

## Evidence that oligoasthenozoospermia may be an etiologic factor for spontaneous abortion after in vitro fertilization-embryo transfer

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**Objective:** To evaluate whether oligoasthenozoospermia may lead to a higher spontaneous abortion (SAB) rate once a pregnancy is established by IVF-ET.

**Design:** Retrospective clinical observational study.

**Setting:** University-based IVF program.

**Patient(s):** Three hundred sixty-four couples with normal semen parameters who underwent IVF-ET with conventional sperm incubation; 70 couples with oligoasthenozoospermia but without marked abnormal sperm morphology (<4% normal forms using strict criteria) who underwent ET after IVF with conventional sperm incubation; and 20 couples with oligoasthenozoospermia but without abnormal sperm morphology who underwent ET after IVF with intracytoplasmic sperm injection (ICSI).

**Main Outcome Measure(s):** Implantation rate, clinical pregnancy rate, SAB rate, and delivery rate after IVF-ET.

**Result(s):** Despite similar pregnancy and implantation rates per ET, as a result of a higher SAB rate (40.0% versus 11.7%), the delivery rates were lower in the female partners of men with oligoasthenozoospermia. Similar patients who used ICSI had a 0% SAB rate.

**Conclusion(s):** Oligoasthenozoospermia should be considered a possible risk factor for SAB in IVF achieved pregnancies. Further studies are needed to determine whether ICSI reduces the risk of SAB associated with oligoasthenozoospermia. (Fertil Steril® 1997;68:545–8. © 1997 by American Society for Reproductive Medicine.)

**Key Words:** Oligoasthenozoospermia, intracytoplasmic sperm injection, spontaneous abortion

Traditionally, pathophysiologic abnormalities in the female partner have been considered etiologic for spontaneous abortions (SABs). These factors include luteal-phase deficiency, genetic defects of the oocyte, uterine structural abnormalities, infection, and immunologic problems, e.g., presence of antiphospholipid antibodies.

Although sperm-related factors have been considered as a possible minor cause of SAB in some instances, e.g., genetic abnormalities, in general, there

is no sperm test to establish or exclude the male partner as a contributor to the cause of SAB.

The objective of the study presented herein was to determine whether oligoasthenozoospermia may be associated with SAB.

### MATERIALS AND METHODS

A retrospective review of IVF cycles using conventional insemination methods performed between February 1, 1994, and February 29, 1996, was conducted. In vitro fertilization cycles were classified by the presence of oligoasthenozoospermia in the semen specimen on the day of retrieval. The couple was

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categorized as oligoasthenozoospermic if the specimen had  $<10 \times 10^6$  motile sperm per mL. Couples whose specimens were teratozoospermic were excluded (normal morphology  $<4\%$  using strict criteria). Only cycles that reached ET were included. Patients were excluded if they had all embryos cryopreserved and the transfer deferred to a later time. Additionally, all cycles using intracytoplasmic sperm injection (ICSI) to attain fertilization performed in the same period were reviewed to identify cases in which the male partner had oligoasthenozoospermia without subnormal morphology. Only cycles in which all the embryos transferred had been fertilized through ICSI were used.

A total of 454 patients were included in this study; 364 patients had normal semen parameters and used standard IVF (group 1), 70 patients had oligoasthenozoospermia and used standard IVF (group 2), and 20 patients had oligoasthenozoospermia and used ICSI (group 3).

For conventional sperm insemination, each oocyte was inseminated with  $2.5 \times 10^4$  (25,000) motile sperm. Sperm concentration was adjusted to include  $1 \times 10^4$  morphologically normal motile sperm per oocyte if the normal morphology score was between 4% and 10%. Up to four oocytes were incubated in the same dish.

Intracytoplasmic sperm injection was performed on all mature oocytes using the method described previously (1). Briefly, for each oocyte a motile sperm was immobilized with an injection pipet in a drop of polyvinylpyrrolidone (Scandinavian IVF Science AB, Göteborg, Sweden) and then injected into the ooplasm. The injected oocytes were placed in human tubal fluid with 10% synthetic serum substitute and incubated for at least 16 hours before evaluating for signs of fertilization. The criteria for recommending ICSI for oligoasthenozoospermia varied somewhat, depending on the clinician seeing the patient; but in general, ICSI was recommended when the motile density was  $<1 \times 10^6$  sperm per mL, if percent motility was  $<20\%$ , or if there was an absence of grade A motile sperm after sperm separation (2). Furthermore, ICSI was used if there was failed or poor ( $<30\%$ ) fertilization from a previous cycle.

