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Early Clinical Outcomes in an IVF Program Using ICSI following Sample Collection in a device Specifically designed for Semen Collection (ProteX) vs a Standard Specimen Cup

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Objective : The advent of ICSI has given rise to the concept that sperm need only intact DNA to complete the fertilization process. The role other semen parameters might play in later embryo development is more controversial. Recently, a new sperm collection device (DISC), specifically designed to maximize the quality of samples used in clinical procedures, was introduced in clinical practice for semen collection. The following is the first report of outcomes from a large-scale retrospective study in an IVF setting using ICSI.

Design: Retrospective cohort comparing outcomes of ICSI cases from semen samples produced in the DISC containing a measured amount of culture media vs. a standard specimen cup (SSC) without media

Materials and Methods: A total of 1056 couples undergoing IVF using ICSI used either an SSC or DISC to collect their semen. Further, approximately 40% of the patients in each group produced their semen samples away from the clinic. Data collected included both partners' ages, standard semen parameters, stimulation and fertilization results, and embryo outcomes. As 92% of the patients were involved in freeze-all protocols, the primary embryo outcome was the percentage of embryos cryopreserved as high-quality expanded blastocysts.

Result: The female partners in the DISC in-clinic arm had the highest average age (38.1; $P < 0.02$), and the lowest average number of oocytes recovered (10.9; $P < 0.03$). Male partners were of similar ages between groups. However, men producing a sample in the DISC device had higher initial counts and motility than men producing in the SSC ($P < 0.001$). In addition, while fertilization and usable blastocyst rates were similar between groups, there was an 11% higher blastocyst rate in the DISC group (43.8% vs. 39.4%) when expressed as embryos frozen/oocyte fertilized ($P < 0.04$).

Discussion: These retrospective data suggest that producing semen in a more physiological collection container (DISC) may provide a larger pool of healthy sperm for IVF procedures and enhance outcomes such as the usable blastocyst rate. Furthermore, when used as designed (including a measured amount of media), it appears semen samples can be produced off-site in the NSCD without compromising IVF outcomes.

INTRODUCTION

It is well documented that as sperm cells are ejaculated, they are subjected to environmental changes, temperature fluctuations, and, in the case of collection for artificial insemination or other ART procedures, exposure to potentially toxic materials (including the collection containers) (1-7). Exposure to these drastic changes leads to activation of a group of proteins referred to as shock proteins, which in turn leads to a programmed death of the cell. The original experimental device, which was dubbed the Texas Tech University Device for Improved Semen Collection (TTU-DISC), was used in experiments with the canine (8). The device was designed to 1) limit exposed surface area, 2) concentrate the sample to maximize internal volume while minimizing total surface area, 3) provide a buffering agent to limit shifts in pH, and 4) provide nutrients. These qualities allow the sample to maintain its temperature, pH, and osmolarity.

A commercial version of the DISC, tradename ProteX, was developed by Reproductive Solutions. (Dallas, TX). Physiological and biochemical studies demonstrated the DISC to produce a superior environment for semen

collection compared to the traditional standard specimen cup, as determined by higher motilities and other semen parameters, delayed acrosome reactions, and demonstration of healthier mitochondria over extended periods of time (9). Further, in a small, FDA-approved trial of equivalence, intrauterine insemination patients had similar conceptions rates, but those using the DISC had significantly more pregnancies continue to term and delivery (10).

The objective of the present study was to conduct the first large-scale clinical trials of the DISC in assisted reproductive procedures. The study not only allowed a comparison of the collection devices but also allowed a comparison of location because of the shift in collection locations due to pandemic conditions. Male outcomes focused on traditional male fertility measurements. Because this program freezes most embryos before transfer, female outcomes focused on the number of embryos reaching blastocyst and cryopreservation.

