

Lower concentration of sperm used for oocyte insemination for IVF may result in higher pregnancy rates: preliminary study

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Abstract

There are some recent studies suggesting that certain semen specimens may be associated with subfertility not because of a fertilization defect but to an implantation disorder related to the extra sperm that attaches to the zona pellucida. Some semen specimens that contain a high percentage of sperm may cause subnormal implantation rates even with the lower sperm numbers found attached to the zona pellucida after normal intercourse. We hypothesized that some semen specimens may have an insufficient number of these abnormal sperm to cause a problem with implantation with normal intercourse but may be of sufficient number to cause a problem with higher concentration of sperm used when inseminating oocytes with *in vitro* fertilization. We hypothesized that a lower sperm inseminating concentration may not sacrifice fertilization rates but improve implantation rates. The study presented herein compared implantation and pregnancy rates with 25×10^3 vs 50×10^3 sperm concentrations. The fertilization, clinical pregnancy, and implantation rates with lower vs higher sperm concentration was 59 vs 62%, 21.2 vs 35.5% and 9.9 vs 12.3%. Despite the trends there was no statistical differences found.

Introduction

The traditional concept is that a male factor is considered etiologic for a couple's

infertility when the sperm is not able to fertilize the oocyte. Thus, sperm concentration, motility, ability to undergo capacitation and acrosome reaction are all necessary to allow one sperm to reach the oocyte and penetrate the zona pellucida.

However, there has been some data suggesting that certain sperm abnormalities can allow what appears to be normal fertilization and cleavage of the embryo, but failure to implant. An example of a dissimilitude of fertilization and implantation rates was seen when comparing matched controls with normal vs subnormal hypo-osmotic swelling (HOS) test scores (Check et al, 1995). The authors hypothesized that some cytokine may have been responsible for impairing the functional integrity of the sperm membrane and these abnormal sperm still attached to the zona pellucida cause some abnormality to the pronuclear embryo leading to future implantation defects. This hypothesis was further supported by demonstrating that avoiding the contact of the supernumerary sperm to the zona pellucida by performing intracytoplasmic sperm injection (ICSI) resulted in normal implantation rates (Katsoff, Check 1997).

A subnormal HOS score (as defined as <50%) is not very common in normospermic samples; the frequency has been estimated to be about 5% (Check et al, 1989). However, there is evidence that implantation disorders possibly related to supernumerary sperm attached to the zona pellucida may exist in a higher frequency than explained by subnormal HOS tests. Alvarez et al, (Alvarez et al, 1996) found that there was a significantly lower pregnancy rate in patients with subnormal stress tests despite the same number of embryos transferred as patients with normal stress tests.

One hypothesis that could explain this phenomenon is that there may be certain cytokines or other growth factors that can adversely affect cell membranes, and that if enough sperm that are associated with this abnormal protein bind to the zona pellucida, some critical amount of damage can occur to the 2 pronuclear embryo or possibly the later stage embryo inhibiting subsequent implantation. Evidence that these adverse protein molecules may exist was provided by the demonstration of improved HOS scores following treatment of the sperm with the protein digestive enzyme chymotrypsin and improved pregnancy rates following insemination (Katsoff, Check 1997). The possibility thus exists that the damaging protein is in such high concentration in specimens with low HOS scores that the relatively small numbers of sperm reaching the zona pellucida after intercourse or insemination can cause implantation abnormalities. However, it may be that some semen specimens contain harmful proteins in a sufficiently low concentration that implantation defects may occur only following the large number of sperm attaching to the zona pellucida when high concentrations of sperm are directly incubated with oocytes as for *in vitro* fertilization.

The study presented herein attempted to see the effect of a lower concentration of sperm used for oocyte insemination on subsequent fertilization rates and pregnancy rates in standard *in vitro* fertilization cycles.

Materials / Methods

The study was a prospective comparison of fertilization, implantation, and pregnancy rates following insemination of oocytes with either the standard concentration of 50,000 sperm versus the lower concentration of 25,000.

After egg retrieval, eggs were incubated at 37°C in organ culture dishes (Falcon) in human tubal fluid (HTF, Irvine Scientific, Santa Ana, CA) containing 10% vol/vol synthetic serum substitute (SSS, Irvine Scientific) and covered with washed, equilibrated mineral oil (Squibb, Princeton, NJ). There were 4 eggs to a dish in 1 mL of HTF.

Sperm was processed through a discontinuous isotonic Percoll gradient: 3 layers of 45, 60, and 90% Percoll (Sigma, St. Louis, MO). The sample was centrifuges through the gradients for 20 minutes, then washed twice in HTF. Sperm was analyzed manually on a MicroCell counting chamber.

Insemination was performed 4 hours after egg retrieval. Sperm was added at concentrations of either 25,000 or 50,000 motile sperm per egg, resulting in sperm concentrations per dish of 100,000 or 200,000 motile sperm.

Fertilization was assessed by coronal removal the next morning, approximately 16 hours post-insemination.

Chi-square analysis was used to compare the pregnancy rates. T-test was used to compare the fertilization rates. P value of .05 was used.

Results

The fertilization rate of all oocytes with the higher standard concentration was 59% versus 62% for the lower concentration of sperm ($p=NS$). The implantation rate per cycle was 9.9% with the higher sperm concentration versus 12.3% with the lower concentration. The clinical pregnancy rate per cycle was 21.2% per cycle versus 35.5% when comparing higher versus lower sperm concentration. This resulted in the live delivery pregnancy rate per cycle to be twice as high (15.1% vs 29%) when less sperm was used to inseminate the oocytes.

Chi-square analysis did not show any statistical differences even though the delivery rate per cycle was doubled when a lower concentration of sperm was used ($p=179$). Post-hoc power analysis demonstrated that a sample size of 169 cycles per cycle would be needed to show a difference in the clinical pregnancy rates per cycle with 80% power at the 5% significance level and 153 cycles per group would be required for significant differences in the delivery rate per cycle.

Discussion

Excess attachment of abnormal sperm to the zona pellucida causing implantation defects could possibly explain the paradox of why increasing the sperm concentration to inseminate the oocytes for severe asthenozoospermia (using strict criteria) significantly improved the fertilization rate (14.5% to 62.6%) but did not improve the extremely low pregnancy rate (Oehinger et al, 1988).

Another study that supports the concept of implantation defects related to some sperm metabolic products especially those attached to the zona pellucida was provided by Gianaroli et al (Gianaroli et al, 1996); they found a 28% per cycle pregnancy rate using an hour sperm-oocyte incubation versus only 11% with a conventional 16 hour exposure (Gianaroli et al, 1996). The implantation rates (15% vs 8%) reflected the same trend.

Unfortunately, our study did not have a sufficient number of cases to attain significance. Therefore, we can only conclude that there was a trend for higher pregnancy rates without sacrificing the ultimate number of embryos when lower sperm concentrations were used for insemination. This study was originally approved by the research review committee of the Cooper Center for IVF. It was agreed that we would not look at the results until the deadline time for submission to the American Society of Andrology. In fact, this manuscript was presented at the February 1997 meeting. Based on the trend, however, the committee voted that it would be unfair to patients to continue using the higher sperm concentration until statistical significance can be reached; thus we will not be able to complete this study, but hope that it will stimulate other centers to corroborate or refute these data. We have presently submitted a proposal to compare 25,000 vs 15,000 sperm with conventional incubation times and also study short incubation.

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