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A Simple One-Step System Enhances the Availability of High-Quality Sperm for Assisted Reproductive Procedures

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Abstract

Over the last forty years, *in vitro* fertilization, which has expanded to assisted reproductive technologies (ART), has gone from an experimental procedure to the mainstay of infertility treatment. A technique that once made news with each birth is now responsible for 2% - 3% of the babies born in several nations of the world. This has happened due to significant advances in hormone therapies, culture techniques, and the specialization of equipment designed to support oocytes and embryos. However, for all the advances made to support female fertility, little has changed in male treatment since the advent of intracytoplasmic sperm injection in the early 1990's. Recently, a number of authors have documented problems with sperm preparation techniques. Some report DNA damage, others membrane and organelle issues, all of which potentially hamper fertilization rates and possibly take-home baby rates. Further, as the clinical workload of ART has increased and staffing shortages have become critical, all labs are looking for simpler, more efficient ways to perform job functions. This study describes a simple, one-step method for preparing semen samples for ART. This new technique minimizes excessive manipulation of the sample compared to current standards and is less likely to cause cell damage. Preliminary results suggest a significant enhancement in recovered sample motility and an optimal sample for ART procedures with minimal sample manipulation.

Keywords

Simple, Enhanced, Sperm, Processing

1. Introduction

As caseloads increase and programs consolidate, assisted reproductive technology (ART) laboratories always look for techniques that improve efficiencies while not sacrificing outcomes. On the female side of procedures, this has led to smaller media volumes (requiring oil overlays), [1] [2] smaller, more efficient incubators, [3] [4] [5] [6] devices like the Stripper to allow fast denuding and manipulation of oocytes and embryos, [7] [8] and new techniques which may eliminate the need for biopsy when performing PGT. [9] [10] [11] Proven equipment and techniques have increased ART efficiency and pregnancy rates. [1]-[8] [12]-[18] In comparison, the effectiveness of other new techniques, such as cell-free PGT, the harvest of extracellular DNA from the media, or other noninvasive embryo assessment tools, remains to be determined but would be a great improvement. [9] [10] [11] [16]-[22]

On the male side of fertility, few would argue against ISCI being the biggest improvement. [23] [24] It has allowed the treatment of males with extremely low counts, low motility, or even those who require surgical sperm extraction to obtain gametes. [25] [26] [27] Because of a reduction in failed fertilization, ICSI has evolved into the method of choice for insemination in the ART laboratory over the last thirty years. [23] [24] [28] [29] [30] [31]

However, while ISCI may be seen as an almost universal means for oocyte insemination, it has limitations. It still requires expensive equipment and well-trained individuals. Further, while many new technologies for embryo selection have developed over the past few years, [16]-[22] there are few options for selecting a "healthy" sperm beyond movement and morphology. Recognizing it has limitations, morphology, and movement have proven the most effective means of producing pregnancies. [28]-[34] Therefore, sperm preparation techniques have targeted producing populations of motile cells with the most normal-looking morphology often sacrificing numbers to ensure the combination of motility and shape. [32] [33] [34]

While it is doubtful any test can be developed to allow assessment of each individual sperm cell, recent advancements in collection technologies have suggested it is possible to increase the odds of obtaining moving, morphological normal cells with intact DNA simply by providing an improved collection environment. [35] [36] Others have shown including a barrier separation device can further increase the odds of capturing a healthy cell for ICSI. [37] [38] [39] The present study provides preliminary results of a new combination of the two concepts, allowing a simple, one-step method of sperm selection, not only for ICSI but which might prove useful for conventional IVF and IUI as well.

