Is Intracytoplasmic Sperm Injection Necessary for Couples Undergoing In Vitro Fertilization–Embryo Transfer with Normal Semen Analyses but Failing Hamster Egg Penetration Assays?¹

ERIC S. SURREY,^{2–5} GRACE S. LEE,³ MARK W. SURREY,^{2–4} and DAVID HILL³

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Purpose: Our purpose was to assess whether in vitro fertilization (IVF)–embryo transfer (ET) candidate couples with basically normal semen analyses but failing zona-free hamster egg penetration assay (HEPA) scores benefit from intracytoplasmic sperm injection (ICSI).

Methods: Twenty consecutive IVF candidate couples with normal–borderline semen analyses and failing HEPA scores were recruited. Mature oocytes obtained from each woman were randomly divided between ICSI (group I; $n = 126$ oocytes) and standard insemination techniques (group II; 138 oocytes). Fertilization (two pronuclei) and cleavage (2–4 cells) rates were assessed for both groups.

Results: There were no statistically significant differences between the two groups with respect to (mean $\pm$ standard error of the mean) fertilization (group I, 63.1 ± 7.75 ; group II, $77.8 \pm 4.7\%$) or cleavage (group I, $87.3 \pm 2.4\%$; group II, $91.2 \pm 3.5\%$) rates.

Conclusions: ICSI is not beneficial for IVF-ET when sperm samples demonstrate a failing HEPA score but have normal or minimally compromised semen analysis parameters.

KEY WORDS: intracytoplasmic sperm injection; hamster egg penetration assay.

INTRODUCTION

The zona-free hamster egg penetration assay (HEPA) has been used to predict defects in the ability of sperm

to undergo capacitation, acrosome reaction, and oocyte penetration *in vitro* (1,2). The value of this assay in predicting outcome for human in vitro fertilization (IVF)–embryo transfer (ET) is extremely controversial (3–6). Several investigators have suggested that the predictive value for failed IVF may be greater in couples with failing HEPA scores and unexplained or tubal as opposed to male-factor infertility (7, 8).

Intracytoplasmic sperm injection (ICSI) techniques clearly represent a major advance in achieving conception for those couples with severe male-factor infertility (9, 10). The benefit of this approach has not been as well established in patients with less severe sperm function abnormalities. The role of HEPA in determining which sperm samples may be best treated with ICSI in couples who are candidates for IVF-ET has also not been established.

In this prospective, randomized investigation, we have attempted to assess the necessity for assisting fertilization by using ICSI in couples undergoing IVF-ET with sperm samples demonstrating a failing HEPA score but with otherwise normal semen analysis parameters.

MATERIALS AND METHODS

This investigation was a prospective, randomized trial. HEPA was performed in our program as part of a routine evaluation prior to IVF-ET. The study population consisted of 20 consecutive IVF candidate couples with semen analyses demonstrating normal–borderline parameters and HEPA with failing scores as described below. Candidates were patients with the following parameters on two semen analyses each performed after 48–136 hr of abstinence and at least 4 weeks apart: concentration, $\geq 20 \times 10^6/\text{ml}$; motility $\geq 40\%$ (grades 2–3); and morphology, $\geq 35\%$ normal forms by World Health Organization criteria.

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² Reproductive Medicine and Surgery Associates, Beverly Hills, California.

³ Center for Reproductive Medicine, Century City Hospital, Los Angeles, California.

⁴ Department of Obstetrics–Gynecology, UCLA School of Medicine, Los Angeles, California.

⁵ To whom correspondence should be addressed at 9675 Brighton Way, Suite 420, Beverly Hills, California 90210.

The zona-free HEPA was performed with sperm samples which were Percoll (Pharmacia, Upsala, Sweden)-washed and incubated overnight in BWW-buffered medium. Twenty cryopreserved and subsequently thawed zona-free hamster eggs (Fertility Technologies, Inc., Natick, MA) were added to the samples for a 4-hr incubation at 37°C under standard culture conditions. Sperm binding and oocyte penetration were subsequently assessed. A penetration score of less than 15% was considered to be failing. Sperm samples from donors with established fertility were used in parallel for assay validation. The assay was considered valid if the control sample penetration score was greater than 50%.

