



INCREASING SPERM CONCENTRATION TO ADJUST FOR SUBNORMAL SPERM MORPHOLOGY DID NOT ADVERSELY AFFECT IMPLANTATION AFTER EMBRYO TRANSFER

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

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

Some sperm specimens contribute to infertility not by impairing the fertilization process but by causing embryo implantation defects. One hypothesis is that a toxic factor attached to the sperm is transferred to the zona pellucida by the supernumerary sperm and then subsequently to the embryo membrane. One abnormality, as manifested by subnormal hypoosmotic swelling test (HOST) scores, causes these problems even with the small number of sperm that attach to the zona pellucida under normal physiologic conditions. This study evaluated whether the technique used to increase the oocyte insemination concentration of sperm to adjust for subnormal morphology values using strict criteria may allow a higher concentration of this purported toxic factor to attach to the zona pellucida, thus resulting in the dissimilitude of normal fertilization rate but low implantation rate. A comparison of the pregnancy and implantation rate of embryos formed by insemination of this adjusted sperm concentration of $\leq 200,000$ sperm vs. those inseminated with the normal concentration of 25,000 sperm failed to demonstrate any differences. Thus, the technique of increasing sperm concentration to adjust for low sperm morphology to improve fertilization rates does not seem to adversely affect implantation rates as long as the HOST scores are normal.

Keywords embryo, implantation, in vitro fertilization, IVF, sperm, strict morphology

Some subfertile males have sperm that allow normal fertilization but the resulting embryos fail to implant [1, 4, 5]. This implantation defect was obviated by fertilizing the oocytes by intracytoplasmic sperm injection (ICSI) [7]. One explanation for these observations is that under some conditions there may be a toxic factor attached to the sperm membrane that gets

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

transferred to the oocyte membrane not by the one spermatozoon that fertilizes the oocyte but by the supernumerary sperm that attach to the zona pellucida.

Before the advent of ICSI [11, 12], various nuances in the sperm-oocyte insemination technique were tried [3]. One technique used with in vitro fertilization (IVF) to improve poor fertilization rates with oligoasthenozoospermia was to try a high oocyte insemination concentration [9]. This technique, despite significantly improving the fertilization rate, still resulted in very poor pregnancy and implantation rates, which, in fact, were no better than what was found with fertilization with lower sperm concentrations [9]. One possible interpretation of these findings could be that the higher concentration of sperm, despite improving the fertilization, did not improve the pregnancy rates because of transferring a toxic factor from the supernumerary sperm to the zona pellucida and then eventually to the embryo. In contrast to the abnormality seen with sperm with low HOS scores, where even the physiologic concentration of supernumerary sperm is able to cause embryo implantation defects, the theory here is that this toxic factor may exist in a much smaller concentration with sperm with normal HOS scores; thus, this problem may be manifested only when inseminating the oocyte with high concentrations of sperm.

The policy at our own IVF center, when presented with a semen specimen with normal morphology using strict criteria of 5-9%, is to adjust the oocyte insemination concentration to 10,000 motile sperm with normal morphology. Thus, many of these patients' oocytes are inseminated by significantly more sperm than the 25,000 reserved for oocytes when the male partner has >10% normal morphology, but usually nowhere near the concentration used by Oehninger et al. of 500,000 [9].

The study presented herein retrospectively evaluated our IVF statistics to see if using a higher sperm concentration for low sperm morphology results in lowered implantation rates.

MATERIALS AND METHODS

All oocyte retrieval cycles between 1 January 1997 and 30 June 1999 in women age ≤ 39 were analyzed. Patients were divided into 4 groups based on % normal morphology (NM): group 1, NM $\leq 4\%$ and ICSI used; group 2, NM 5-9%, ICSI used; group 3, NM 5-9%, conventional insemination with increased concentration of sperm used; and group 4, NM $\geq 10\%$ with conventional insemination of each oocyte with 25,000 motile sperm. For group 3, the final insemination concentration was 10,000 NM motile sperm/oocyte. NM was based on strict criteria. Within each group, patients were further classified by the presence of oligospermia (< 20 million/mL) or asthenozoospermia $< 40\%$ motile (SART criteria). Patients were excluded if the HOS test was $< 50\%$.

RESULTS

Tables 1 and 2 show the pregnancy and fertilization rates for patients undergoing the luteal phase leuprolide acetate/gonadotropin protocol and Tables 3 and 4 show the results for patients undergoing the follicular phase leuprolide acetate/gonadotropin protocol. Tables 5 and 6 show the results for all cycles combined. Within each protocol, there is no significant difference in the pregnancy rates by normal morphology and treatment used for either patients with male factor or normal sperm. When all cycles are combined, there is no difference in pregnancy

Table 1. Clinical pregnancy rates for luteal phase leuprolide acetate/gonadotropin cycles

	Male factor*	Normal sperm*
NM ≤ 4%, ICSI	60% (18/30) [42-77]	40% (4/10) [9.8-70]
NM 5-9%, ICSI	61% (22/36) [45-77]	37% (11/29) [20-55]
NM 5-9%, increased concentration	71% (10/14) [47-95]	62% (23/37) [46-77]
NM ≥ 10%, standard IVF	42% (6/14) [17-68]	49% (42/85) [38-60]

Note. Values are pregnancy rates [95% confidence intervals].

