SHORT COMMUNICATION

NORFOLK, VIRGINIA

Comparison of Various Preparation Methods for the Use of Cryopreserved-Thawed Spermatozoa in Insemination Therapy

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INTRODUCTION

Different approaches have been implemented to improve the recovery and/or the quality of motile sperm after cryopreservation-thawing. Gradient separation of the motile fraction before freezing offers the possibility of selecting spermatozoa that retain motility for up to 24 h (1). An intrauterine insemination (IUI)-ready cryopreservation method using sucrose and glycerol-based cryoprotectant with Percoll processing produced improved results when compared to conventional cryopreservation (2). Additionally, selection of a highly motile sperm population by swim-up was shown to improve postthaw acrosomal integrity, motility, and other functional parameters (3). The purpose of this prospective study was to assess the impact of different semen processing methods (i.e., samples cryopreserved as whole semen and IUI-ready, and those washed for IUI after thawing) on motility parameters postthaw.

MATERIALS AND METHODS

Semen from 26 men undergoing infertility evaluation (study group) and five fertile donors (control group) were examined. Samples with $>15 \times 10^6/\text{mL}$ motile sperm were included. For each independent ejaculate three preparative methods were tested: whole semen, IUI-ready, and IUI-wash. Each sample

was divided into two equal fractions. One portion was cryopreserved as whole semen. The other part was subjected to a 20 min/ $380 \times g$ gradient centrifugation, using 90 or 40% Percoll (Sigma, St. Louis, MO), to separate the motile sperm for cryopreservation (IUI-ready). The motile fraction was washed ($380 \times g$, 10 min) once with human tubal fluid supplemented with 0.3% human serum albumin (HTF-HSA; Irvine Scientific, Santa Ana, CA) before cryopreservation.

To cryopreserve, each fraction was slowly and gently mixed with the freezing medium (TYB-G, Irvine)(4). Volume ratio of semen to TYB-G was 1:1 and for the motile fraction was 1:1/2. The mixture was transferred into cryovials, refrigerated for 1 h, and cryopreserved in liquid nitrogen vapor for 20 min.

The specimens were thawed in a water bath at 40°C for 3 min and analyzed. The thawed whole semen fraction was evaluated and further washed once with HTF-HSA to prepare it for an IUI (IUI-wash). All dilutions changes were factored in calculating final parameters reported. Cryosurvival rate was determined as the ratio of postthaw/prefreeze percentage of motility.

Repeated analysis of variance (ANOVA) followed by Tukey-Kramer for motion analyses, Barlett's method for analysis of standard deviation differences, and the Kolmogorov-Smirnov test for assessment of Gaussian distribution were used. Data are presented as mean $\pm$ standard deviation. The level of significance was set at $P < 0.05$.

RESULTS

The sperm motion parameters after subjecting the samples to the various sperm preparation methods are summarized in Table I. In the patient group, the concentration of motile spermatozoa, progressive motility, and cryosurvival rate in IUI-ready samples were significantly higher than in the samples cryopreserved as whole semen ($P < 0.05$, $P < 0.001$, $P < 0.001$, respectively) and in the IUI-wash samples ($P < 0.01$, $P < 0.05$, $P < 0.01$, respectively). In the donor

Table 1. Comparison of Motion Parameters of Cryopreserved-Thawed Spermatozoa Subjected to the Different Sperm Preparation Methods

Preparation methods	Motion parameters					
	Conc. motile ($\times 10^6/\text{mL}$)	Motility (%)	Cryosurvival (%)	VCL ($\mu\text{m/s}$)	Rapid sperm (%)	Linearity
Patients						
Whole semen	10 ± 10^a	23 ± 14^a	35 ± 18^a	34 ± 6	18 ± 11^a	6 ± 2
IUI-ready	14 ± 16^b	37 ± 18^b	59 ± 25^b	39 ± 5	29 ± 8^b	6 ± 1
IUI-wash	9 ± 12^a	28 ± 21^a	40 ± 28^a	37 ± 14	31 ± 21^b	6 ± 2
Donors						
Whole semen	54 ± 39	51 ± 20	57 ± 23	36 ± 4^a	26 ± 12^a	7 ± 1
IUI-ready	42 ± 16	60 ± 7	68 ± 7	40 ± 4^a	39 ± 4	7 ± 1
IUI-wash	56 ± 41	71 ± 15	80 ± 18	50 ± 8^b	55 ± 17^b	7 ± 1

Note. Superscripts *a* and *b* indicate statistically significant difference between values.

group, the various methods showed no significant differences.

In the patient group, the percentage of rapid spermatozoa was significantly higher in the IUI-ready and IUI-wash when compared to whole semen samples ($P < 0.05$ and $P < 0.01$, respectively). In the donor group, this percentage was significantly higher only in IUI-wash compared to whole semen ($P < 0.01$).

DISCUSSION

Sperm cryopreservation still represents a valuable clinical aid in the management of infertility. Here, we compared postthaw outcome of three different cryopreservation/processing techniques routinely used to prepare sperm for IUIs. We also used a commonly practiced conventional manual vapor freezing method. It was noted that in patient samples, gradient separation of spermatozoa with high motility prior to cryopreservation resulted in superior postthaw motion parameters including the total recovery of motile cells and progressive motility. It may be speculated that such outcome is due to the exclusion of abnormal sperm and other cells and the elimination of their possible detrimental effects including, among others, the generation of reactive oxygen species. It is acknowledged that the population of patients studied herein included men consulting for infertility with normal or only slightly subnormal sperm parameters. Whether this is also true for samples with more severe degrees of oligoasthenoteratozoospermia remains to be established.

Additionally, it is also recognized that the processing methods employed have inherent differences. However, the methodologies employed did not

deviate from what routinely and clinically is daily performed in most centers to process cryopreserved samples for IUIs. We aimed to explore if any of these methods exhibits improved outcome that make it more suitable for optimized IUI therapy. Investigating the pregnancy outcome of such methods is desirable. However, in the absence of such outcome, it may be plausible to assume that samples with higher number of motile sperm and with better motion characteristics may be superior to those lacking such features for achieving pregnancy.

Percoll has been replaced with safer gradient centrifugation products. Nevertheless, several studies have been published indicating that the quality of samples processed using Percoll is comparable to the approved gradients currently in clinical use (5). Gradient processing selects spermatozoa with highest motility and superior morphology, and removes leukocytes that are known to induce damage during processing. Cryopreservation as IUI-ready also adds convenience in the clinical setting. As mentioned, if motility and the number of motile sperm are considered crucial for success, either IUI-ready or IUI-wash could be recommended for patient and donor cryopreserved semen preparations for IUI therapy. Our donor sperm program currently use IUI-ready samples processed with ISolate™ (ISolate, Irvine). The cumulative pregnancy rate has been 60% after a 6-month treatment (6).

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