ProteX

SUMMARY OF SUPPORTING EVIDENCE FOR THE USE OF PROTEX

REPRODUCTIVE SOLUTIONS

ProteX

REPRODUCTIVE SOLUTIONS

TABLE OF CONTENTS

Patent US 6,864,046 B1, Patent Pending: US 17/364,792, PCT/US2021/040017

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REPRODUCTIVE SOLUTIONS

INTRODUCTION

From the earliest uses of artificial insemination, through the development of the advanced techniques in ART (assisted reproductive technologies) used today, collecting and preserving the fertilizing capabilities of semen has been challenging.

It is generally understood that unprotected, freshly ejaculated semen loses motility and fertilizing capability, rapidly rendering it virtually useless in a matter of a few minutes to a few hours (time varies depending on the species).

This loss in motility and fertilizing capability can be attributed to the fact the sperm are easily susceptible to shock due to temperature shifts, pH imbalances, and osmotic stress. This shock results in structural changes to membranes and leads to the acrosome reaction, premature capacitation, and decreased cell function. These biochemical changes are manifested physiologically in the sample as decreased motility, decreased fertility, and ultimately cell death.

Traditional methods of collecting semen into a dry specimen cup allow drastic shifts in temperature and pH, resulting in damage to the sperm.

Previous research shows protecting the semen from temperature, pH, and osmotic stress upon collection extends the functional life and fertilizing capability of the sperm.

The development of sperm-specific media has allowed semen to be preserved for use for a considerable time post-collection and almost indefinitely with cryopreservation. In practice, the media is added anywhere from a few minutes to a few hours post-collection to help sperm survival, but it is currently not standard practice to add media at the time of collection. This time is critical when considering the shock that can occur to sperm.

ProteX™ is scientifically engineered to better protect the biochemical health of the sperm at the precise moment of collection and for a much longer term thereafter.

ProteX is an insulated container made from unique materials that prevent damaging temperature shocks. ProteX is designed to funnel the semen sample to a well to reduce surface area-to-volume ratio, vastly improving thermal efficiency over time. In addition, ProteX includes 1 mL of media, which is added prior to collection.

The buffers in the media help protect the sperm from shifts in pH and allow the sample to begin osmoregulating before labaratory processing. The result is significantly higher motility for an extended period, plus, as seen in the biochemical analyses in Study 2, FIGURE 2 and STUDY 6, FIGURE 1, ProteX provides better protection of sperm acrosomes over all time points.

BACKGROUND

The device for improved semen collection (DISC), which would eventually become ProteX, was invented by Dr. Samuel Prien and Dr. Dustie Johnson. The inventors sought to design a new species-specific semen collection container and collection technique to provide a physiologically stable environment for sperm function by controlling the temperature, surface area-to-volume ratio, pH, and osmotic stress.

A number of studies were performed to prove that the new container design and method reduces the shock to the sperm upon collection, allowing the sample to maintain sperm function and fertilizing capability much longer than traditional methods. No current U.S. standard exists stating samples must be processed within an hour of collection, however it is widely known that the traditional method of collection is a race against rapid sample degradation.

Across the studies, a variety of species, including canine, bovine, equine, and humans were utilized to demonstrate the effect of ProteX in different circumstances. Device designs specifically tailored to the needs of each species were used.

The included studies date back 20 years, and therefore some of the materials and methods were naturally phased out over time as standards shifted or advancements became available. For example, in some studies, samples were left in incubation over time because this was standard practice. It is now understood that incubation increases cell metabolism, accelerating activation and apoptosis. It is now our recommendation to leave samples and media at ambient room temperature for best results.

Direct insights into the research, methodology, and results have been added to this summary document by the co-inventors themselves. This additional information is intended to provide helpful context to professional practitioners and does not fundamentally change the outcomes or interpretation of the published results.

REPRODUCTIVE SOLUTIONS

1 A Novel Collection Technique for Improved Semen Quality

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PUBLICATIONS

The Journal of the Society for Gynecologic Investigation 8 Supplement: 229A, 2001 - poster presentation; *Journal of Dairy, Veterinary and Animal Research* 1: 2014 - full publication.