2. Materials and Methods

2.1. Design of System

The system, defined as the sperm isolation device using a barrier mesh between fluids (SID; known commercially as the NovaSort; Reproductive Solutions, Inc.,

Dallas, TX), was designed to work in any properly shaped holding vessel, but ideally in tandem with the device for improved sperm collection (DISC; known commercially as the ProteX; Reproductive Solutions, Inc.), a specimen cup explicitly designed for semen collection, with the goal of maximizing the quality of the sperm in the collected sample. By design, the cup funnels the collected sample into a central well that contains a measured amount of media. The combined effects of the cup are to 1) decrease the area of the sample left exposed to either the plastic of the cup or air, 2) increase the internal volume (maximize volume to surface area ratio, 3) insulate the sample from outward environment temperature extremes, and 4) by adding the measured amount of media, protect the sample from pH and osmotic shifts [35] [36].

Based upon previous patented work with sperm separation, [40] the SID for enhanced sperm selection was designed to nest within the DISC. It contains a woven mesh to produce 10-um openings. The size of the hole being large enough for motile sperm cells to swim through but small enough to hold surface tension at the SID/sample interface. The use of the system is extremely simple. **Figure 1(a)** diagrammatically demonstrates the DISC (left) and SID (right). The male is provided a DISC containing one milliliter and allowed to collect his sample by masturbation, Once the DISC is returned to the laboratory, the SID is prepared by adding 0.75 mL of media (**Figure 1(b)**), The SID is the lowered into the semen sample (**Figure 1(c)**) and incubated at ambient temperature to allow sperm to swim into SID chamber (**Figure 1(d)**).

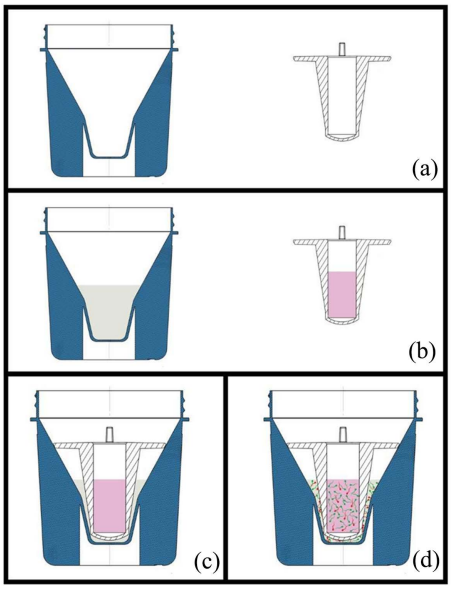

Figure 1. Methodology of a new sperm isolation device (SID) used in combination with the device for improved semen collection (DISC), (a) diagrammatically demonstrates the DISC (left) and SID (right). The male is provided a DISC containing one milliliter and allowed to collect his sample by masturbation, once the DISC is returned to the laboratory, the SID is prepared by adding 0.75 mL of media (b), The SID is the lowered into the semen sample (c) and incubated at ambient temperature to allow sperm to swim into SID chamber (d).

2.2. Study Design

To evaluate the SID/DISC system, studies were conducted at two private fertility clinics. Deidentified semen samples were obtained from two clinical laboratory facilities following routine sample collection and evaluation for fertility assessment as allowed by the standard patient treatment consent. Prior to collection, a sterile DISC was preloaded with a one-milliliter volume of media used for sperm preparation in that clinic. The patient then collected their sample by masturbation and the specimen was assessed clinically using standard lab protocols. Once the clinical semen analysis was complete, the sample was deidentified and handed off for research. The sample underwent a second, abbreviated semen analysis consisting of volume, concentration, and motility using a computer-assisted semen analyzer to determine eligibility for inclusion in the study. To enter the study, the sample had to meet inclusion criteria of a minimum of two milliliters of raw semen (a total of three mL with 1 mL of additional media), 30 million total cells, and a minimal initial motility of 30%. Samples meeting the inclusion criteria were then split in half; half remaining in the DISC, and half being processed using a swim-up procedure (control). While it can be argued that there are better preparatory techniques for use as a control, the swim-up was the only available procedure that would allow for multiple evaluations across the processing time points and a direct comparison of the efficiency of the new device.