Sperm concentration and motility were measured using the MicroCell Counting Chamber (Conception Technologies, La Jolla, CA).

Embryos were graded at time of transfer as either "good," if they had  $<20\%$  fragmentation with equal blastomeres, or "poor," if they had  $\geq 20\%$  fragmentation and/or unequal blastomeres.

Clinical pregnancy was defined as ultrasound confirmation of a gestational sac in the uterus. Implantation rates were defined as the ratio of the number

**Table 1** Comparison of IVF-ET Outcome by Oligoasthenozoospermia

Variable	Group 1, normal semen* (n = 364)	Group 2, oligoasthenozoospermia† (n = 70)
Women's age (y)‡	35.2 ± 4.8	35.5 ± 5.6
Ovarian stimulation‡		
Oocytes retrieved	12.6 ± 7.2	12.5 ± 7.8
Fertilization rate	65.8 ± 20.6	66.5 ± 20.4
E <sub>2</sub> day of hCG (pg/mL)§	2010 ± 1208	2152 ± 1176
Embryos transferred	3.5 ± .8	3.5 ± 1.0
Embryo quality		
Good (%)	67.5	65.5
Poor (%)	32.5	34.5
Sperm parameters¶		
Motile density (10 <sup>6</sup> /mL)**	10.1 to 256.5 (32.7)	0.1 to 9.9 (6.2)
Normal morphology (%)‡‡	4 to 32 (11)	4 to 38 (9)
Insemination concentration per oocyte‡‡		
2.5 × 10 <sup>3</sup>	148 (40.7)	38 (54.3)
1 × 10 <sup>4</sup> normal morphology	216 (59.3)	32 (45.7)
Outcome§§		
Clinical pregnancies	25.8 (94/364)	21.4 (15/70)
Multiple pregnancies	42.5 (40/94)	33.3 (5/15)
Implantation rates	11.9 (147/1237)	8.8 (22/249)
Spontaneous abortions	11.7 (11/94)	40.0 (6/15)
Delivery rates¶¶	22.8 (83/364)	12.8 (9/70)

\* Defined as  $\geq 10 \times 10^6$  motile sperm per mL.

† Defined as  $<10 \times 10^6$  motile sperm per mL.

‡ Values are means ± SD.

§ Conversion factor to SI unit, 3.671.

|| Good is defined as  $<20\%$  fragmentation and equal blastomeres; poor is defined as  $\geq 20\%$  fragmentation and/or unequal blastomeres.

¶ Values are presented as range with median in parentheses.

\*\*  $P < 0.05$ .

‡‡  $P < 0.05$ .

‡‡‡ Values are numbers with percentages in parentheses.

§§ Values are percentages with numbers in parentheses.

|||  $P < 0.05$ .

¶¶  $P < 0.05$ .

of gestational sacs per embryo transferred. Spontaneous abortion rates were the percentage of clinical pregnancies that experienced pregnancy loss. Chi-square analysis was used to compare the pregnancy rates (PRs) and implantation rates per ET and SAB rates by presence of oligoasthenozoospermia. Logistic regression was used to assess the relationship between motile density and SAB rates, controlling for confounding factors. The Mann-Whitney *U* test was used to determine whether there was a difference in morphology scores between the groups. A *P* value of 0.05 was used.

## RESULTS

There were no differences in the female characteristics of the couples in groups 1 and 2 (Table 1). Both were of similar age and had responded to ovarian stimulation with the same mean number of oocytes retrieved, sera E<sub>2</sub> levels on day of hCG, similar fertilization rates, and number of embryos transferred.

Embryo quality was similar in the two groups because the distribution of embryo grades did not differ by group.

The median motile densities in the three groups were  $32.7 \times 10^6$ ,  $6.2 \times 10^6$ , and  $3.0 \times 10^6$  motile sperm/mL. The median normal morphology scores using strict criteria were 11.0%, 9.0%, and 7.0%, respectively. The Mann-Whitney *U* test showed a significant difference in morphology scores between groups 1 and 2 ( $P = 0.002$ ). In group 1, 40.7% of the group used the  $2.5 \times 10^4$  insemination concentration compared with 54.3% of group 2; 59.3% of group 1 used the adjusted insemination concentration as compared with 45.7% of group 2 ( $P = 0.035$ ,  $\chi^2$ ).

A comparison of the sperm parameters and insemination concentrations used in groups 1 and 2 is presented in Table 1. Although the median normal morphology score was lower in the low motile density group ( $P = 0.002$ , Mann-Whitney *U* test), the proportion of patients using the adjusted insemination concentration was larger in the normal motile density group ( $P = 0.035$ ,  $\chi^2$ ).