OBJECTIVE

To date, the common thread in the use of semen extenders/collection techniques, whether they are being used for fresh extended semen, chilled semen, or cryopreserved semen, is that the extenders have all traditionally been added post-collection. The objective of this study was to determine if modifying the method of collection / extension of semen to include a warmed media environment would improve semen parameters by lessening cold and pH shock.

MATERIALS AND METHODS

Ten canine semen samples were collected with a modified artificial vagina to allow for a true split collection into two collection containers at one time. The treatment half of the sample was collected into a measured amount of warmed extension media. The control half was collected into a dry container and no attempt to maintain temperature was used. Standard semen parameters, available sperm pool, and number of inseminations were evaluated at specific time intervals. Evaluations continued until samples reached zero percent motility. Data analysis was performed with SPSS using the general linear model and appropriate t-tests.

RESULTS

There was a difference between the treatment and control groups for motility ($p < 0.001$), motility by time ($p < 0.001$), time to zero motility ($p < 0.001$), time to last full insemination ($p < 0.03$), forward progression ($p < 0.001$), acrosome reaction ($p < 0.001$), acrosome reaction by time ($p < 0.02$), and viability ($p < 0.001$). There was no difference in morphology between the treatment and control groups ($p > 0.05$).

CONCLUSIONS

Modification of the semen collection / extension procedure resulted in improved semen parameters for extended time periods post-collection. The data suggest the described collection technique can yield significantly more motile sperm by placing the sample into a physiologically favorable environment (eliminating pH and cold shock and allowing osmoregulation to begin), thus providing more available sperm for breeding.

REPRODUCTIVE SOLUTIONS

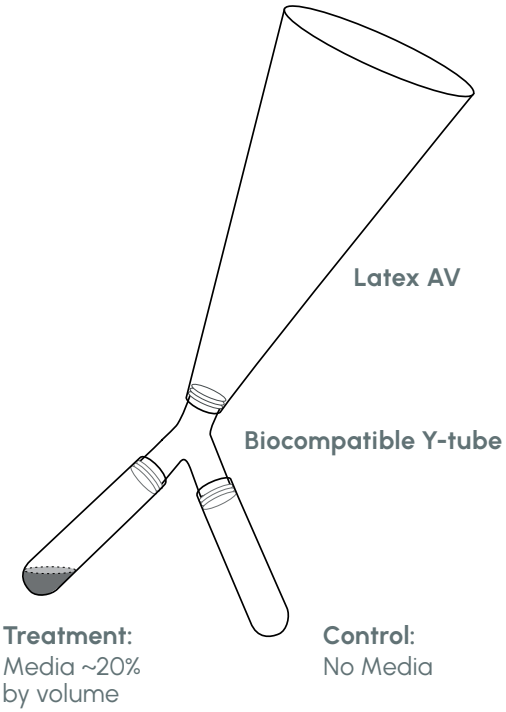
1 A Novel Collection Technique for Improved Semen Quality

INSIGHTS

This is the original canine study and is the first to describe the concept of a modified collection method, Device for Improved Semen Collection (DISC), that would eventually become ProteX.

Canines were chosen as the initial model because canine sperm are one of the more difficult to maintain for any length of time outside the body. In addition, canine sperm have similar volume, concentration, and sperm physiology to humans. Unlike other animal models, there are infertile canines, creating another correlation to human diagnoses.

Prior research by the investigators determined that the specific amount of media to be used in the new device prior to collection should be ~20% by volume of the expected ejaculate. This ensures the sperm are osmotically balanced. In the case of canines and humans, media should be 1 mL. This amount was determined to be optimal for providing the pH buffering capacity and allowing the sample to begin osmoregulating, without causing osmotic stress.



Modified Artificial Vagina

The modified artificial vagina was developed by the investigators to allow for a true split collection as opposed to a fractionated collection. This allowed for a direct comparison of the DISC to traditional collection using the same ejaculate.