The indications for IVF-ET in this patient population are displayed in Table I. All patients had a circulating serum follicle-stimulating hormone (FSH) level of less than 10 mIU/ml and an estradiol (E_2) level of less than 80 pg/ml on cycle day 2 or 3. Patients underwent gonadotropin down-regulation with a gonadotropin-releasing hormone agonist (Lupron; TAP Pharmaceuticals, Waukegan, IL), 1.0 mg subcutaneously daily, initiated during the midluteal phase. After 10 days of agonist therapy and once serum E_2 levels were noted to be less than 50 pg/ml, the dose was decreased to 0.5 mg daily and gonadotropin stimulation (Metrodin or Fertilinex; Serono Laboratories, Randolph, MA) was initiated at a dose ranging from 225 to 450 IU im daily. Follicular development was monitored with serial ultrasound examinations and serum E_2 and progesterone (P_4) levels with gonadotropin doses adjusted as appropriate. Human chorionic gonadotropin (Profasin, Serono), 10,000 U intramuscularly, was administered when at least two follicles were greater than 18 mm in mean diameter, a serum E_2 level of ≥ 500 pg/mL, and a P_4 level of ≤ 1.9 mg/ml were noted.

Oocytes were collected by transvaginal ultrasound-guided aspiration techniques using intravenous sedation and cultured in either protein-supplemented human tubal fluid (Irvine Scientific, Irvine, CA) or Basal-XI (Biocare Corp., San Diego, CA) medium prepared as microdrops under sterile mineral oil (Squibb, Princeton, NJ).

Table I. Indications for IVF

Endometriosis	4 (20%)
Peritoneal-tubal factor	6 (30%)
Unexplained ^a	8 (40%)
Ovulatory factor	2 (10%)

^a Only abnormality was failing HEPA.

After approved informed consent was obtained from each couple, all morphologically normal metaphase-II oocytes aspirated from each patient were randomly divided by the embryologist into two equal groups on which either ICSI or standard insemination techniques were performed. ICSI was undertaken on 126 oocytes in group I by introducing a single motile sperm into each oocyte by filter-isolated mouth pressure in glass depression slides using Nomarski optics. This procedure was performed 1–6 hr following oocyte aspiration. Group II consisted of 138 oocytes on which conventional insemination was performed with 10,000–20,000 motile sperm added to microdrops of medium each of which contained 1–6 oocytes. Unequal numbers in the overall groups stem from the fact that some oocytes initially felt to be mature and randomized to group I were not deemed to be so at the time of ICSI.

Oocytes were checked for the appearance of pronuclei (PN) 16–18 hr and for embryo cleavage 40–42 hr post insemination. Normal fertilization (two PN) and cleavage rates were assessed for both groups. Data were analyzed by paired *t* tests and chi-square analysis where appropriate. A *P* value of less than 0.05 was considered to be statistically significant.

RESULTS

The mean precycle sperm parameters and HEPA scores are displayed in Table II. The mean $\pm$ standard error of the mean HEPA score of $3.8 \pm 1.6\%$ for the patients was well below the passing level of 15%. No penetration at all was appreciated in 10 patients. Basic clinical data regarding controlled ovarian stimulation and oocyte aspiration are presented in Table III. These patients were relatively young and responded well to stimulation, resulting in a high enough number of mature oocytes obtained per aspiration to result in at least four oocytes to be allocated to each group for each patient.

Normal fertilization and cleavage rates (2- to 4-cell stage) were similar between the two groups (Fig. 1). Fertilization rates were higher with ICSI than standard insemination in only four patients. The converse was true in only two patients. Fertilization rates of less than 50% were noted in 20% of ICSI procedures and 10% of standard insemination procedures. These differences were not statistically significant. Due to transfer and cryopreservation of embryos pooled from both groups in several patients, comparative pregnancy rates for each group cannot be calculated.