Traditional semen samples collected for fertility procedures have been collected in sterile specimen cups designed initially for urine and preserved specimens for pathology (standard specimen collection cup – SSC). Unfortunately, this type of cup provides little to no protection of the sample from the environment. In fact, the large diameter of the base, 4 to 6.5 cm, allows the typical semen sample to spread over a very wide surface area as well as exposing a large percentage of the sample to the plastic of the container: potentially allowing a large percentage of the cells to absorb toxins in the plastics.

By comparison, the DISC was designed to minimize the exposed surface area by funneling the sample into a central well to minimize the exposed surface, maximizing the sample volume held away from the air-exposed or plastic-exposed surfaces. The DISC is insulated, allowing the sample to dictate its cooling rate and slowing that rate to approximately 0.3 ºC/min, slowing activation of physiological and biochemical pathways resulting in a larger pool of healthier sperm at the time of fertilization. Further, by including a measured amount of media in the bottom of the central well, there is additional pH regulation and controlled activation of cellular pumps, again slowing the activation of physiological and biochemical pathways critical to normal fertilization.

Materials and Methods

A large East Coast academic fertility center made a change to collection procedures at their clinic to incorporate DISC due to pandemic conditions and began offering offsite collection. This change allowed for a direct comparison of DISC to the standard specimen container (SSC), in patients undergoing some form of fertility treatment who were collecting semen samples via masturbation.

As designed, the DISC contained 1mL media to controlled initial physiological and biochemical reactions. Samples collected in the SSC were collected in a dry cup per clinic protocols. Due to COVID protocols during the ART study, samples were further divided between those collected in a clinical setting and those collected off-site (at-home collection).

A total of 1085 ICSC cycles were reviewed. Cycles not using exclusively ICSI or missing either collection device type or location were removed, resulting in a total of 1007 cycles meeting the criteria of the study (430 collected in the DISC and 577 in the SSC). Data collected for the patients (female) included: age, oocytes recovery, fertilization, and a total number of frozen embryos. In addition, to allow a more direct measurement of sperm source on outcomes, embryo reaching cryopreservation was also expressed as a percentage of embryos fertilized. For the male patients, data were collected for age, day of abstinence, initial volume, initial concentration of cells/mL, % initial motility, final concentration, and final motility.

Prior to analysis, data were further broken down by location so that the final analysis was a comparison 1) DISC in-clinic, 2) DISC at-home, 3) SSC in-clinic, 4) SSC at home. Finally, data were analyzed by ANOVA with mean separation as appropriate OR Chi-squared using the statistical software for the social sciences Ver. 25.

Results

Focusing first on the male partners, males in all groups were of similar ages (P =0.12), observed similar days of abstinence (P = 0.91) and had essentially equal volumes (P = 0.86, Table 1). These parameters suggest a very similar population of males across the four treatments and suggest the differences seen in preprocessing motility and concentration (P < 0.001) related to the collection environment. Samples collected in the in-clinic DISC had higher motility than any other combination of device and location. This was similar to results reported in earlier studies (ref). In addition, using the DISC in at-home collection yielded results either similar or superior to SSC in-clinic collections (Table 1). Interestingly, post-processing motility, which was expected to be equivalent due to the processing technique, remained significantly higher in the DISC in-clinic group.

Table 1. A comparison of demographic and pre-and post-processing semen parameters from men using a DISC versus a standard specimen container (SSC) for semen collection for use in assisted reproductive technology procedures. Patients collected either in-clinic or at-home.

	ProteX in-clinic	STD (+/-)	ProteX at-home	STD (+/-)	SSC in-clinic	STD (+/-)	SSC at-home	STD (+/-)	P-value
	N=277		N=153		N=327		N=250		
Age	39.9	5.9	38.8	5.7	40	6.3	40.15	5.6	0.16
Days of Abstinance	2.3	1.6	2.4	2.7	2.2	1.7	2.2	1.3	0.86
Initial Volume (mL)	2.3	1.2	2.3	1.2	2.3	1.2	2.3	1.2	0.99
Initial Concentration (mil/mL)	69.3[a,b]	59.6	74.2[a]	96.3	54.7[c]	47.1	58.9[b,c]	45.5	0.001
Initial Motility (%)	50.8[a]	16.4	45[a,b]	18.5	43.4[b,c]	17.2	40.3[c]	17.7	0.001
Final Concentration (mil/mL)	5.3	3.9	5	3.4	5.4	4.1	5.8	5.2	0.34
Final Motility (%)	92.7[a]	18.8	86.3[b]	27.8	89.7[a,b]	22.9	86.6[b]	23.5	0.008

*means followed by different superscripts are significantly different.

