# First Use of a Simple One-Step Device for Enhanced Sperm Motility in a Clinical Setting: a comparison of clinical outcomes to a commonly used isolation technique

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Assisted reproductive technologies have been used to treat-infertility for almost 50 years. Since the field's inception, the focus for improvement has been mainly on female gametes and their resulting embryos. However, there is more and more evidence that the selection of the male gametes and how they are processed can significantly impact the success of an IVF cycle. ~~success~~. Recently, a new system was developed that combines sample collection and a simple, one-step method for motile sperm selection: the ProteX$^{tm}$ (PX) with NovoSort$^{tm}$ (NS; Reproductive Solutions, Dallas, TX). Preliminary lab-based trials demonstrated the system could yield a significant number of motile cells with minimal effort. The objective of the current study was to determine how sperm harvested from the system performed in a clinical environment. The PX/NS system was incorporated into clinical practice for IVF sperm processing for a period of one month and outcomes were compared to those of the previous month using standard techniques. The use of the PX/NS system did not adversely affect the rate of fertilization, blastocyst formation, or the % of usable blastocysts produced from the ART procedures. In addition, initial pregnancy rates were also equivalent. The PX/NS is a simple-to-use one-step system for the selection of sperm for ART. It does not require centrifugation, gradient use and is easy to implement in a clinical setting with minimal training. The process itself requires no expensive lab equipment and required only minimal technician time for completion. Data suggest that this simple technique produces sufficient high-quality sperm to achieve equivalent clinical outcomes to traditional preparatory techniques without labor costs and preparatory time in processing. Furthermore, there is an absolute chain of custody from collection until use because all work is done in the PX collection cup, an important necessity for each IVF cycle involving male and female gametes.



## Introduction

It seems almost hard to believe that the practice of in vitro fertilization started almost fifty years ago. (1). Since then, much in this field has changed. We use significantly different equipment, culture media, follicle stimulation medications and we now have the ability to do genetics testing at the

earliest stage of embryo development (2-5). Yet for all these major advances, until quite recently, little has changed on the male side of infertility treatment since the advent of intracytoplasmic sperm injection (ICSI) in the early 1990's (6,7).

Until quite recently, the most common practice for preparation of sperm for assisted reproductive techniques has involved either a simple swim up procedure or a density gradient (8), both of which expose sperm to centrifugation. However, there now exists evidence suggesting centrifugation is not a benign treatment and may, in fact, damage the sperm cell membranes, mitochondria and DNA (8-12). Such damage would lead to lower physiological and biochemical function, which may result in lower motility and fertilization rates and possibly affect long-term pregnancy outcome (12).

Most recently a series of devices have been developed which allow for the harvest of motile sperm populations without centrifugation (8,9,1,13,14). Each works by requiring sperm to pass through a physical barrier between the native sample and a neat media preparation. Each system purports the benefit of isolating increased motile sperm populations without centrifugation. Previous work from this program demonstrated that one of these systems, the NovoSort (NS; Reproductive Solutions; Dallas, TX) provided a simple means of sperm isolation (14). The objective of the current study was to compare its use to that of a traditional density gradient technique in a clinical setting.



## Materials and Methods:

### Specimen Collection

The NovoSort is part of an integrated system which combines collection and processing in a single container. Samples are initially collected within the ProteX (PS; Reproductive Solutions), a collection device specifically designed for semen samples, and which has been shown to provide protective effect on the sample at the time of collection, including higher motility parameters (15, 16) as well as potentially limiting the negative effects on sperm biochemical function; including DNA fragmentation

(17). The device is designed to be thermal protective, limit oxygen exposure, and because of the including of 1 mL of sperm wash media (provide type) prior to collection, prevent pH and osmotic shifts. (what about ROSD generation?) The system also guarantees a complete Chain-of-Custody as the sample remains in the single container from collection until use in the ART procedure within the ART laboratory.

In contrast, semen in the control group was collected in a standard specimen cup (SSC) and processed in a separate laboratory.

**Semen Processing**

**a) NS Processing**

Once the semen sample was collected, the NS device was prefilled with 0.75 mL of fresh sperm wash media and then lowered into the PX being careful not to disrupt the static tension on the NS mesh. Samples were then incubated for 15 minutes at room temperature. At the end of the incubation, a small volume of the media (approximately 30 uL) was extracted from the center of the media in the NS and transferred to the insemination dish for ICSI injection.

