A Redesign of the ProteX to Harvest Free Oxygen Species Appears to Improve Overall Semen Parameters

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Abstract

Objective: Previous research from this laboratory has demonstrated that semen quality, both physiological and biochemical, can be improved via a simple redesign of the semen collection cup. The redesign, termed the Device for Improved Semen Collection (DISC – trade name ProteX), maintains a stable environment during and after collection, preventing cell shock and preserving cell function. However, the original design did nothing to prevent DNA fragmentation. Recently the system underwent a redesign incorporating a proprietary method to harvest free oxygen radicals, an identified cause of DNA fragmentation. The object of this study was to determine the effects of the redesign on all cellular functions.

Design: Lab-based study of standard semen parameter

Materials and Methods: In these initial studies, frozen bovine semen was thawed and processed similarly to an IUI sample to yield a final 6 mL containing approximately 20 million cells/mL. Two milliliters of the cell suspension were then transferred to a standard specimen cup (SSC), the original ProteX device (PRO), or the redesigned system (Pro+), and the sample maintained on a benchtop at room temperature. At times 0, 1, 3, 6, 9, 24, and 48 hrs, the samples were briefly vortexed, and aliquots used to prepare slides for semen analysis, acrosome reactions, and DNA fragmentation. Semen analyses were performed on a Hamilton Thorn IVOS unit, and acrosome and DNA fragmentation determined using standard techniques. Resulting data were analyzed using ANOVA with repeated measures.

Result: As expected, all SA parameters decreased over time ($P < 0.001$). However, cells stored in the Pro and Pro+ maintained significantly higher cellular activity for all semen parameters when compared to the SSC after as little as 6 hrs ($P < 0.005$). This can be seen easily at 48 hrs how were the Pro and Pro+ maintained over 20% of the initial motility and rapid cells while the SSC had dropped to 7 and 1%, respectively ($P < 0.001$). Further, while the motility and rapid cells were similar between the Pro and Pro+, other semen parameters indicated the cell stored in the Pro+ possessed better overall activity than in the Pro ($P < 0.01$). Acrosome and DNA fragmentation data are pending.

Discussion: The ProteX semen collection system continues to be a superior collection system for andrology samples that a standard specimen cup. Further, the redesigned ProteX appears to provide additional protection to overall sample quality, which should result in healthier cells for all ART procedures.

Introduction

It is well documented that a certain level of free-oxygen radical generation is necessary for normal semen function. However, too high a level of free-oxygen radical concentration within the fluid environment (native seminal fluid, media, or any combination) can lead to biochemical damage, including disruption of membrane integrity, loss of organelle function, and DNA fragmentation. Therefore maintain a stable concentration of free-oxygen radical in necessary for normal sperm cell function.

Current technologies are adding free-oxygen radical scavengers directly to the media the cells are cultured with. There are two disadvantages to this approach. First, by adding the scavengers to the media solution, they are free to be absorbed inside the cell, which may have unintended consequences on cell function. A number of scavengers are mild to severely toxic, so embedding them within the sperm cell could potentially damage the embryo formed at conception. Second, by adding media with scavengers to the semen sample, there will be a rapid absorption of most of the free-oxygen species, followed by a rebound as the metabolizing cells create more. The fluctuation in free-oxygen species may actually trigger chemical pathways prematurely.

Previous research from this laboratory resulted in the creation of a new collection system, referred to as the ProteX (Reproductive Solutions, Inc., Lubbock, TX). This system provided superior semen sample by providing a collection environment that minimizes cellular stress, through 1) minimizing exposed surface area maximized the volume to surface area ratio, 2) Providing a thermal barrier to the outside environment that allows the sample to drive its own temperature and 3) when used as recommended, lessens osmotic shock. Together, these properties provide a more motile, more biochemically, and physiological normal population of sperm cells. While the original system provides some antioxidant protection, we recently redesigned the system to include direct antioxidant protection to further improve the collection environment.