INSIGHTS

REPRODUCTIVE SOLUTIONS

1 A Novel Collection Technique for Improved Semen Quality

FIGURE 1

MOTILITY OVER TIME – ALL CANINES

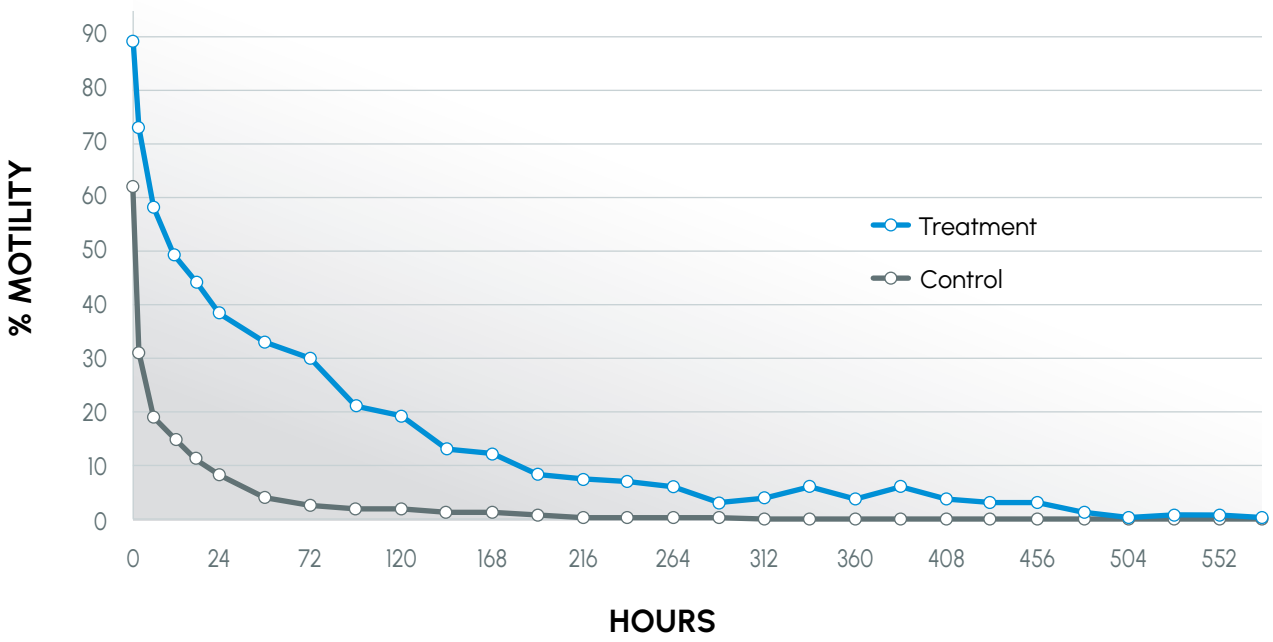
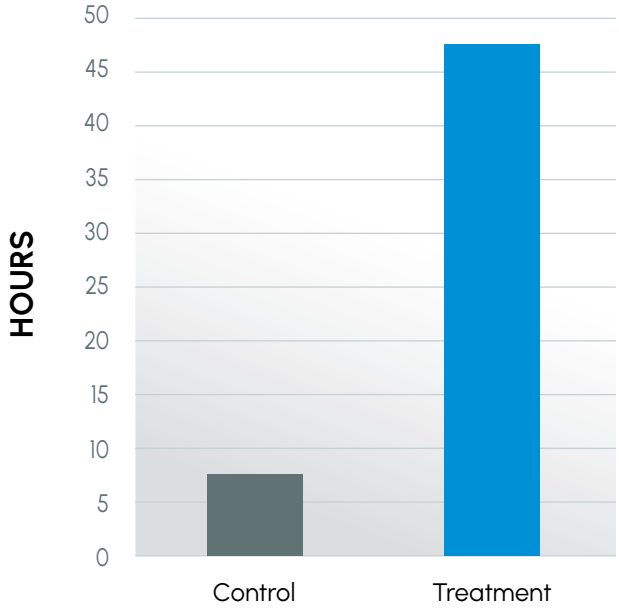