**b) Standard Technique**

In this study the NS was compared to the standard clinical practice of isolating sperm using a density gradient. Once sperm collection was complete the sample was allowed 30 minutes to liquify prior to processing at a separate laboratory. In brief, samples were processed using the ISolate technique (Fuji Films – Irvine Scientific; Santa Anna, CA). Gradient tubes were prepared by placing 1.0 mL of the 90% ISolate solution in the bottom of a standard 15 mL conical centrifuge tube. The semen sample was then mixed and up to 2.0 mL overlayed on the 90% ISolate solution, again being careful not to cause mixing at the interface. The tube was then transferred to a centrifuge and spun at 200-300xG for 10 minutes. The gradient supernatant was then carefully removed, and the pellet resuspended in 2mL fresh sperm wash media (Global Total Fert, Cooper). The pellet then underwent a second

centrifuge step at 200-300xG for five minutes. The final pellet was resuspended in 0.25 to 0.5 mL of fresh sperm wash media (Global Total Fert, Cooper). and transfer to the ART laboratory. Approximately 2-3 uL of sample were transfer to insemination dish for ICSI procedures.

**Design of the Study**

This was a retrospective clinical trial study. Cycles in September 2023 using the ISolate procedure were compared to cycles using sperm processed with the new PX/NS procedure done in October 2023. Ninety six percent of the cycles where ICSI only, there for cycles which were combined conventional IVF/ICSI or conventional IVF were eliminated from the analysis. Additionally, both arms of the study had three patients whose oocytes failed to fertilize and were eliminated, meaning all remaining cases had at least one, confirmed fertilized oocyte. Initial data collected included patient SART age demographic, numbers of mature oocytes inseminated, fertilization rates, blastocyst development, and number of usable blastocyst (defined as embryos qualified for cryopreservation). Preliminary data on pregnancy outcomes were included. Data were subject to statistical analysis using student's T, Chi-Square analysis, or ANOVA as appropriate.

**Results**

The PX/NS group collected in October contained 93 patients, whereas the ISolate group collected in September contained 168 samplers. However, the overall patient populations in both groups appeared similar based on the SART age demographics (Table 1; P = 0.498), with the mean age of the female patients using sperm derived from the NovoSort was 37.12 (+/- 4.54 STD) versus 37.22 (+/- 4.3 STD) for female patients using sperm derived by ISolate treatment.

**Table 1.** A comparison of female patient ages broken down by SART age demographics between female patients using sperm derived from the NovoSort versus the ISolate Technique

| Technique | | < 35 | 35-37 | 38-40 | > 40 | Total |
|---|---|---|---|---|---|---|
| NovoSort | # | 28 | 23 | 18 | 24 | 93 |
| | (%) | 30.1% | 24.7% | 19.4% | 25.8% | 100.0% |
| ISolate | # | 46 | 35 | 42 | 45 | 168 |
| | (%) | 27.4% | 20.8% | 25.0% | 26.8% | 100.0% |

As there was no other means for matching patients in this analysis, and splitting by age group would lead to small group numbers (< 20/group) data were combined for further analysis.

Initial motilities in the NovoSort group ranged from 19-67%. Of the 95 samples processed, five had worse motility after processing (5%), ten remained unchanged (10.5%) but fully 84.5% demonstrated recovery of increased motility which demonstrated an average increase of 54% over the native sample and all produced more than sufficient cells for ICSI.

A total of 2,243 oocytes were deemed mature and injected. While the Female patients were of similar ages, there was a trend toward fewer oocytes being retrieved and injected per patient in the NovoSort arm of the study (Table 2; P =0.08). However, even with fewer oocytes to work with, sperm recovered using the simple NovoSort technique, demonstrated similar rates of fertilization, blastocyst formation, and usable blastocyst available for fertility treatment. Further a comparison of early outcome data from 44 transfers suggests identical positive pregnancy rates between the two sperm treatments (Figure 1). A total of 12/20 transfers with embryos in the NovoSort group demonstrated a positive pregnancy (60%) versus 14/24 (58.3%; P = 0.338) when the sperm were derived from the Isolate procedure.

Table 2. A comparison of fertilization blastocyst formation rates between female patients using sperm derived from the NovoSort versus the ISolate Technique in ISCI procedures

| | NovoSort | Isolate/Wash |
|---|---|---|
| **n ICSI** | 817 | 1715 |
| **n Fertilized** | 632 | 1350 |
| **% Fertilized** | **77.4%** | **78.7%** |
| **n Blastocyst** | 365 | 808 |
| **% Blastocyst/2pn** | **57.8%** | **59.8%** |
| **n Usable Blastocyst** | 257 | 556 |
| **% Usable Blastocyst/2pn** | **40.7%** | **41.1%** |

