Identification of Male-Factor Semen Samples Prior to Insemination and in Vitro Fertilization

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Semen analyses carried out as part of the clinical in vitro fertilization or intrauterine insemination protocols provide important information that determine the type of clinical treatment of the male partner and the sperm processing method. It is postulated that the sperm of male-factor patients cannot survive hypoosmotic stress conditions because of defective sperm membrane function. To test this, 0.1 ml of semen from each of 102 patients was placed in 1.0 ml of 150 mosmol/liter eosin citrate fructose solution and incubated for 30 min at 37°C. The percentage viability of the sperm cells was then determined. The results indicated that patients with two or more abnormal semen parameters had a significantly lower percentage viability while in the hypoosmotic solution (40.6 ± 4.7%), in contrast to non-male-factor patients (69.0 ± 1.6%). Donor sperm (N = 32) serving as controls (73.3 ± 2.1%) had a viability in hypoosmotic solution similar to that of non-male-factor patients. The data suggest that sperm of male-factor patients are less able to survive the hypoosmotic stress conditions as shown by the percentage viability in hypoosmotic solution and emphasize the importance of using less stressful sperm processing methods for in vitro fertilization or insemination in these patients.

KEY WORDS: sperm; semen analysis; in vitro fertilization; insemination; hypoosmotic swelling; viability.

INTRODUCTION

Semen analyses are routinely carried out as part of the clinical workup prior to in vitro fertilization or intrauterine insemination procedures and the analyses provide important information that determine the type of clinical treatment of the male partner and the sperm processing method. It is postulated that the sperm of male factor patients cannot survive hypoosmotic stress conditions because of defective sperm membrane function or "leaky" membrane. Evidence supporting this premise comes from studies showing a correlation between the in vitro fertilizability of human sperm and the percentage of sperm swelling as determined through the hypoosmotic swelling test (1,2). However, these studies were based on the determination of specific patterns of sperm tail swelling and a study of the sperm viability in the hypoosmotic solution is lacking. Such a study would provide valuable information on the integrity of the membranes at the sperm head. The objective of the present study was to compare male-factor and non-male-factor semen samples' percentages of sperm viability after stressing the sperm with the hypoosmotic solution and determine if there were differences in sperm viability.

MATERIALS AND METHODS

Semen were collected from 90 infertility patients and 8 donors at the Fertility Center and liquefied at room temperature for 30 min. Routine semen anal-
ysis was then carried out on each specimen in accordance with guidelines set by the WHO (3). Donor samples were from donors screened in accordance with the guidelines set by the American Fertility Society (4). Supravital staining to determine the percentage viability of sperm was performed using 0.5% eosin Y stain (5). The percentage normal morphology of the sperm was determined using the Diff-Quik staining procedure. Sperm hyperactivation motility was assessed under the light microscope after diluting the semen sample 1:20 with Hams' F-10 supplemented with 3.5% human serum albumin and incubating at 37°C for 30 min (6). Semen samples were classified as belonging to the male-factor category when two or more of the parameters (count, motility, morphology) were abnormal.

Eosin Y at 0.5% (w/v) concentration was added to the citrate and fructose mixture (1) and the final osmolarity adjusted to 150 mosmol/liter using MilliQ/UF water. The pH was set at 7.9 and the mixture was filter-sterilized. Aliquots (0.1 ml) of the semen samples from donors (N1 = 32) and patients (N2 = 102) were individually mixed with 1.0 ml of the prewarmed stain mixture and incubated at 37°C for 30 min. At the end of the 30-min period, an aliquot (5 μl) of each sample was pipetted onto a glass slide, a coverslip placed on top of the droplet, and the slide examined under the light microscope (400x magnification). The percentage viability was calculated from the number of sperm with clear, non-red-colored heads divided by the total number of sperm (sperm with non-red-colored heads plus red-colored heads) multiplied by 100. The percentage of sperm cells undergoing hypoosmotic swelling (types B to G collectively) (1) was also determined under phase contrast. A total of 100 sperm cells (irrespective of whether the cells were swollen or not) in several random fields was analyzed for viability in the hypoosmotic solution and another 100 cells were examined for hypoosmotic swelling. The cutoff value of 45% or more for normal samples was based on the lowest percentage sperm viability in hypoosmotic solution in donor samples. The normal criterion for the hypoosmotic swelling test has previously been established at 60% or more sperm swelling (7).

Using the male-factor criterion as the fixed variable, the data on percentage viability in hypoosmotic solution were analyzed and compared with data from the non-male-factor and donor control groups. The relation between the viability in hypoosmotic solution parameter and the other semen parameters were examined using linear regression correlation coefficient and the significance was tested using the t test. A P < 0.05 value was considered significant.

RESULTS

The data indicated that male-factor patients characterized by two or more abnormal semen parameters had a significantly lower (P < 0.05) percentage viability in hypoosmotic solution (40.6 ± 4.7%) in comparison with non-male-factor patients (69.0 ± 1.6%). Donor sperm serving as controls (76.2 ± 2.1%) had a percentage viability in hypoosmotic solution similar to that of non-male-factor patients. The intraassay coefficient of variation in the percentage viability in hypoosmotic solution for donor semen (n = 10) was previously determined to be 4.7%. The interassay coefficient of variation based on different samples from the same donor ranged from 3.0 to 25.2% over a 3-month period.

The percentage viability in hypoosmotic solution parameter correlated the strongest with the total sperm motility parameter (r = 0.572, P < 0.005, n = 102). Strong correlations were also observed for the percentage rapid progression or type A sperm motility pattern (r = 0.452, P < 0.005, n = 102), percentage supravital staining viability (r = 0.442, P < 0.005, n = 100), and percentage sperm hyperactivation motility (r = 0.436, P < 0.005, n = 102). A low correlation was observed between the percentage viability in hypoosmotic solution and the percentage normal morphology (r = 0.280, P < 0.01, n = 102) or the sperm count (r = 0.256, P < 0.01, n = 102). There was a lack of correlation between the viability in hypoosmotic solution parameter and the percentage hypoosmotic swelling (r = 0.150, n = 102), the semen volume (r = 0.117, n = 102), and the length of abstinence (r = 0.043, n = 96). Altogether, there were 13 semen samples categorized as male-factor samples of the 134 samples based on two or more abnormal semen analysis parameters. The viability in hypoosmotic solution parameter using the 45% or more criterion for a normal sample identified 10 of these 13 samples, while the hypoosmotic swelling test (60% or more swelling) identified 7 of the 13 samples.