The new design, term ProteX+ was aimed at increasing cell stability while limiting any possible toxic effects of the scavenger molecules. First, by "fixing" the scavenger molecule within the container, the scavenger molecules are not free to enter the cell. Second, unlike having the scavengers in media, where they rapidly absorb the majority of free-oxygen species at first introduction, by having the scavengers within the plastic wall, they will create a constant but limited absorption point and an equilibrium within the semen sample. By preventing either a rapid rise or fall in free-oxygen species, the sample should remain more biochemically stable. More intact membranes means a longer functional cell life. Fewer reactive species could also mean less DNA damage.

Acknowledgements

1. The authors wish to thank Reproductive Solutions Inc. (RSI) for supplying the Protex and Protex+ used in this study.
2. The authors want to thank Fuji-Irvine for supply the media used in this study.
3. The authors also wish to thank RSI and the Innovation HUB at Texas Tech University for providing funds for this study via a HUB prototyping grant.

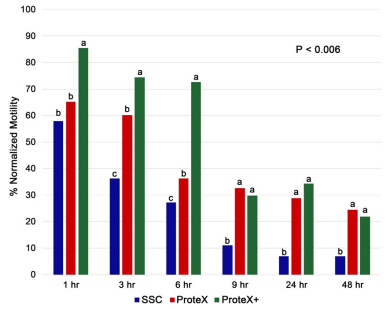

Figure 1. Normalized motility of cryopreserved bovine semen samples incubated in a Standard Specimen Cup (SSC), ProteX or ProteX+ over 48 hrs, demonstrated higher motility in the ProteX+ for at least the first 6 hrs post-thaw ($P < 0.006$). Individual means with different superscripts are different within a time point.

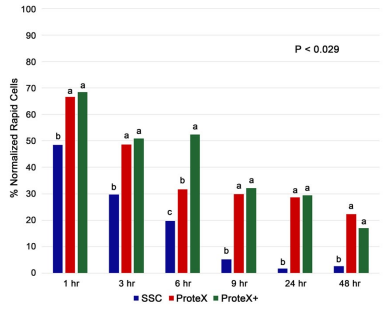

Figure 2. Normalized motility of cryopreserved bovine semen samples incubated in a Standard Specimen Cup (SSC), ProteX, or ProteX+ over 48 hrs ($P < 0.029$). Individual means with different superscripts are different within a time point.

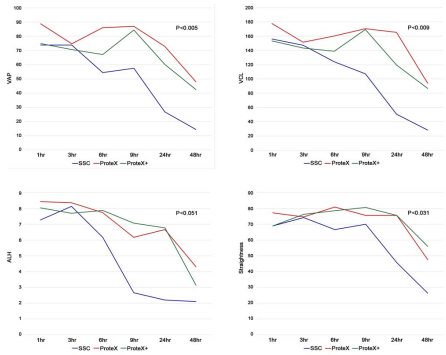

Figure 3. Motility parameters of cryopreserved bovine semen samples incubated in a Standard Specimen Cup (SSC), ProteX or ProteX+ over 48 hrs

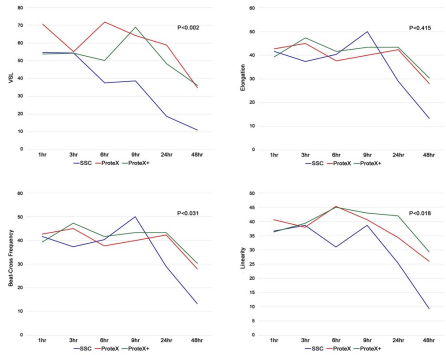

Figure 4. Additional motility parameters of cryopreserved bovine semen samples incubated in a Standard Specimen Cup (SSC), ProteX or ProteX+ over 48 hrs.

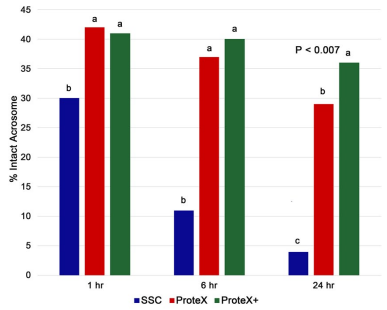

Figure 5. Acrosome status of cryopreserved bovine semen samples at 1, 6, and 24 hrs post-thaw after incubation in a Standard Specimen Cup (SSC), ProteX or ProteX+ over 48 hrs ($P < 0.007$). Individual means with different superscripts are different within a time point.

