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Research Article

Semen Collection in a Device Specifically Designed for Human Semen Improves Sample Physiological and Morphological Parameters

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Abstract

Objectives: The current series of studies examined the use of the device for improved semen collection (DISC) and its effects on semen quality specifically designed for human semen collection. The system was designed to lessen cellular shock by providing an environment that optimized conditions for sperm survival.

Materials and Methods: A series of experiments were conducted to examine the effects of the DISC, on sperm cell physiology, biochemistry and, in a small FDA-approved clinical trial, to prove equivalence for pregnancy outcomes.

Results: Samples collected into the DISC maintained sample motility as well as other movement parameters for extended periods of time when compared to samples collected in a traditional standard specimen cup ($P < 0.05$). Further, the samples appeared to maintain biochemical functions, such as the acrosome reaction ($P < 0.001$) and mitochondrial function ($P < .001$), suggesting the DISC improved sample environment, which protects cells during the collection process.

Conclusions: The DISC was designed to provide a superior collection environment for human semen by limiting environmental shock to the sperm during and after the collection process. Data from the present study suggest the system helps maintain both sample physiological and biochemical parameters for extended periods of time, making more healthy cells available for clinical procedures, which should translate into higher pregnancy rates.

Introduction

It has been forty years since the announcement of the first birth from in vitro fertilization (IVF)[1]. While this single event opened the door to continuous improvements in infertility treatments, the improvements have focused primarily on the female partner[2-3], her gametes[4-6,] and the embryos she will carry[4.5.7]. The one exception might have been the development of intracytoplasmic sperm injection ICSI in the late 1980s[8-10]. However, it can be argued that ICSI was developed to bypass issues with spermatozoa rather than trying to maintain the natural fecundity of the sperm cells used in IUI and ART procedures. In fact, with the advent of ICSI, the attitude has developed, "we only need one normal-looking cell."

However, during the same forty-year period, significant research has demonstrated that choosing sperm cells,

like embryos, based solely on morphology, is to risk physiological, biochemical, or DNA damage, which might prevent the cell from providing normal fertilization[11-14].

Materials and Methods

The DISC

The device for improved semen collection was designed to minimize environmental shifts during semen collection. The device tapers from the width of a standard specimen cup to form a centralized collection zone, which minimizes the outer exposed surface area while maximizing the internal volume. This arrangement supports sample stabilizing and maintenance of its temperature at the point of collection. The outer shell of the device forms an insulating cocoon around the sample allowing for a slower post-collection cooling rate, limiting activation of shock proteins and maintaining cellular activity. Finally, the container is optimized by adding a measured amount of media, optimally no more than 20% of the expected volume, to provide pH control and activate osmotic pumps preventing osmotic shock (Figure 1). The design allows the sample to create its own stable environment, which is maintained for extended periods. The slow cooling rates prevent shock proteins or other cellular pathways leading to the acrosome reaction and premature capacitation.

To determine if the DISC maintained a superior collection environment compared to a standard specimen container, a series of experiments were conducted to assess the environment created, and its ability to maintain standard semen parameters, and cellular biochemistry and physiology. Finally, a small FDA approved fertility trial was conducted to prove equivalence to current standard collection techniques.

Thermal Studies

The thermal properties of the DISC were compared to those of a standard specimen cup (SSC; Fisher Scientific; St. Louis, MO) before the start of experiments with viable semen samples. Room temperature (21-23^{o}C) and warmed (37^{o}C) standard specimen cups and DISC were fitted with thermocouples to measure the temperature of specimens. With the thermocouples in place, 5 mL of water, previously warmed to between 39-40^{o}C, were added simultaneously to both the SSC and the DISC to simulate semen samples. Both containers were then placed unprotected on a standard laboratory counter on non-thermal conductive absorbent pads at ambient room temperature (21-23^{o}C). To standardize temperature measurements, all measurements were started at 37^{o}C. Measurements were continued at one-minute intervals until the specimen had dropped a total of 10^{o}C (to 27^{o}C). The experiments were repeated ten times. The resulting data were compared using ANOVA with repeated measures.