FIGURE 2

TIME TO LAST FULL INSEMINATION DOSE - CANINES



REPRODUCTIVE SOLUTIONS

1 A Novel Collection Technique for Improved Semen Quality

FIGURE 3

MOTILITY OVER TIME **TOLERANT** CANINES

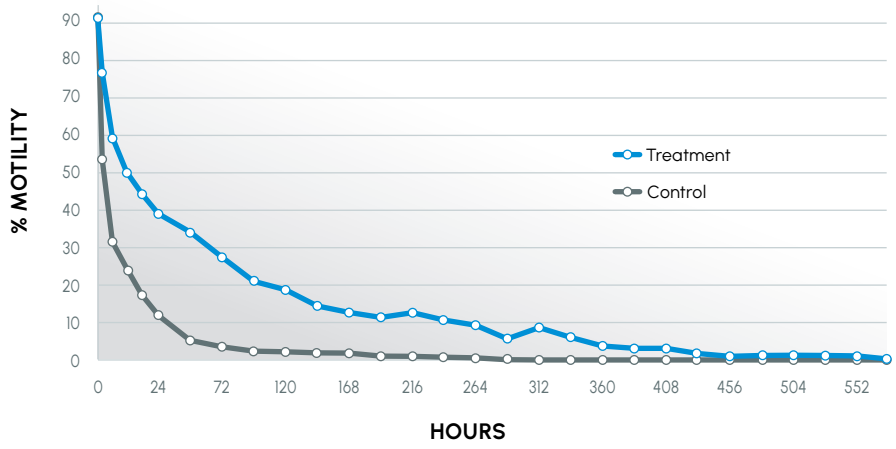
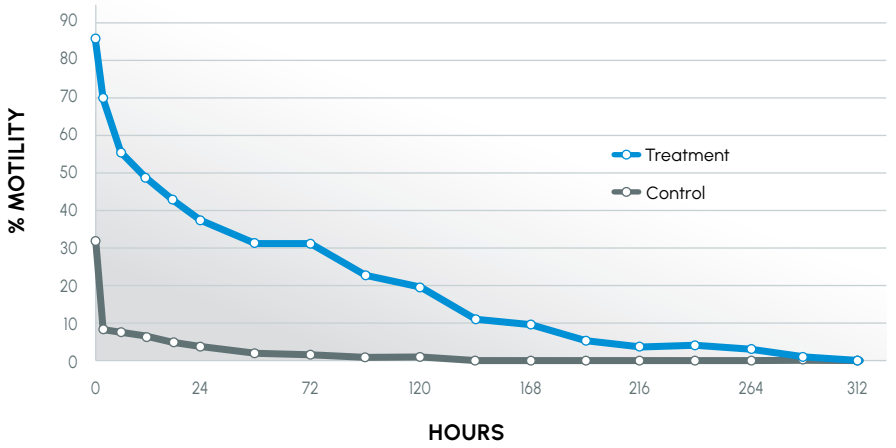


FIGURE 4

MOTILITY OVER TIME **INTOLERANT** CANINES

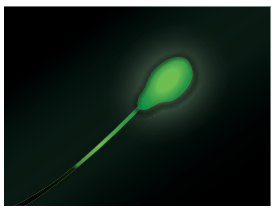


INSIGHTS

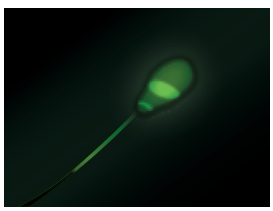
Method Tolerance and Acrosomes

Animals used in the study were recognized as being tolerant (normal) or intolerant (infertile) to traditional collection methods. The animals that were intolerant to traditional methods had the most improvement.

This study also showed that more motile, acrosomally intact sperm were maintained in the treatment group. The result indicated sperm better maintained their fertilizing capability.