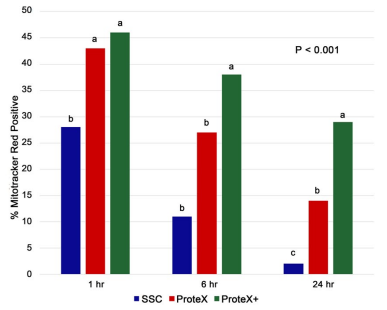

Figure 6. Use of Mitotracker red to suggest mitochondrial status of cryopreserved bovine semen samples at 1, 6, and 24 hrs post-thaw after incubation in a Standard Specimen Cup (SSC), ProteX or ProteX+ over 48 hrs ($P < 0.001$). Individual means with different superscripts are different within a time point.

Materials & Methods

Leaching Testing:

While the proprietary material has an FDA-approved application, there are reports they can have toxic effects in high concentrations. Therefore a study was conducted to determine if leached occurred from the Protex+ device.

1. 5 mL of media was placed in standard specimen cups (SSC), ProteX and ProteX+
2. At times 0, 1, 3, 6, 9, and 24 hrs, 0.5mL of media was tested for the presents of the antioxidant agents
3. At no time where the antioxidant agents known to be present in the ProteX+ detected above background leaves seen in the SSC or original ProteX design.

Physiological and Biochemical Studies:

1. Due to restrictions during the current COVID Pandemic, initial studies were conducted with frozen bovine semen. The lab had sufficient quantities of semen from three bulls to create three samples (each representing a single animal) containing 20-28 million cells /mL in fifteen mL of media.
2. Cells were thaw and prepped using standard techniques and resuspended in 6 mL of Fuji-Irvine Multipurpose Handling Media- Complete. Each sample was then tested for initial motility and rapid cell movement to allow normalization of these data for statistical analysis.
3. Five milliliters of the suspension was transferred to an SSC, ProteX, and ProteX plus and incubated on the counter for 48 hrs.
4. At times 1, 3, 6, 9, 24, and 48 hr, the samples were gently mixed and an aliquot analyzed for standard motile parameters using a Hamilton-Thorne IVOC Computer-assisted semen analyzer with bovine specific software.
5. Additionally, slides were prepared for acrosome reactivity, mitotracker red detection of mitochondrial activity, and Halo detection of DNA Fragmentation using standard techniques at 1, 6, and 24 hr.
6. The resulting data were analyzed using ANOVA with mean comparison at analysis time point.

Results

1. As mentioned above, no antioxidant compounds were detected in the media, suggesting all scavenging took place at the media/ container interface as designed.
2. As expected, there was a rapid decrease of all motility parameters from samples stored in the SSC as early as 3 hrs post-thaw (Figures 1-4).
3. As reported in earlier studies, the ProteX maintained most cell activity significantly longer that the SSC (Figures 1-4).
4. The ProteX+ demonstrated higher normalized motility and rapid cell movement than either the ProteX or the SSC (Figures 1 and 2), and equivalent or higher values for all other parameters tested (Figures 3 and 4).
5. Additionally, cells incubated in the ProteX+ demonstrated significantly more intact acrosomes and functional mitochondria that the SSC at all times past 1 hrs, and better than the ProteX (Figures 5).
6. Further, the ProteX+ maintained significantly higher numbers of intact acrosomes thru 24 hrs and more functional mitochondria from 6 hrs on when compared to the original ProteX Design (Figure 6).
7. Halo analysis is pending.

Discussion

1. Data from this preliminary study supports previous work that suggests the ProteX a better system for semen collection.
2. Further, data suggest the ProteX+, with its redesign to include antioxidant proper ties, is superior to the ProteX or SSC in maintaining cell function and viability.
3. Further study in a more controlled environment will be needed to verify these observations.
4. A prospective, randomized controlled study is underway using human donors with normal semen parameters.

Conflict of Interest

The authors wish to acknowledge potential conflicts of interest as both SP and LP are co-founders, members of the board, and company officers at RSI. Further, SP is an inventor of the technologies developed into ProteX and Protex+. Such conflicts are managed through an official conflict management plan at TTUHSC.