Preliminary Studies to Test the Effect of the DISC on Semen Parameters

In a preliminary study, nine semen samples were collected from each of the three donors (N=27). Using a Latin square design, each sample was assigned to one of three different collection techniques: 1) SSC at room temperature(control), 2) DISC at room temperature, and 3) the DISC warmed to 37^{o}C, using one of three media 1) Ham's F-10 (Sigma Chemical; St. Louis, Missouri) alone, 2) Ham's F-10 with Human Serum Substitute (Irvine) or 3) Human Tubal Fluid (Irvine) for processing. Control SSC were simply an empty container provided at the time of the collection. Both DISC treatments included 1 mL of the assigned media added prior to collection. All samples were collected by masturbation every three days until the completion of the process. Once each sample was collected, it was prepared for IUI using a standard simple centrifugation technique[14]. Once prepared, the samples were transferred to a 37^{o}C incubator with 95% humidity and 5%CO_2. Standard semen parameters including concentration, motility, viability, linearity, and velocity were evaluated at 1, 3, 6, 12, 18, and 24 hours post-processing. In addition, acrosome data were collected at all time points. All standard semen parameters were evaluated on a Nikon Alphaphot microscope equipped with phase optics (Nikon Inc.; NY, NY) and the computer semen analysis program Cell Soft.

Acrosome measurements were made on a Zeiss Standard microscope equipped with fluorescence (Carl Zeiss Inc., NY, NY) and using a Chlorotetracycline staining technique[15]. When viewed under a fluorescent microscope, equipped with a 520μm excitation filter and a 570μm barrier filter, the intact acrosome cap appears a bright fluorescent yellow.

Biochemical Studies

As initial studies suggested maintenance of semen parameters over time, a series of biochemical studies were conducted to determine how the DISC affected sperm quality. Nine donors supplied three samples, each collected in a standard specimen cup (SSC), the DISC or the DISC with 1 mL of media. Following collection, each sample was processed using a sperm washing technique and then placed in HAM's F-10 media and cultured for 12 h. At predetermined intervals of 0, 3, 6, 9, 12, and 24hr, aliquots were taken for standard semen analysis and biochemical assessment, including intactness of acrosomal membranes, apoptosis, lipid per oxidation level, mitochondrial membrane potential, and DNA Fragmentation. Standard semen parameters of motility, velocity, and linearity were determined using an IVOS computer-assisted semen analyzer (Hamilton Thorne; Beverly, MA). Viability was determined using eosin stain and counted manually using a Nikon. The state of the acrosome was determined using the specific labeling by peanut agglutinin (PNA) conjugated with fluoresce in (FITC) described by Mortimer *et al.*[16]

During apoptosis, phosphatidylserine (PS) is externalized from the inner leaflet to the external leaflet of the phospholipid bilayer. Annexin V is a protein that binds to PS. Phosphatidylserine externalization was determined using an assay containing annexin V conjugated to FITC. Apoptosis (positive control) was induced by incubating with 2.5 mM Staurosporine in Ham's F-10 for one hour. Necrosis (positive control) was by incubating with 10 mM H_2O_2 for one hour. The quantity of lipid per oxidation was determined by using a thiobarbituric acid reactive substance (TBARS) quantification assay. Mitochondrial membrane potential was determined using Mito Tracker Red CMXRox, which accumulates in mitochondria with high membrane potential. The amount of DNA fragmentation was determined using a HALO assay based on the sperm chromatin dispersion test[9]. The resulting data were subjected to ANOVA with repeated measures.

Fertility Trial

An FDA-approved, prospective, randomized controlled trial of the DISC was conducted in the treatment of IUI patients. Couples were recruited and randomized in a crossover study alternating the DISC and SSC. Patients were recruited from two different fertility centers, with separate labs, which serve different patient populations across the West Texas region. Site A was at the Texas Tech Health Sciences Center in Amarillo, TX. Site B was at the Texas Tech Health Sciences Center in Lubbock, TX. Each site maintained its traditional culture media.

Further, Site A performed semen analysis manually while Site B performed a number of the analysis with the IVOS-CASA. Patients remained in the study for a maximum of 6 cycles (3 cycles in each device) or until pregnancy. Twenty-four couples completed a total of 51 treatment cycles. The resulting data were analyzed using Statistical Program for Social Sciences (SPSS) to detect differences in semen parameters and pregnancy rates when using the DISC or SSC using t-Test or Chi-square as appropriate.

Results

Preliminary temperature trials demonstrated the DISC performed as designed. The 5 mL of fluid in the ambient temperature SSC lost heat at a rate of 2-3^{0}C per minute. However, in comparison, the DISC lost heat at a rate of only .3-.5^{0}C per minute (Figure 1, $P < 0.001$). Therefore, while the unprotected sample in SSC dropped 10^{o}C in under 5 min, the simulated sample in the ambient temperature DISC took over 20 min to cool to the sample point.