The outcomes of the ETs are summarized in Table 1. The pregnancy and implantation rates per transfer did not differ by presence of oligoasthenozoospermia (PRs, 25.8% for group 1 and 21.4% for group 2;  $P = 0.437$ ,  $\chi^2$ ). The implantation rates were 11.9% for group 1 and 8.8% for group 2 ( $P = 0.167$ ). The delivery rates, however, were significantly lower (22.8% versus 12.8% ( $P = 0.050$ )) because of a higher SAB rate in the oligoasthenozoospermic group (40% versus 11.7%  $P = 0.005$ ), respectively. There were 40 (42.5%) multiple pregnancies in group 1 and 5 (33.3%) in group 2 ( $P = 0.501$ ).

Because the normal morphology differed in the first two groups, logistic regression was used to control for differences in normal morphology and insemination concentration. The logistic regression showed that motile density was significantly associated with SAB after controlling for normal morphology and insemination concentration. The odds ratio of having an SAB with low motile density compared with that with normal motile density was 3.1 ( $P < 0.05$ ).

The patients in the ICSI group were of similar age (mean, 35.4 years) and had a similar number of oocytes retrieved (mean, 13.1) but attained lower fertilization rates (mean, 38.5%) and, therefore, had fewer embryos transferred (mean, 2.9), compared with groups 1 and 2; 65.5% of the embryos transferred were of good quality and 34.5% were graded poor. Four patients achieved a clinical pregnancy, for a PR of 20% per transfer and an implantation rate of 13.8%. There were three multiple pregnancies (75%). There were no SABs (0%), so the delivery

rate was also 20%. This small group showed similar pregnancy and implantation rates compared with the standard IVF groups, but lower abortion rates. Statistical significance was not attained.

## DISCUSSION

The higher SAB rates after oocyte insemination with sperm from men with oligoasthenozoospermia suggests that this sperm parameter can be considered as one of the etiologic factors for SAB after undergoing IVF-ET. The mechanism by which oligoasthenozoospermia causes SAB is not known.

Because there have been reports of an increased incidence of chromosomal abnormalities with poor-quality semen specimens (3), a genetic etiology could be speculated to explain the higher SAB rate in women undergoing IVF whose oocytes were inseminated using conventional techniques with poor-quality sperm. This hypothesis seems less likely in view of the low SAB rate in the group with oligoasthenozoospermia in which ICSI was used. Theoretically, there should be the same chance that a sperm with an abnormal chromosome would fertilize the oocyte whether there was natural fertilization or ICSI. Nevertheless, the data do not totally exclude the genetic mechanism because the group receiving ICSI was small and the low SAB rate did not reach statistical significance; thus, just having a fortuitously low SAB rate was possible.

The concept that would best fit the data would be that the sperm (other than the one causing fertilization) that attach to the oocyte or 2 pronuclear zygote can somehow inhibit secure implantation when a male has oligoasthenozoospermia. This "toxic factor" theory would explain how ICSI can overcome the mechanism by which oligoasthenozoospermia causes a higher risk of SAB. The small numbers in the group receiving ICSI precludes definite conclusions but does suggest a trend that, it is hoped, will be confirmed with larger numbers in the study.

Toxic sperm factors, in fact, have been hypothesized to explain the dissimilitude between fertilization and implantation rates in couples in whom the male partner has a hypo-osmotic swelling test score  $< 50\%$  (4) or in IVF cycles where a poor "stress test" result was found (5).

There is, however, still one possibility that could detract from the conclusion that oligoasthenozoospermia is an etiologic cause of SAB. Men requiring ICSI clearly had a more severe sperm abnormality. One could hypothesize that the infertility in the couples with men with only moderate oligoasthenozoospermia may have had the infertility problem in some cases not related to the sperm but to a more

cryptic oocyte or uterine defect. The latter could lead to a higher rate of SAB.

Future attempts to corroborate or refute these data should include couples in whom the woman has tubal factor in both groups being compared. This may, however, require a cooperative study with several institutions, because there would not be as many couples needing ICSI in which tubal occlusion is also present. On the basis of the data presented here, it may be fair to randomize patients who have only moderate oligoasthenozoospermia into two groups in which one half has conventional insemination of the oocytes and the other half has ICSI.

Thus, these data strongly suggest that oligoasthenozoospermia should be considered as a cause of spontaneous abortion. By only selecting males without marked abnormal morphology (<4% normal forms using strict criteria), sperm shape was excluded as a confounding variable. This does not mean that teratozoospermia could not increase the risk of SAB independently.

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