



A STUDY TO DETERMINE IF LIMITING THE CONTACT OF SPERM WITH ZONA PELLUCIDA REDUCES THE RATE OF SPONTANEOUS ABORTIONS

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A recent study suggested that oligoasthenozoospermia may be an etiologic factor for spontaneous abortion (SAB) after in vitro fertilization-embryo transfer (IVF-ET). However, IVF-ET with intracytoplasmic sperm injection (ICSI) did not seem to be associated with an increased SAB rate. The study presented herein compared the rate of SAB in pregnancies achieved by IVF-ET according to the type of oocyte insemination process. The 3 types evaluated were conventional insemination, which exposed the oocyte to 25,000 sperm with prolonged contact (16-24 h), intermediate contact with a short insemination protocol where contact with 25,000 sperm was limited to 2 h, and very limited contact with ICSI, where only 1 sperm was injected into the oocyte thus not exposing the zona pellucida to any sperm. The patients were further subdivided into age groups of ≤ 39 or ≥ 40 . SAB rates after frozen ET were also evaluated. The clinical pregnancy and SAB rates following fresh or frozen ET for conventional, ICSI, and short insemination techniques for the 2 age groups were comparable. These data question whether oligoasthenozoospermia may be a factor in causing SAB, and whether avoidance of contact with the zona pellucida by using ICSI can negate this effect. A larger study is needed.

Keywords miscarriage, supernumerary sperm, toxic factors

The possibility exists that the presence of oligoasthenozoospermia and/or teratozoospermia could be associated with sperm having a higher frequency of chromosome anomalies leading to an increased risk of spontaneous abortions (SABs). In fact, one study by Kiefer et al. suggests that oligoasthenozoospermia may correlate with a higher frequency of SABs at least following in vitro fertilization-embryo transfer (IVF-ET) [5].

Since some sperm abnormalities, e.g., low hypoosmotic swelling (HOS) test scores, have been found to be associated with infertility related to supernumerary sperm attaching to the

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zona pellucida (ZP) leading to implantation abnormalities [2], one could also envision milder implantation disorders allowing conception but ending in SAB. Based on the proposed mechanism of some toxic factor transferred to the ZP from the attachment of supernumerary sperm causing functional impairment of the oocyte membrane, it would not be surprising if intracytoplasmic sperm injection (ICSI) could overcome the block to fertility with sperm with subnormal HOS scores [3].

Implantation defects have been attributed to sperm without necessarily having low HOS scores. Alvarez et al. found marked reduced implantation rates in couples where the male partner had a subnormal stress test [1]. Further support for the concept of supernumerary sperm possibly increasing the risk of SAB was the demonstration of a marked reduced SAB rate when ICSI was performed for males with oligoasthenozoospermia [5].

The study presented herein attempted to corroborate or refute in a larger study the data suggesting that ICSI can lower SABs. Furthermore, the study would determine if limiting the time exposure of sperm with oocyte by shortening the incubation period could lower the rate of SABs.

MATERIALS AND METHODS

During a 1½-year-time period, all IVF cycles were evaluated where a male factor related to oligoasthenozoospermia (SART, count $<20 \times 10^6/\text{mL}$; % motility $<40\%$) was present. Included in this study was a subset where the oocytes were inseminated with sperm using a short incubation time, which was part of a prospective randomized study comparing pregnancy rates (PRs) and implantation rates to conventional insemination (Table 1).

Fertilization, clinical pregnancy, implantation, and SAB rates were determined according to the method of insemination. These parameters (except fertilization rates) were also determined for frozen ETs. The data were analyzed according to two age groups: ≤ 39 or ≥ 40 . Chi-square analysis was used to compare the rates. A *p* value of .05 was used.

The ICSI procedure was performed similar to the method published by Van Steirteghem et al [8]. For each oocyte, a motile sperm was immobilized with an injection pipet in a drop of

Table 1. Insemination protocols

Conventional insemination	Short-term 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 post-retrieval using 25,000 motile sperm per oocyte.	Inseminate mature oocytes 4 h post-retrieval 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 two 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. Clinical pregnancy rates (PRs) and spontaneous abortion (SAB) rates following IVF-ET

	Type of insemination method		
	Conventional	ICSI	Short insemination
	≤39 years old		
Clinical PR/transfer (%)	48	40	62
% of SABs	9.1	17.2	27.8
	≥40 years old		
Clinical PR/transfer (%)	50	33	30
% of SABs	0	40	67

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 ≥16 h before evaluation for signs of fertilization (two pronuclei).