Table II. Precycle Sperm Parameters^a

Concentration	Motility (%)	Normal morphology (%)	HEPA score (%)
51.3 ± 8.3 × 10 ⁶ /ml	48.2 ± 2.8	45.7 ± 6.5	3.8 ± 1.6 ^b

^a Results expressed as mean ± standard error of the mean.^b Ten patients with 0% penetration.**Table III.** Clinical Data^a

n (Cycles)	Age (years)	Days 2–3		Gonadotropins		Mature oocytes retrieved
		FSH (mIU/ml)	E ₂ (pg/ml)	Ampoules (75 IU)	Days	
20	34.4 ± 1.2	5.6 ± 0.5	42.8 ± 4.5	33.9 ± 2.3	10.8 ± 0.4	14.3 ± 1.7

^a Results expressed as mean ± standard error of the mean.

DISCUSSION

In this study, we have demonstrated that ICSI is not beneficial for IVF-ET when sperm samples demonstrate a failing HEPA score but have otherwise normal or minimally compromised parameters on traditional semen analyses. Neither fertilization nor cleavage rates were enhanced.

The role of the HEPA in predicting the benefit of ICSI over conventional insemination in IVF-ET has not, to our knowledge, been previously addressed. Chen and colleagues have previously reported that in normal males, the rate of sperm penetration by subzonal insertion of sperm was significantly lower with

failing HEPA results (11). Vazquez-Levin *et al.* had demonstrated that failing HEPA scores had no predictive value for success with partial zona pellucida drilling techniques (12).

The fertility potential for men with normal semen analyses, yet failing HEPA scores, is somewhat controversial. Investigators have reported that a high correlation exists between failed IVF and failing HEPA scores primarily in couples with unexplained infertility or tubal-factor infertility and partners with normal semen analyses (7,8). Kuzan and colleagues have demonstrated little or no correlation between HEPA scores and IVF cycle outcome (4). Others have shown that spontaneous conception also correlates with HEPA results (13,14). However, these findings have been refuted by O'Shea *et al.* (15).

The high incidence of false-positive results represents one of the weaknesses of the HEPA assay (12, 16,17). A variety of techniques including Test-Yolk buffer, pentoxifylline, and follicular fluid incubation as well as alteration of time and temperature has been used in an effort to decrease the incidence of false/positive results and enhance the sensitivity of the assay (6,18–23). These techniques were not used in the HEPA employed in this investigation. Their use may have theoretically improved the poor predictive value associated with this study. Similarly, the relatively small sample size may have limited the power of this investigation resulting in a type-II error. A larger-scale investigation would clearly be of value.

In summary, therefore, the HEPA provides little indication for the need for ICSI in couples with otherwise normal semen analysis parameters. These findings do

Fig. 1. Comparative normal fertilization (two pronuclei) and embryo cleavage (2 to 4-cell-stage) rates in oocytes in group I undergoing ICSI compared with those in group II undergoing standard in vitro insemination techniques.

not address the issue of whether a failing HEPA score suggests a need for IVF-ET per se in couples with otherwise unexplained infertility. However, the value of the HEPA as currently performed in the standard evaluation of the patient with infertility must be seriously questioned.