**p* = NS comparing NM groups within each sperm category.

rates when male factor is present. However, when sperm is normal the pregnancy rates are higher in the group with the increased insemination concentration.

DISCUSSION

The data presented here demonstrate at the modest increase in sperm concentration used to adjust the concentration to 10,000 motile sperm with normal morphology using strict criteria did not seem to be associated with embryo implantation defects. Using this system, the maximum number of sperm used for insemination even with only 5% normal morphology was 200,000, and possibly when the HOS test is normal, the toxicity of sperm causing embryo implantation defects does not manifest until one uses even higher concentrations (e.g., 500,000 sperm).

Other factors may have been responsible for the very poor pregnancy rate found in the previous study when high concentrations of sperm were used for oocyte insemination [9]. A more recent study from the same group found no significant difference in pregnancy or implantation rates comparing one group using high sperm concentration and another group using ICSI (although ICSI did result in a statistically higher percentage of embryos with better embryo quality [10]). Similar conclusions about pregnancy and implantation rates were reached by Hall et al. [6].

Table 2. Fertilization rates for luteal phase leuprolide acetate/gonadotropin cycles

	Male factor*	Normal sperm*
NM ≤ 4%, ICSI	(0-100) 66 (61 ± 27) (<i>n</i> = 40)	(28.6-100) 77 (74 ± 18.1) (<i>n</i> = 14)
NM 5-9%, ICSI	(10-100) 71 (69 ± 15.9) (<i>n</i> = 50)	(0-100) 65 (63 ± 23.2) (<i>n</i> = 38)
NM 5-9%, increased concentration	(28.6-100) 74 (68 ± 21.4) (<i>n</i> = 16)	(25-100) 66 (65 ± 19.1) (<i>n</i> = 44)
NM ≥ 10%, standard IVF	(27-95) 71 (66 ± 20) (<i>n</i> = 21)	(0-100) 66 (63 ± 24.2) (<i>n</i> = 121)

Note. Values are ranges, medians, means ± standard deviations.

**p* = NS comparing NM groups within each sperm category.

Table 3. Clinical pregnancy rates for follicular phase leuprolide acetate/gonadotropin cycles

	Male factor*	Normal sperm*
NM ≤ 4%, ICSI	27% (6/22) [8.8–45.8]	33% (2/6) [–4.2–70.9]
NM 5–9%, ICSI	31% (7/22) [125.4–51.2]	46% (7/15) [21.5–71.8]
NM 5–9%, increased concentration	34% (9/26) [16.4–52.8]	54% (6/11) [25.3–83.8]
NM ≥ 10%, standard IVF	50% (5/10) [19.2–80.8]	25% (18/71) [15.3–35.4]

Note. Values are pregnancy rates [95% confidence intervals].

**p* = NS comparing NM groups within each sperm category.

Table 4. Fertilization rates for follicular phase leuprolide acetate/gonadotropin cycles

	Male factor*	Normal sperm*
NM ≤ 4%, ICSI	(0–100) 55 (52 ± 30.3) (<i>n</i> = 33)	(69.0–100) 77 (78 ± 9.8) (<i>n</i> = 8)
NM 5–9%, ICSI	(0–100) 55 (60 ± 28.8) (<i>n</i> = 30)	(14.3–100) 60 (60 ± 21.4) (<i>n</i> = 21)
NM 5–9%, increased concentration	(0–100) 77 (72 ± 30.3) (<i>n</i> = 12)	(25–100) 66 (66 ± 21.9) (<i>n</i> = 31)
NM ≥ 10%, standard IVF	(33.3–100) 55 (59 ± 22.2) (<i>n</i> = 13)	(0–100) 66 (65 ± 23.8) (<i>n</i> = 91)

Note. Values are ranges, medians, means ± standard deviations.

**p* = NS comparing NM groups within each sperm category.

Table 5. Clinical pregnancy rates for all cycles

	Male factor*	Normal sperm*
NM ≤ 4%, ICSI	46% (24/52) [32–59]	37% (6/16) [13.9–61]
NM 5–9%, ICSI	50% (29/58) [37–62]	41% (18/44) [26–55]
NM 5–9%, increased concentration	47% (19/40) [32–62]	60% (29/48) [46–74]***
NM ≥ 10%, standard IVF	45% (11/24) [26–65]	38% (60/156) [30–46]

Note. Values are pregnancy rates [95% confidence intervals].