Control samples were prepared by transferring half of the sample into a test tube and centrifuged for 10 minutes. Once the sample had been centrifuged, the supernatant was removed, and the pellet of sperm was kept in the bottom of the test tube. Then, 0.75 mL of sperm-washing medium was layered over the sample. The tube was placed in the incubator (37˚C). At times 0, 5, 15, 30, and 60 minutes, a small aliquot was sampled from the top layer of the specimen and underwent semen analysis in the CASA system.

Once the control portion of the sample was removed, the experimental arm was prepared within the DISC. Preparation was quite simple. While holding the SID vertically, 0.75 mL of fresh sperm wash media was added to the SID central cord. The SID unit was then gently lowered and nested in the DISC, placing the mesh in direct contact with the semen sample and a path for sperm to leave the native sample and enter the media in the SID core. The sample was then allowed to incubate at room temperature for one hour. At times 0, 5, 15, 30, and 60 minutes, a small (4 μL) aliquot of sample was taken from the middle of the media in the inner core, being careful not to allow the tip of the media to contact the SID mesh. The aliquot underwent an abbreviated semen analysis for concentration and motility, allowing the determination of the total motile sperm count inside the SID. In total 26 samples met the inclusion criteria and were processed for the study.

2.3. Statistical Analysis

Initial comparisons were made using a two-way analysis of variance comparing

the two processing methods and the method over time. If differences were established, each combination of method and time was treated as an individual observation to allow a means of comparison for both method and time using either one-way analysis of variance or Student's t-tests as appropriate.

3. Results

As expected by the system's design, the motile concentration increased with time because of cells swimming into the SID ($P < 0.001$; Figure 2). The native samples in this study had motilities ranging from 29% - 88%. There is an obvious difference in recovered motility due to method ($P < 0.001$) and time ($P < 0.001$). There is also an interaction between time and treatment, with the SID trending upward over time while the swim-up prepared samples trended downward ($P < 0.001$). While samples processed using the swim-up technique demonstrated an average motility of under 60% across the hour, once a significant number of cells had entered the SID (≥5 minutes), there was always s a higher percentage of motile cells in the SID device.

Further, the SID produced a much "cleaner" sample compared to the swim-up. The swim-up produced samples containing up to 40% nonmotile cells. Further, both the concentration of cells and the motility of cells peaked processed using swim-up peaked at 5 minutes and then decreased over the hour. By contrast, samples processed in the SID had a linear increase in motile cells for at least the first hour after processing and demonstrated increased motility ($P < 0.001$; Figure 3).

These outcomes were reflected in the percentage of recovered motile cells as a percentage of motile cells available from the native sample. While the highest number of motile cells in the swim-up occurred at 15 minutes, there was also the presence of 40% nonmotile cells in the sample (Figure 4). By contrast, the

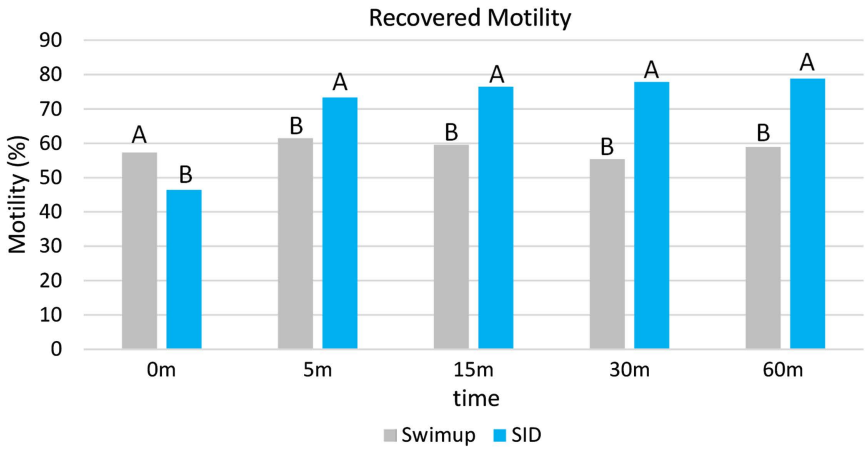

Figure 2. A comparison of the percentage of motile sperm cells seen over a one-hour time period in twenty-six paired samples split and processed using a swim-up procedure versus a new sperm isolation device using a barrier mesh between fluids (SID) one-step preparatory technique. Means with different superscripts within a time point are significantly different ($P < 0.001$).

