SPERM DNA FRAGMENTATION (SDF) WAS MOST EFFECTIVELY IMPROVED BY A SPERM SEPARATION DEVICE COMPARED TO DIFFERENT GRADIENT AND SWIMUP METHODS

Broussard A[1,2,3], Leader B[2], Tirado E[2], Colver R[1], Reuter L[1], Bopp B[1], Will M[1], Anspach Will E[1], Adaniya G[4]

1.Midwest Fertility Specialists 2. ReproSource 3. Eastern Virginia Medical School 4. Ovation Fertility Indianapolis

OBJECTIVES

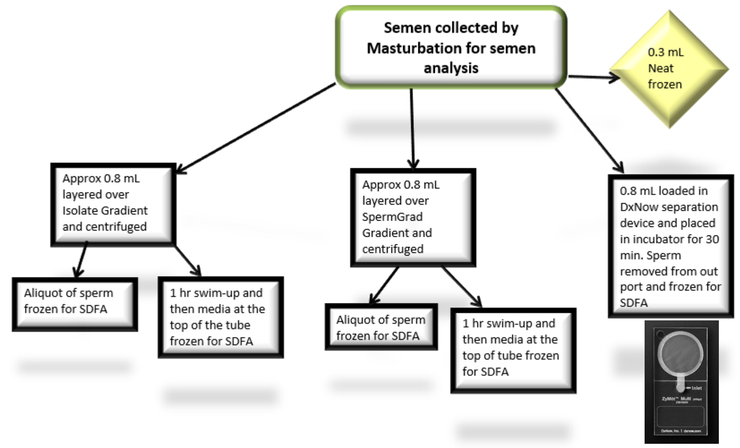
This was a prospective study to compare different commercially available sperm preparation techniques on the same semen sample to determine which methods most effectively improves sperm DNA fragmentation index (DFI) and other sperm health biomarkers, such as oxidative stress adducts (OSA) and high DNA stainability (HDS) index.

ABSTRACT

Sperm DNA fragmentation (SDF) has previously been correlated with adverse outcomes in the in vitro fertilization (IVF) laboratory, poor implantation and increased pregnancy loss in multiple studies. In a previous study we determined that sperm DNA fragmentation index (DFI), oxidative stress adducts (OSA), and high DNA stainability (HDS) at the time of insemination was negatively correlated with fertilization rates, with both standard insemination and intracytoplasmic sperm injection (ICSI). We concluded that different sperm preparation methods should be evaluated to determine which yielded the best sperm quality.

In this current study we evaluated different methods to improve DFI, OSA, and HDS. The methods used were ZyMōt Sperm Separation Device (1,DxNow) Isolate gradient (2), SpermGrad gradient (3), Isolate + Swim-up(4), and SpermGrad + Swim-up (5). Semen samples of 30 men with the mean age of 37±8.3 years were processed with each method. Neat sperm concentrations ranged from 7 to 104 million/mL with 23.3% oligospermic. The motility of the samples ranged from 15% to 80% motility with an average of 52.2±16.9%. The sperm separation device consistently outperformed the other methods to improve DFI, HDS, and OSA.

METHODS & TABLES

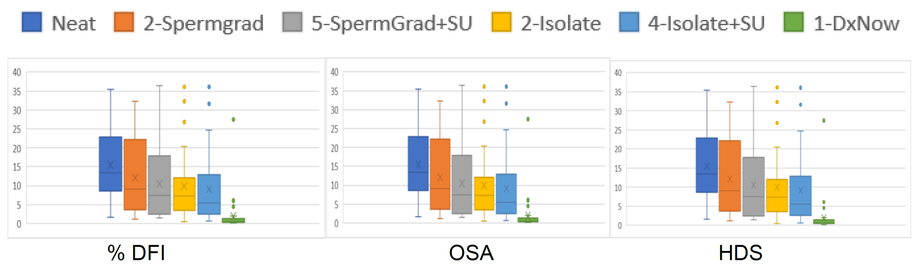


Coded specimens were sent to an external clinical laboratory (ReproSource, Woburn, MA) for blinded SDF measurement, reported as % DNA Fragmentation Index (%DFI), using the acridine orange and flow cytometry as described in the SCSA© method. DFI, OSA, and HDS were compared between preparation methods on the same ejaculate using Wilcoxon Rank Sums between each pair with $P<0.05$ considered significant (See table). Box and whisker plots were used to display the variation of DFI, HDS, and OSA post-processing.

Prep Method 1	Prep Method 2	DFI p-value	OSA p-value	HDS p-value
Neat	Isolate	0.0052	0.0002	0.0011
Neat	Isolate + SU	0.0023	0.0002	<0.0001
Neat	SpermGrad	0.074	0.0574	0.2837
Neat	SpermGrad + SU	0.0184	0.0024	0.0002
Neat	DxNow	<0.0001	<0.0001	<0.0001
Isolate	Isolate + SU	0.6789	0.8360	0.0389
Isolate	SpermGrad	0.3750	0.0656	0.0326
Isolate	SpermGrad + SU	0.6520	0.7394	0.1808
Isolate	DxNow	<0.0001	0.0657	<0.0001
SpermGrad	SpermGrad + SU	0.4464	0.3183	0.0044
SpermGrad	Isolate + SU	0.1761	0.0656	0.0005
SpermGrad	DxNow	<0.0001	0.0004	<0.0001
DxNow	Isolate + SU	<0.0001	0.0224	<0.0001
DxNow	SpermGrad + SU	<0.0001	0.0428	<0.0001
SpermGrad + SU	Isolate + SU	0.4779	0.9764	0.0044

DFI, OSA, and HDS were compared between preparation methods on the same ejaculate using Wilcoxon Rank Sums between each preparation method.

RESULTS



CONCLUSIONS

The sperm separation device is a novel method that effectively reduced DFI ($p<0.0001$) compared to two different gradients (2,3) and gradients followed by swim-up (4,5). The device also has eliminated centrifugation, a step known to increase oxidative stress. The device reduced OSA levels, a measurement of oxidative stress, and HDS, which is a measurement of immature cells and high histone retention. These are both indicators of sperm health and function. Overall, the quality of the sperm obtained post-processing was improved by the use of the separation device, which may increase the chance of a healthy sperm being used for fertilization.

SUPPORT

This study was possible by the support of a ReproSource Investigator Reward and the donation of the ZyMōt devices from DxNow.