The pre-fertilization parameters for the female patients in the study were more variable and obviously unaffected by the collection device their partner used. Table 2 compares the female patients who participated in this study. On average, patients in the DISC at-home arm were 1.5 years younger than those whose partners used DISC in the clinical setting (P < 0.02). However, the ages of both groups were similar for patients collecting in the SSC. As might be expected, the older age group, patients in the DISC in-clinic group, averaged fewer oocytes retrieved (P < 0.02). However, the four groups demonstrated equal rates of fertilization (P = 0.32) and averaged equal numbers of embryos reaching blastocyst and being cryopreserved (P = 0.13).

Table 2. Table 1. A comparison of demographic and outcomes data from woman whose partners used a DISC versus a standard specimen container (SSC) for semen collection during their assisted reproductive technology procedures. Patients collected either in-clinic or at-home

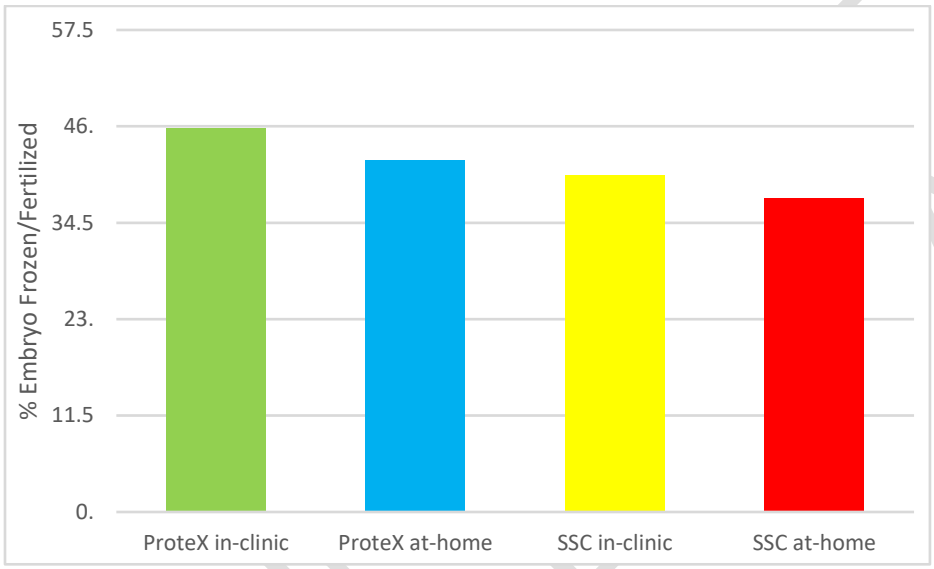
	ProteX in-clinic	STD (+/-)	ProteX at-home	STD (+/-)	SSC in-clinic	STD (+/-)	SSC at-home	STD (+/-)	P-value
	N=277		N=153		N=327		N=250		
Age	38.2[a]	4.7	36.8[b]	4.6	37.7[a,b]	4.5	37.8[a,b]	4.1	0.02
Oocytes Recovered	10.7[b]	9.3	13.4[a]	10.6	11.6[a,b]	9.6	13[a,b]	10.3	0.02
ICSI attempted	9.8	7.2	10.2	7.5	9	7.1	9.9	7.3	0.3
Fertilized	7.7	6.1	8.1	6.4	7.1	6.1	7.8	5.9	0.32
Cryopreservation	3.8	4.1	3.5	3.8	3.1	3.7	3.4	3.7	0.13

*means followed by different superscripts are significantly different.