**p* = NS comparing NM groups within each sperm category.

***p* = .05 comparing NM groups for normal sperm.

****p* < .001 comparing increased concentration vs. the other treatment.

Table 6. Fertilization rates for all cycles

	Male factor*	Normal sperm*
NM \leq 4%, ICSI	(0-100) 61 (57 \pm 28.7) (n = 73)	(28.6-100) 77 (75 \pm 15.5) (n = 22)
NM 5-9%, ICSI	(10-100) 71 (66.1 \pm 21.9) (n = 80)	(0-100) 62.4 (62.8 \pm 22.4) (n = 59)
NM 5-9%, increased concentration	(0-100) 75.0 (70 \pm 25.1) (n = 28)	(25-100) 66 (66 \pm 20.2) (n = 75)
NM \geq 10%, standard IVF	(27.3-100) 68 (64 \pm 20.9) (n = 34)	(0-100) 66 (64 \pm 23.9) (n = 212)

Note. Values are ranges, medians, means \pm standard deviations.

**p* = NS comparing NM groups within each sperm category.

Thus, studies by Hall et al. [6] and Oehninger et al. [10], coupled with the data presented here, lead us to believe that neither teratozoospermia or high insemination concentrations are causes of embryo implantation defects. Thus, our method of adjusting the sperm concentration to 10,000 motile sperm with normal morphology for specimens with only 5-9% normal forms does not appear to have an adverse effect on outcome (though we cannot prove that it improves fertilization rates, since no controlled study was performed). Impairment of the functional integrity of the sperm membrane as manifested by subnormal HOS scores [8] and possibly the defect determined by an abnormal stress test [1], remain unique for sperm resulting in embryo implantation defects. To date, embryo implantation defects with the smaller numbers of sperm attaching to the zona pellucida under normal in vivo conditions have been demonstrated only with sperm with HOS scores less than 50% [2].

REFERENCES

1. Alvarez JG, Minaretzis D, Barrett CB, Mortola JF (1996): The sperm stress test: a novel test that predicts pregnancy in assisted reproductive technologies. *Fertil Steril* 65:400-405.
2. Check JH, Epstein R, Nowroozi K, Shanis BS, Wu CH, Bollendorf A (1989): The hypoosmotic swelling test as a useful adjunct to the semen analysis to predict fertility potential. *Fertil Steril* 52:159-161.
3. Check JH, Hourani C, Goldsmith G (1994): The use of non-micromanipulation techniques for male factor and in vitro fertilization outcome. In: *Frontiers in Endocrinology: Male Factor in Human Infertility*. Tesarik J (Ed). Rome: Ares-Serono Publications, pp. 271-286.
4. Check JH, Katsoff D, Check ML (in press): Some semen abnormalities may cause infertility by impairing implantation rather than fertilization. *Med Hypotheses*.
5. Check JH, Stumpo L, Lurie D, Benfer K, Callan C (1995): A comparative prospective study using matched samples to determine the influence of subnormal hypoosmotic test scores of spermatozoa on subsequent fertilization and pregnancy rates following in vitro fertilization. *Hum Reprod* 10:1197-1200.
6. Hall J, Fishel S, Green S, Fleming S, Hunter A, Stoddart N, Dowell K, Thornton S (1995): Intracytoplasmic sperm injection versus high insemination concentration in cases of severe teratozoospermia. *Hum Reprod* 10:493-496.

7. Katsoff D, Check JH (1997): Two methods of achieving pregnancies despite subnormal hypoosmotic swelling test scores. *Fertil Steril* 68:549-551.
8. Kiefer D, Check JH, Katsoff D (1996): The value of motile density, strict morphology, and the hypoosmotic swelling test in in vitro fertilization-embryo transfer. *Arch Androl* 37:57-60.
9. Oehninger S, Acosta AA, Morshedi M, Veeck L, Swanson RJ, Simmons K, Rosenwaks Z (1988): Corrective measures and pregnancy outcome in in vitro fertilization in patients with severe sperm morphology abnormalities. *Fertil Steril* 50:283-287.
10. Oehninger S, Kruger TF, Simon T, Jones D, Mayer J, Lanzendorf S, Toner JP, Muasher SJ (1996): A comparative analysis of embryo implantation potential in patients with severe teratozoospermia undergoing in-vitro fertilization with a high insemination concentration or intracytoplasmic sperm injection. *Hum Reprod* 11:1086-1089.
11. Palermo G, Joris H, Derde M-P, Lanlus M, Devroey P, Van Steirteghem AC (1993): Sperm characteristics and outcome of human assisted fertilization by subzonal insemination and intracytoplasmic sperm injection. *Fertil Steril* 59:826-851.
12. Van Steirteghem AC, Nagy Z, Joris H, Liu J, Staessen C, Smitz J, Wisanto A, Devroey P (1993): High fertilization and implantation rates after intracytoplasmic sperm injection. *Hum Reprod* 8:1061-1066.