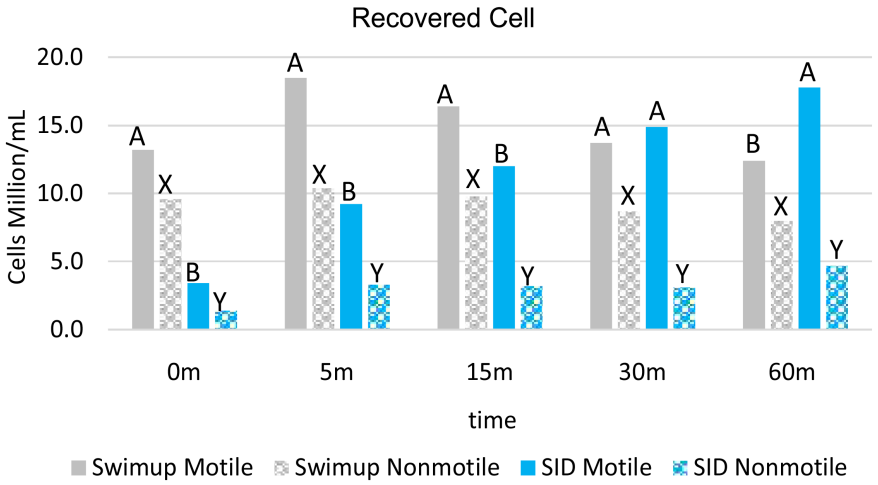

Figure 3. A comparison of the total number of motile and nonmotile sperm cells recovered over a one-hour time period in twenty-six paired samples, split and processed using a swim-up procedure versus a new sperm isolation device using a barrier mesh between fluids (SID) one-step preparatory technique demonstrating differences in recovery patterns between the two techniques ($P < 0.001$). Means with different superscripts within a time point and cell type [motile (A, B) vs nonmotile (X, Y)] are significantly different ($P < 0.001$).

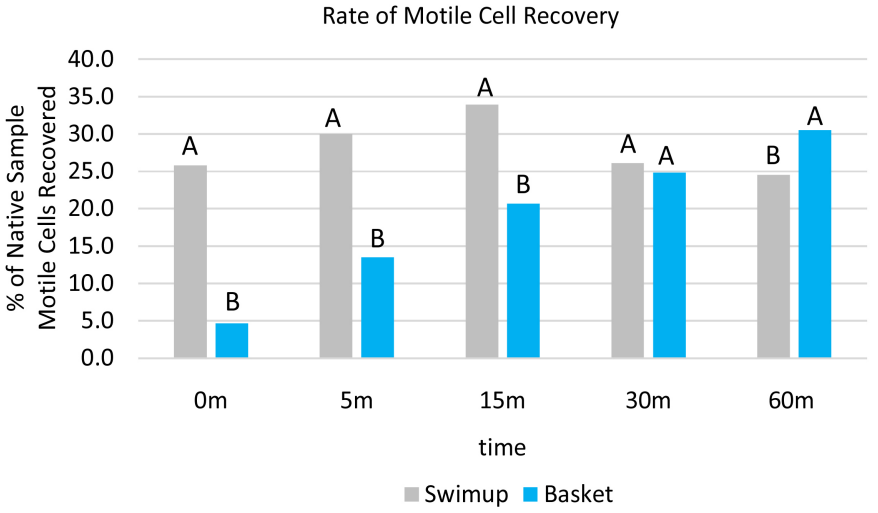

Figure 4. A comparison of the percentage of motile sperm cells recovered from the available population in the native sample seen over a one-hour time period from twenty-six paired samples split and processed using a swim-up procedure versus a new sperm isolation device using a barrier mesh between fluids (SID) one-step preparatory technique demonstrated different patterns of sperm recovery (linear in the SID; $P < 0.001$), Means with different superscripts within a time point are significantly different ($P < 0.001$).

percentage of motile cells recovered with the SID increased in a linear fashion and was over 80% motile, further, because of the barrier nature of the SID device. The SID eliminated all round cells, and preliminary data (not shown suggests a higher percentage of normal cells, suggesting the SID favors normal motile cells.