Acrosomally Intact Sperm



Acrosomally Reacted Sperm

REPRODUCTIVE SOLUTIONS

2 Improving Semen Quality Using a Modified Collection Technique

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PUBLICATIONS

Fertility and Sterility Vol. 78 Supplement S226-S227. Published in issue: September 2002 - poster presentation.

OBJECTIVE

The common practice of semen collection / extension techniques in humans, whether being used for intrauterine insemination, cryopreservation, or an advanced reproductive technique, is to collect the sample into a dry, unprotected specimen cup. Empirically, the environment provided by this container would allow drastic shifts in specimen temperature and pH resulting in spermatozoa damage. Previous studies in the canine have demonstrated long-term (days) improvement in semen parameters when the semen is collected into a modified collection device that provides a warm environment that is both pH and osmotically balanced. The objective of the present study was to determine if the same modification could be used to improve semen parameters in the human.

DESIGN

Comparison of three semen collection techniques using a Latin Square to account for the variations between collections within the same donor.

MATERIALS AND METHODS

Eight donors provided three semen samples each for the study. The study design was randomized by first collection. Control samples were collected into a standard, dry specimen cup. No attempt was made to hold the sample at body temperature prior to processing. The remaining two samples were collected into a modified container (DISC) that funneled the sample into a reduced volume central well, which contained 1 mL of a pH-buffered media and which was insulated from temperature fluctuations. In one case, the media was at 37°C, in the other the media was held at room temperature (23°C). Once collected, the samples were held for 15 minutes at 37°C prior to processing using a standard semen washing technique.

The samples were then placed in 5 mL of fresh media and incubated at 37°C with 95% relative humidity and 5% CO_2. Standard semen parameters and biochemical markers were evaluated at times 0, 1, 3, 6, 12, 18 & 24 hours post-processing. Data analysis was performed with SPSS using the general linear model and appropriate t-tests.

2 Improving Semen Quality Using a Modified Collection Technique

RESULTS

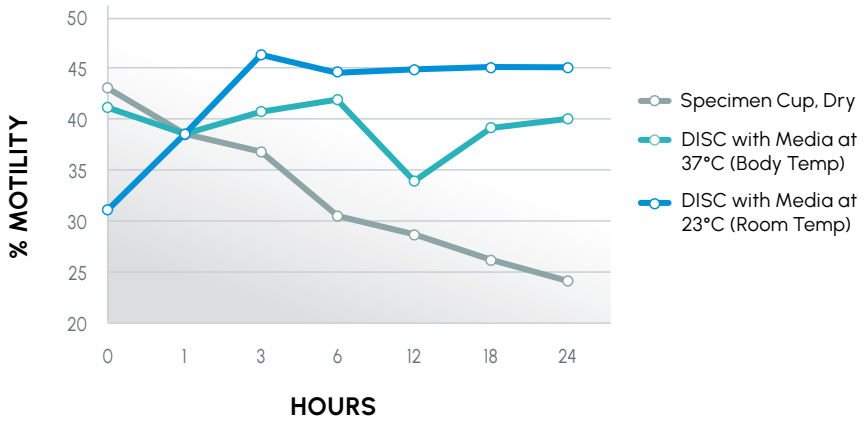
Both treatment groups maintained greater motility ($p < 0.001$), viability ($p < 0.001$), linearity ($p < 0.001$), and velocity ($p < 0.001$) with a significant increase of intact acrosomes over time as compared to the control over the 24-hour period. Motility in the two treatment groups was 6-fold higher than the control at 24 hours. The two treatment groups had 45% more intact acrosomes at 24 hours.

CONCLUSIONS

Modification of the semen collection / extension procedure resulted in improved semen parameters for extended time periods post-collection. The data suggests the described technique can yield significantly more motile sperm by placing the sample into a physiologically favorable environment, thus making those sperm available for use in a variety of infertility treatments.

FIGURE 1

MOTILITY OVER TIME USING HAM'S F-10 PLUS SERUM



INSIGHTS

This is the only study where serum was added to the media to measure the impacts of media content to semen samples. We discovered the best performing combination was Ham's F-10 plus serum. Today, most commercial media developed specifically for sperm washing already contains ~10% serum. In later studies, our researchers utilize third-generation media that includes pH-buffering agents and controls metabolite production.