While these data appear to suggest little difference in fertility outcomes, if one re-exams the rate of embryos frozen as a percentage of the oocytes fertilized (Figure 1), couples using the DISC have a higher percentage of

their fertilized embryos frozen (Figure 1; $P < 0.02$). This would suggest that overall, the cohorts of embryos moving through the ART process are healthier. Further, in this study, the group with the highest percentage of embryos stored was the in-clinic DISC group, the oldest women with the fewest oocytes recovered.

Figure 1. A comparison of the influence of semen collection device (DISC versus the Standard Specimen Cup – SSC) and collection location on the eventual number of cryopreserved embryos stored in an assisted reproductive technologies program.



DISCUSSION

The goal of infertility treatment, be it relatively low-tech IUI or highly sophisticated ART treatments, is not simply pregnancy. Instead, the goal is to send the couple home with the child they have invested so much time, emotional energy, and financial resources as they try to become parents. It is well recognized that there are many steps in the process, from normal fertilization to blastocyst formation to implantation and establishment of heartbeat, that can make or doom a pregnancy in the early stages. Further, it has become very apparent that the health of the individual gametes which form that fertilization event may have a significant impact on final outcome (11-14).

The DISC is the first semen collection device specifically targeting the collection environment to maximize the number of healthy sperm available for infertility treatment. The system's design attempts to minimize cellular shock during the collection process and slow the processes that prematurely activate both fertilization and apoptotic pathways, which eventually lead to cells becoming non-functional (8, 9).

In this first, large-scale use of the DISC in fertility trials, sperm collected in the DISC were compared to sperm collected in the traditional SSC and its effects on semen parameters, fertilization rates, and the percentage of the fertilized embryos reacting blastocyst stage and freezing. Sperm collected in the DISC appeared to outperform sperm collected in the SSC at all levels. First, the was an average 18% higher motility rate in samples collected in the DISC regardless of collection location or time between collection and the initial motility assessment. Further, there was an average of 15% higher motility when comparing similar collection sites with similar delays in processing between collection and the sperm washing procedure. This would suggest an additional pool of healthy sperm available for the procedure, which would be non-motile when collected traditionally.

Obviously, pregnancy and pregnancy outcomes would be the "gold standard" measurement of any new fertility procedure. However, the reality of the modern ART world is that embryos may sit in cryostorage for months or years before the first attempt at transfer. Given this reality, an alternative approach to assessing the success of a new technology is to determine how many fertilized embryos are stored as blastocysts. In the present study, there was an overall increase of 11% more embryos frozen in patients using the DISC for semen collection than those using the traditional SSC (44.4% vs. 39.4%; $P < 0.01$). The data also suggest the collection site location and the possible delay in semen processing will play a role in the percentage of embryos reaching storage. However, overall failed fertilization was only 3% in both groups, regardless of the location of sperm collection.

Together, these data support the idea that the DISC produces a superior environment for sperm collection. As a result, cells collected in the DISC appear more physiologically and biochemically active and potentially more competent to produce a viable human embryo. This is supported by both the semen parameter data and the number of embryos in the ART arm that made it to the blastocyst stage and met the criteria for freezing.

The study may be limited in that only ICSI cases were included. However, only 6% of the available data was collected using conventional or a mixture of conventional and ICSI fertilization (70 cases). An insufficient group for any meaningful comparison. Which suggests a follow-up study with conventional insemination is warranted.

While it must be stressed that even healthy embryos can fail to result in live births, there can be no doubt that a healthy embryo conceived from the healthiest of oocytes and the healthiest of spermatozoa would have the best chance of resulting in a healthy baby. Early pregnancy data appears to support this concept. However, as of the end of data collection, < 10% of patients had received an embryo transfer, yielding far less data than necessary for statistical power, suggesting the need for an outcomes study. Overall, the data continue to support the DISC supplies an improved collection environment to support the physiologically and biochemically healthiest sperm.

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