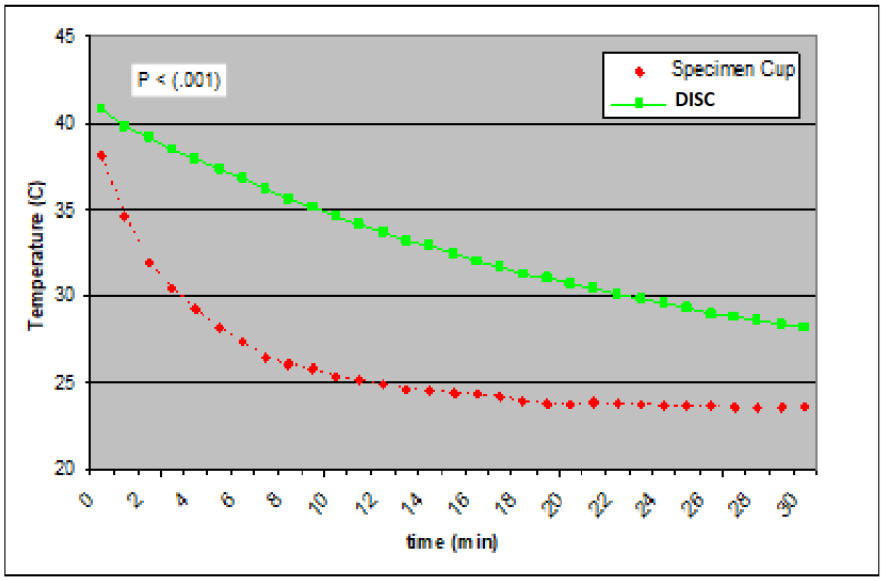
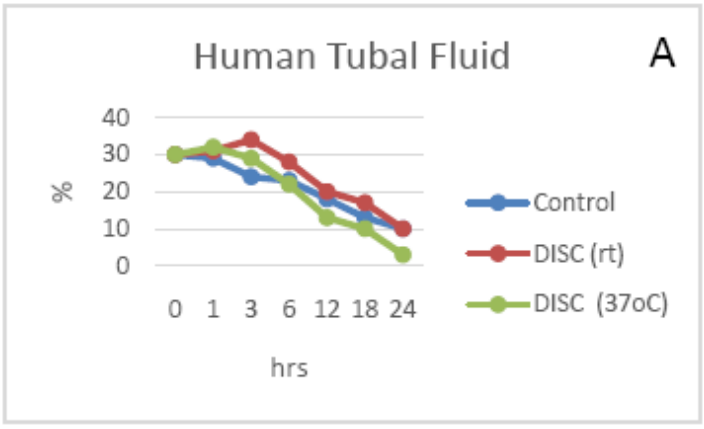


Figure1. Temperature loss over time from 5 mL of fluid stored in a Standard Specimen Cup versus a new semen collection device (P <.001).

Further, while prewarming the SSC to 37^{0}C only slightly slowed the cooling rate (1.8 – 2.5^{0}C/min), warming the DISC increased the cooling time from 20 to 38 minutes (P < 0.001). Together, these data support the concept that the DISC maintains a stable temperature for much longer than the SSC and prevents cold shock due to exposure to surfaces found in a clinical or transport environment.

Given the results of the temperature trials, studies progressed to laboratory-based comparisons of sample quality using standard semen parameters over time. Studies were conducted comparing standard semen parameters from three donors in 9 different combinations of collection devices and media. As expected, all semen parameters changed over time (P < 0.001), and these changes were both media and device-dependent (P < 0.05). Looking at specific parameters, Figure 2 shows the changes in motility over time. Those samples collected in Ham's F-10 with or without serum performed better than samples collected in HTF. Further, those samples collected in the DISC in either Ham's media maintained motility for longer periods versus their controls (P < 0.04).

Both viability and track speed were maintained well in all three treatments for the 24 hr time-period (Table 1). This might be expected given the length of the study; however, while control samples demonstrated increased acrosome reactions over time (P < 0.001), samples collected in either DISC device maintained significantly higher intact acrosomes over the 24 hr period. Collectively these data support the concept that the DISC maintains semen quality over extended periods, which might indicate healthier cells for assisted reproductive procedures, including IUI.