4. Discussion

There is ever-increasing pressure on assisted reproductive technology laboratories to become more efficient and cost-effective while not sacrificing the lab's primary mission, sending couples home with a healthy infant. The Center for Disease Control and the Society for Assisted Reproductive Technologies long ago abandoned pregnancy alone as the sole means of measuring a program's effectiveness. They have recognized that simply achieving pregnancy does not equate with live birth. In fact, before the advent of PGT, it was estimated that as much as 30% of ART pregnancies would be lost prior to birth [41].

While numerous potential issues can result in pregnancy loss, the advent of PGT has taught us that a significant number of embryos that reach the blastocyst stage carry genetic defects [42]. Yet the "gold standard" for gamete and embryo selection remains little more than a beauty contest. Our selection criteria still rely highly on morphological appearance. While there are strong correlations, and morphology is a good tool, better techniques are needed if we are to increase take-home baby rates.

On the embryo side of the equation, new, noninvasive techniques based on metabolites, morphometrics, cell-free DNA, and other determinations show great promise in further identifying the healthiest embryos. [9] [10] [11] [16]-[22] However, on the male side, currently, there is little beyond morphology and motility available for the selection of sperm.

Therefore, if we have no means of directly assessing a single sperm, then the SID option appears to be to produce the "cleanest" population of cells that meet the selection criteria. While a number of different techniques have developed for sperm processing, including commonly used simple wash, swim-up, density and centrifugation, and, more recently, barrier techniques, [37] [38] [39] each presents challenges to obtaining a high-quality sample. The older techniques all rely on a centrifuged sample, which has been demonstrated to potentially disrupt cellular DNA through increasing reactive oxygen species. [43] [44] Further compounds used in density gradients may have issues with toxicity. Newer barrier techniques process only portions of the native sample, return only a small portion of the motile cells, and may require additional processing steps post-sample harvest.

Data from the present study suggests that allowing cells to swim into the center well containing almost pure media preparation dramatically enhances the ratio of motile to nonmotile cells while confining larger round cells in the original collection vessel away from the motile population to be used in fertility treatment procedures with minimal technician effort. Further, preliminary data (not shown) suggest the system favors the transit of cells with normal morphology over motile cells with abnormal shapes. Because the system appears to allow recovery of a significant portion of the native sample's motile cells, this would suggest the system would provide a large population of normal motile cells for use in ART.

Additionally, the new combined system described here has many other potential advantages. The system is designed to work with an entire native ejaculate, thus increasing the total number of cells over methods that take only a portion of the sample for processing. It requires no centrifugation steps, and the original collection vessel limits oxygen exposure, lessening the chances of excess reactive oxygen species generation. Further, the collection container and SID materials contain embedded compounds known to scavenge excess reactive species, thus lowering the chances of the processing step causing DNA, membrane, or organelle damage during processing when compared to a standard specimen cup. [45] [46] [47]

There are also two logistically solved problems with this "all-in-one" system. All collection and processing steps occur in a container verified by the patient, ensuring the chain of custody from the point of collection through the insemination procedure. Finally, this simple one-step system requires no significant training or tech time, potentially improving efficiency without sacrificing quality in the ART laboratory.

While the results of the study are promising, further work is needed. Now that there is data available suggesting the optimum time to harvest cells for various ART procedures, comparisons to newer sperm processing systems which allow only a single time point for cell harvest. These studies should be expanded to not only look at semen parameters but also the quality of sperm cell DNA and even pregnancy outcomes.

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Conflicts of Interest

This is to acknowledge that SP and LP are inventors of the SID technology and have financial interests in RSI. Further, JL and MV serve on the scientific advisory board of RSI.

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