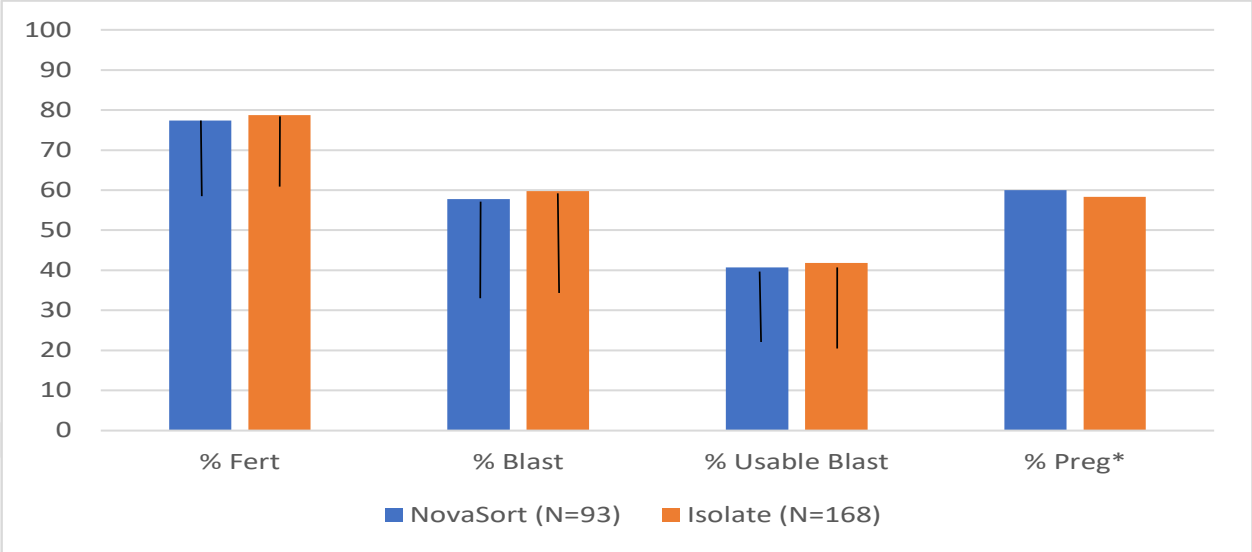


Figure 1. A comparison of clinical outcomes of patients using either the NovoSort (N = 93) versus the traditional ISolate technique (N =168) for sperm isolation prior to oocyte insemination demonstrates equivalence between the two techniques. Note, preliminary pregnancy data is based on 44 transfers – 24 in the ISolate group versus 20 in the NovoSort group (P = 0.338)

## Discussion

There is an expected 10-fold increase in the next 10 years in ART cases in the USA. One out of 6 couples requires infertility treatment to fulfill their dream of parenthood. With this development there

will be increasing pressure on IVF laboratories to be more efficient and cost-effective (14,15). However, there is an equal amount of pressure to ensure quality and accuracy in the laboratory, as mistakes have lifelong consequences (16).

The PX/NS system represents a solution to both simplifying and speeding up the processing of male gametes but also to ensure a complete chain of custody of the sample from its collection through its use in ART procedures. Previous studies (17-19) have shown the PX system to be a superior collection environment, reducing the stress on the male gametes (limited ROS generation) and possibly eliminating gamete damage, which would cause abnormal embryo development.

The addition of the NS to the PX has allowed a simpler means of producing an enhanced motile sperm population for ART procedures. While previous laboratory-based studies have suggested the system can be used to produce samples for ART (20), this study focused on if the system could produce similar clinical outcomes to the traditional sperm gradient commonly used in ART laboratories for ICSI procedure. While the study did show that the system cannot enhance motility in all cases (approximately 5% of the cases demonstrated reduced motility after processing), it did demonstrate that the motile cells acquired from the PX/NS produced equivalent rates of fertilization, blastocyst formation and early pregnancy outcomes with a single processing step compared to multi-step methods such as the gradient technique.

In conclusion, while there are at least two additional systems for sperm isolation using a barrier technique, the NS appears to have at least two advantages. First, unlike the PX/NS combination, the other two systems require movement of samples between devices and use of centrifugation. Further, except in those cases of extremely large volumes (> 7 mL), the PX/NS system allows processing of full samples within a single device. In contrast, other systems recommend the use of multiple devices. Finally, recent procedural modifications made after these trials might lead to even purer populations of motile cells.

Finally, in this trial the NS performed equally well to the traditional gradient technique and did so with a minimum of training and sample processing time. Further studies will help refine the technique and might offer insight into modifications necessary to allow processing that 5% of samples which were negatively impacted by this processing technique.

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