significant cost to the procedure. Thus, it does not seem reasonable to perform ICSI on all patients routinely to cover this theoretical, but not fully proven, abnormality leading to implantation defects.

Dirnfeld et al. found that decreasing the incubation period from 16 to 3 h significantly improved implantation rates [4]. They speculated that shorter exposure of the oocyte to sperm prevented zona hardening. Gianaroli et al. also showed a higher implantation rate when co-incubating sperm and oocyte for 1 h as opposed to 16 h [5]. They also found higher fertilization rates with the shorter incubation time. They suggested that a reduced exposure of oocyte to sperm improved embryo viability, possibly due to a decrease in potential damage from sperm metabolic waste products [5].

The study presented here prospectively and randomly compared IVF outcome following co-incubation with conventional overnight co-incubation vs our own short co-incubation procedure.

MATERIALS AND METHODS

There was randomization by the last digit of female partner's social security number (odd/even) to either conventional or short incubation techniques for couples where male factor appeared. These techniques are described in Table 1.

Table 1. Insemination protocols

Conventional insemination	Short insemination
Place 4 oocytes in 1.0 mL of culture media covered with oil.	Place 4 oocytes in 1.0 mL of culture media covered with oil.
Inseminate mature oocytes 4 h postretrieval using 25,000 motile sperm per oocyte.	Inseminate mature oocytes 4 h postretrieval using 25,000 motile sperm per oocyte.
Incubate gametes overnight.	Incubate gametes for 2 h.
Coronal removal is performed the following morning and fertilized oocytes are recorded and moved to clean dishes.	After 2-h incubation gently wash the cumulus complex through 2 changes of media, changing the pipet each time. Place the oocytes in clean dish and incubate overnight.
Cryopreservation is done in the usual manner noting type of insemination protocol on cryo sheet.	Coronal removal is performed the following morning and fertilized oocytes are recorded and moved to clean dishes. Cryopreservation is done in the usual manner, noting type of insemination protocol on cryo sheet.

Table 2. Comparison of IVF parameters according to type of co-incubation procedure

IVF parameters	Conventional co-incubation	Short incubation
Sperm concentration ($\times 10^6/\text{mL}$)	68 \pm 54.8	75 \pm 52.3
Percent motility	63 \pm 11.0	63 \pm 10.0
Normal morphology (strict criteria)	15 \pm 4.0	11 \pm 4.6
Mean HOS score	71 \pm 9.0	72 \pm 9.0
Number of oocytes retrieved	10 \pm 10.9	13 \pm 11.4
Number of mature oocytes	9 \pm 5.5	12 \pm 10.9
% fertilization all oocytes	63 \pm 28.3	66 \pm 20.9
% fertilization of mature oocytes	66 \pm 28.1	70 \pm 20.1
Mean number of embryos transferred	3 \pm 1.3	3 \pm 1.1

RESULTS

There were 35 patients having conventional insemination vs 31 with short co-incubation. The groups were comparable in terms of stimulation characteristics, age, and baseline semen parameters. Only the first oocyte retrieval cycle was used. Patients were eliminated from the study for deferred transfer related to inadequate endometrial thickness, endometrial echo pattern, or risk of ovarian hyperstimulation syndrome. Various parameters are compared in Table 2. PRs are compared according to co-incubation procedure in Table 3. No differences according to type of co-incubation technique was demonstrated.

DISCUSSION

For this study there did not appear to be any improvement in PRs or implantation rates using the short incubation procedure. The data do not seem to corroborate the conclusions of Dirnfeld et al. and Gianaroli et al. that short incubation improves the implantation rate [4, 5]. However, differences in experimental design could account for the different conclusions. The study presented herein was looking for possible use of short co-incubation for occult male factor problems causing implantation defects. Most males had normal semen parameters and those below SART standards were only mildly abnormal. More severe problems were treated with ICSI. In contrast, Gianaroli et al. evaluated male factor problems. The possibility exists that the presence of transferable toxic factors associated with the sperm is more likely in patients with oligoasthenozoospermia or tetrazoospermia [5]. Evidence to support this

Table 3. Pregnancy rates according to co-incubation technique

	Conventional co-incubation	Short co-incubation
Number of embryo transfers	26	24
Number deferred for frozen ET	9	7
β -hCG levels > 500 mIU/mL	10 (41%)	11 (42%)
Clinical PR (ultrasound evidence)	10 (41%)	8 (30%)
Viable PR (past first trimester)	7 (29%)	8 (30%)
Implantation rates	17.9%	17.8%

possibility was provided by Oehninger et al., who found that increasing the sperm concentration improved fertilization rates using sperm with poor morphology by strict criteria but actually lowered PRs [7, 8].

There were some differences in the actual technique that we used compared to that of Gianaroli et al. [5]. We used communal growth dishes instead of microdroplets, because Almagor et al. reported higher PRs after communal growth [1]. Also, our incubation time 2 h versus 1 h for Gianaroli et al. [5] and 3 h for Dirnfeld et al. [4].

Future studies should consider a 3-way randomization of treatment assignment for mild male factor cases: conventional insemination, short insemination, and ICSI. Subsequent results would compare relative fertilization, PRs, and implantation rates. The data should be analyzed separately for 3 semen parameters: concentration, morphology, and percent motility.

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