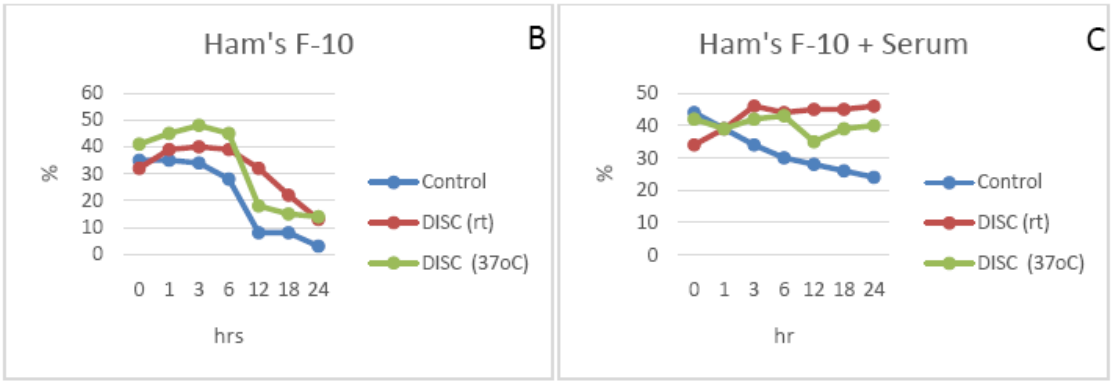


Figure 2. Comparison of motility maintenance over time of semen samples collected in a standard specimen cup (control), the device for improved semen collection (DISC) or a DISC warmed to 37oC, from 9 human donors.

Table 1. Maintenance of track speed and viability over the 24 hr culture period

	Track Speed (um)		Viability (%)	
Treatment Group	Initial	24 hr	Initial	24 hr
Control				
HTF	51	40	80	61
Ham F-10	59	32	62	37
Ham's + Serum	71	70	87	80
DISC room temp				
HTF	52	20	88	65
Ham F-10	62	63	69	48
Ham's + Serum	62	60	93	90
DISC 37°C				
HTF	68	69	87	64
Ham F-10	63	60	70	48
Ham's + Serum	71	70	98	90

Similar results were seen between devices for viability, motility, and acrosomes in the biochemical studies. While the data suggests similar apoptotic activity in all cultures, the MitoTracker Red studies suggest that the mitochondria from the DISC with media (DISC+; $P < 0.001$)remained membrane intact and functional much longer than samples collected in the SSC (Figure 3). However, the percentage of DNA fragmentation was similar in all treatments ($P = 0.896$).

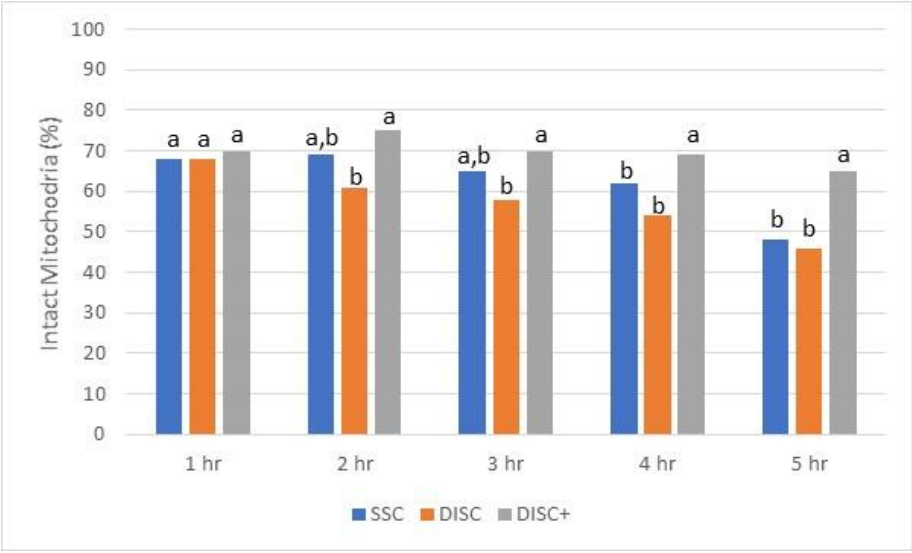


Figure 3. A comparison of mitochondrial membrane integrity over time in semen collected in a standard specimen cup (SC), dry DISC, or a DISC containing 1 mL of Ham's F10 media with serum. Columns with different subscripts are sstatisitical different within a time point ($P< 0.001$).

The final study was an FDA-approved clinical trial of equivalents in IUI treatments. Twenty-four couples enrolled in the study and completed a total of 51 cycles completed; 25 with the DISC and 26 with the SSC. Nine couples completed only a single cycle, 8 couples completed two cycles, 3 couples completed three cycles, 3 couples completed four cycles, and one couple completed five cycles. Seven couples opted for in-vitro fertilization (IVF) after one cycle. All outcomes were included in the data analysis.

There have been 9 pregnancies in 51 cycles (17.6%), four in the DISC (16%) versus five in the SSC (Figure 4; 19.2%). Interestingly, all four pregnancies (100%) using the DISC continued to term while only 2/5 pregnancies (40%) using sperm collected in the SSCreached delivery (Figure 4). Further, all three miscarriages occurred in the first trimester.