RESULTS

These data do not support the hypothesis that limiting the contact of the sperm with the ZP will decrease the risk of SAB (Tables 2 and 3). The numbers in each group were not large enough to show statistical differences between any groups. However, if there was any trend at all, it was that the group with the most prolonged sperm-ZP contact (conventional insemination group) had the lowest SAB rate.

In only 11 patients age ≤39 having conventional insemination was male factor present according to SART criteria ($<20 \times 10^6/\text{mL}$ concentration $<40\%$ motility). Comparable numbers for ICSI and short incubation were 51 and 4. The SAB rates were 16.7, 11.1, and 0%. The SAB rates where male factor existed in the older group were 0.0% ($n = 4$), 50% ($n = 12$), and 0.0% ($n = 2$).

Table 3. Clinical pregnancy rates (PRs) and spontaneous abortion (SAB) rates following frozen ET

	Type of insemination method		
	Conventional	ICSI	Short insemination
	≤39 years old		
Clinical PR/transfer (%)	49	53	27
% of SABs	27	18	0
	≥40 years old		
Clinical PR/transfer (%)	24	29	30
% of SABs	40	0	100

DISCUSSION

A previous publication found similar PRs and implantation rates following IVF-ET using conventional insemination techniques when comparing males with mild to moderate oligoasthenozoospermia to those with normal semen specimens [5]. However, the SAB rate was 40.0% in the former and only 11.7% in the latter [5]. That study mentioned 4 patients having conceived by IVF-ET with ICSI, and there were no SABs. Thus, the mechanism for SAB may not be related to a higher frequency of chromosome abnormalities in the sperm but may be related to the supernumerary sperm in some way damaging the ZP [5]. The hypothesis continued that in these cases the damage to the ZP was not enough to prevent implantation but would cause eventual SAB [5].

The data presented here do not suggest that shortening the incubation time of sperm and oocyte reduces the SAB rate, nor do these data show that ICSI seems to reduce it either. The results of this study seem to refute the previous publication that there is a higher SAB rate following IVF-ET when oligoasthenozoospermia is present [5]. However, a change in protocol may explain the differences. With the demonstration of lower fertilization rates when sperm had a lower percentage of normal forms as evidenced by strict morphologic criteria [6], the Cooper Center for IVF began to increase the usual concentration of the sperm used for incubation from the standard 25,000 because of evidence that this could increase the fertilization rate [7]. The sperm was adjusted in the aforementioned study if the normal morphology was 4–10% so that the sperm concentration was 1×10^4 normal motile sperm per oocyte [5] (couples with male partners with marked teratozoospermia with <4% normal forms were excluded from that study). Interestingly, in the study by Oehninger et al., despite significantly improving the oocyte fertilization rate by increasing sperm concentration for inseminating oocytes with sperm with poor morphology, the PRs actually decreased, which would be consistent with this explanation [7]. The lower SAB rate in the 11 couples using standard insemination with oligoasthenozoospermic sperm could be related to the small study group since the previous study evaluated 70 couples [5]. However, we think that a strong possibility to explain the lower SAB rate is that we no longer adjust the sperm concentration for morphology with 4–10% normal forms but use the standard 25,000 concentration. For samples with <4% normal forms we just do ICSI.

Data have failed to support any increase in SAB rate with *in vivo* pregnancy when oligoasthenozoospermia existed [4]. Based on all these studies we suspect that the sperm itself is not a frequent cause of SAB with *in vivo* pregnancies, or IVF, with an insemination concentration of 25,000 sperm or less, or IVF with ICSI. However, when excessive sperm are used for incubation, the supernumerary sperm attached to the ZP may cause implantation abnormalities that can eventually lead to SAB. We hope that these data will encourage other IVF centers that also increase sperm concentration for lower morphology to compare SAB rates in this group versus patients having ICSI to see if this hypothesis can be substantiated.

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