REFERENCES

1. Yanagamachi R, Yanagamachi H, Rogers BJ: The use of zona free animal ova as a test system for the assessment of the fertilizing capacity of human spermatozoa. *Biol Reprod* 1976;15:471-476
2. Rogers BJ: The sperm penetration assay: its usefulness reevaluated. *Fertil Steril* 1985;43:821-840
3. Aitken RJ, Best FSM, Warner P, Templeton A: A prospective study of the relationship between semen quality and fertility in cases of unexplained fertility. *J Androl* 1984;5:297-301
4. Kuzan FB, Muller CA, Zarutskie PW, Piron LL, Soules MP: Human sperm penetration assay is an indicator of sperm function in human in vitro fertilization. *Fertil Steril* 1987;48:282-286
5. Coetzee K, Kruger TF, Meinkveld R, Swanson RJ, Lombard CJ, Acosta AA: Usefulness of sperm penetration assay in fertility predictions. *Arch Androl* 1989;23:207-212
6. Soffer Y, Golan A, Herman A, Pansky M, Caspi E, Ron-El R: Prediction of in vitro fertilization outcome by sperm penetration assay with Test-Yolk buffer preincubation. *Fertil Steril* 1992;58:556-562
7. Margalioth EJ, Feinmesser M, Navot D, Mordel N, Bronson RA: The long-term predictive value of the zona-free hamster ova sperm penetration assay. *Fertil Steril* 1989;52:490-494
8. Ibrahim ME, Moussa MA, Pedersen H: Efficacy of zona-free hamster egg sperm penetration assay as a prediction of in vitro fertilization. *Arch Androl* 1989;23:267-274
9. Palermo G, Joris H, Devereoy P, Van Stierteghem AC: Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet* 1992;7:248-254
10. Palermo G, Joris M, Deide M-P, Camus M, Devroey P, Van Stierteghem A: Sperm characteristics and outcomes of assisted fertilization by subzonal insemination and intracytoplasmic sperm injection. *Fertil Steril* 1993;59:826-835
11. Chen SU, Yang YS, Ho HN, Hwang JL, Hong TS, Lin HR, Huang SC, Lee TY: Microinjection of human sperm into the perivitelline space of hamster eggs: Comparison with zona-free hamster egg penetration of human sperm. *Arch Androl* 1993;30:201-207
12. Vazquez-Levin M, Kaplan P, Sandler B, Garrisi GJ, Gordon J, Navot D: The predictive value of zona-free hamster egg sperm penetration assay for failure of human in vitro fertilization and subsequent successful zona drilling. *Fertil Steril* 1990;53:1055-1059
13. Shy KK, Stenchever MA, Muller CH: Sperm penetration assay and subsequent pregnancy: A prospective study of 74 infertile men. *Obstet Gynecol* 1988;71:685-690
14. Gwatkin RB, Collins JA, Jarrell JF, Kohut J, Milner RA: The value of semen analysis and sperm function assays in predicting pregnancy among infertile women. *Fertil Steril* 1990;53:693-699
15. O'Shea DL, Odem RR, Cholewa C, Gast MJ: Long term follow-up of couples after hamster penetration testing. *Fertil Steril* 1993;60:1040-1045
16. Aitken J: On the future of the hamster oocyte penetration assay. *Fertil Steril* 1994;62:17-19
17. Mao C, Grimes DA: The sperm penetration assay: Can it discriminate between fertile and infertile men? *Am J Obstet Gynecol* 1988;159:279-286
18. Yee B, Cummings LM: Modification of the sperm penetration assay using human follicular fluid to minimize false negative results. *Fertil Steril* 1988;50:123-128
19. Morales P, Llanes M, Yovich JL, Cummings JM, Vigil P: Pentoxifylline increases sperm penetration into zona-free hamster oocytes without increasing the acrosome reaction. *Andrologia* 1993;25:359-362
20. Lambert H, Steinleitner A, Eisermann J, Serpa N, Cantor B: Enhanced gamete interaction in the sperm penetration assay after incubation with pentoxifylline and human follicular fluid. *Fertil Steril* 1992;58:1205-1208
21. Sanchez R, Schill WB: Influence of incubation time/temperature on acrosome reaction/sperm penetration assay. *Arch Androl* 1991;27:35-42
22. Falk RM, Silverberg KM, Fetterolf PM, Kirchner FK, Rogers BJ: Establishment of TEST-Yolk buffer enhanced sperm penetration assay limits for fertile males. *Fertil Steril* 1990;54:121-126
23. McClure RD, Tom RA, Dandekar PV: Optimizing the sperm penetration assay with human follicular fluid. *Fertil Steril* 1990;53:546-550