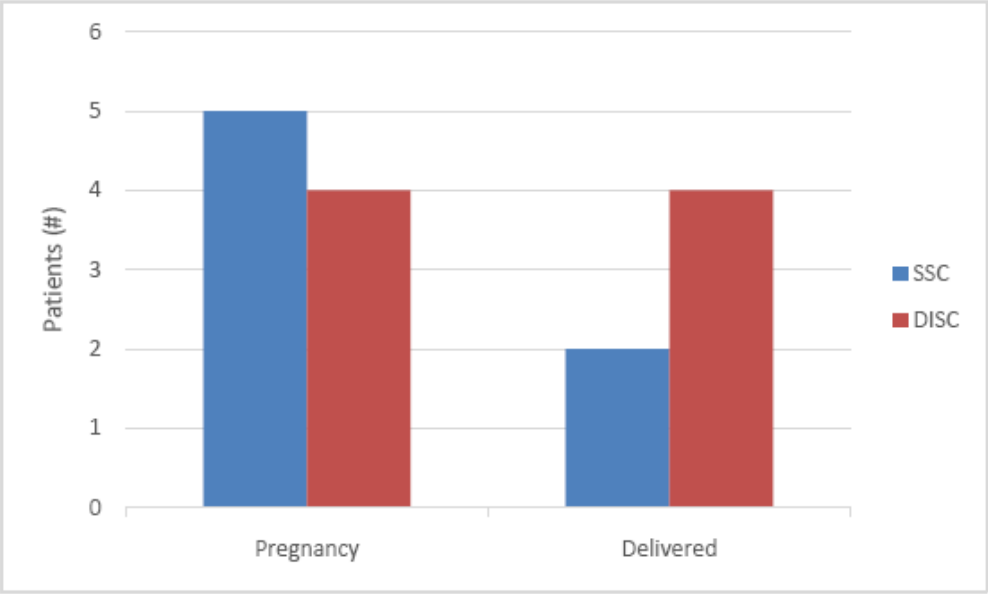


Figure 4. Pregnancy results and deliveries from a preliminary fertility trial of the device for improved semen collection (DISC) versus a standard specimen cup (SSC).

It should be noted that this was a relatively small clinical study. Seven of the couples opted to advance their treatment plan to IVF before completing the recommended IUI cycles in hopes of conceiving more quickly. However, of the remaining 17 couples, nine conceived (52.9). Thus, conception was achieved equally in the DISC and SSC.

Discussion

The world of infertility treatment has changed significantly in the last forty years. However, outside of surgical procedures to recover sperm cells[17-18], little has changed in the way in which sperm cells are collected. However, a significant number of recent studies have suggested that traditional collection techniques might lead to issues with sperm function[19-22], including DNA issues[11-14]. The presences of such issues in the sperm to be used for fertilization would lead to concerns over embryo quality.

The concept that "we only need one sperm" is a fallacy. Much as it has come to be understood that a morphologically perfect embryo cannot guarantee pregnancy due to a number of potential biochemical and physiological issues beyond simple appearance, neither can simple normal morphology guarantee a functional sperm[23-25].

The present study presents an alternative collection technique based on the physiological needs of sperm cells at the time of collection. The concept of the DISC is to slow or prevent the activation of cellular pathways by preventing shock-related events during the collection process. The device provides better temperature, and, when used with a measured amount of media, osmotic regulation of cells lessening

the chances shock pathways will set off at the time of collection.

As in earlier animal trials[26], the present series of studies demonstrate that the semen samples collected in the DISC maintain normal semen parameters for extended periods of time compared to the SSC. Further, biochemical assays demonstrated that the cells remain acrosomally intact, appear to have extended periods before undergoing capacitation in vitro, and maintain mitochondrial function longer than cells collected in the SSC, suggesting healthier cells for any treatment procedure.

While the clinical trial did not demonstrate a difference in pregnancy rates seen in previous animal trials[26], it was small and only meant to prove equivalency with the current collection techniques. Further, even though there are a number of factors that can impact pregnancy outcomes from conception to birth, it is interesting that all conceptions in the DISC reached delivery while those in the SSC had conception losses similar to those reported in healthy populations.

Together, the data suggest the DISC to be a superior collection device for semen collection. Cells appear to be more physiologically and biochemically active and potentially more viable than those collected in the traditional SSC. Further study will be needed to assess if pregnancy outcomes in humans are similar to those seen in animal trials. However, current data would appear to suggest that the device provides a better collection environment for the cells, leading to better overall cellular activity.

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