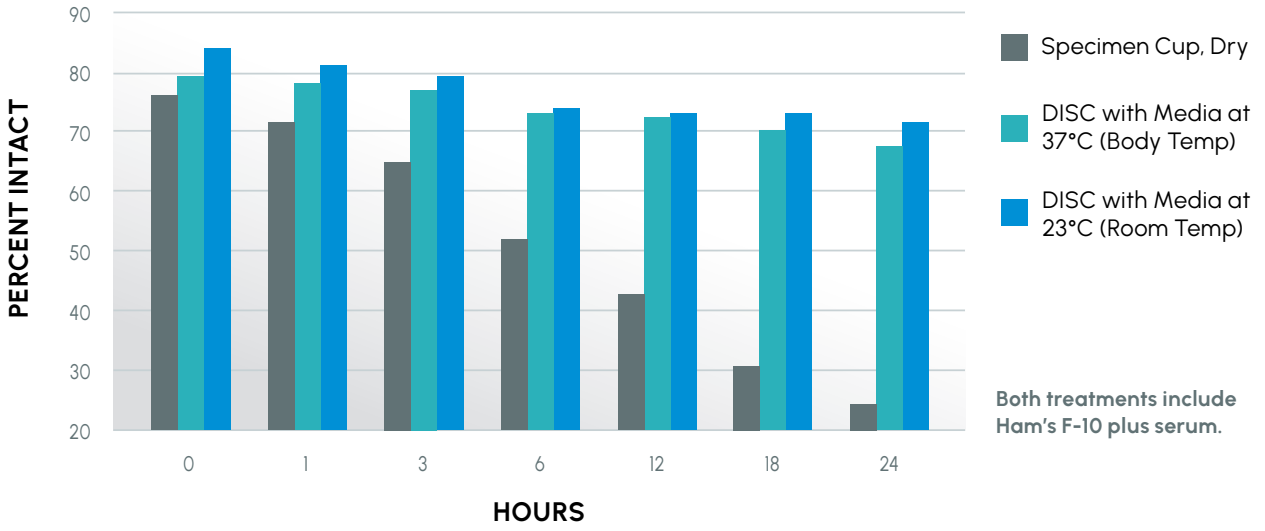
Today, many researchers are discovering the crucial role of media for at-home collection. The design of the DISC well allows for an optimal volume of media (1 mL) without the sample pooling to one side of the container. It is widely known that adding media prior to collection in a specimen cup requires ~5 mL of media to properly cover the sample, due to the larger area of the specimen cup's bottom surface. That volume of media causes osmotic stress, damaging the sample before and after processing.

REPRODUCTIVE SOLUTIONS

2 Improving Semen Quality Using a Modified Collection Technique

FIGURE 2

PERCENTAGE OF INTACT ACROSOMES OVER TIME



INSIGHTS

This study is the first to include a deeper review of acrosome membrane data, the leading indicator of biochemical health of the sperm and the potential for fertilization. Both DISC treatments follow a similar trendline that indicates the DISC itself vastly improves the percentage of intact acrosomes at all time points, regardless of media temperature. Samples within DISC at 23°C have 72% intact acrosomes at 24 hours, which is over 45% more intact acrosomes than a specimen cup. As with FIGURE 1 in this study, room temperature media outperforms media warmed to body temperature.

There is a severe downward trendline for the dry specimen cup, confirming the necessity to process specimen cup samples within an hour before further cellular and membrane damage occurs. Even so, at the initial reading, the specimen cup sample has 3% and 8% fewer intact acrosomes than DISC with media at 37°C and DISC with media at 23°C respectively. Intact acrosomes decreased 24% at 6 hours, and over 50% at 24 hours within the specimen cup sample. Biochemical marker research continues in STUDY 6 (pp. 20-22), comparing human samples collected in specimen cup, DISC dry, and DISC with media.

REPRODUCTIVE SOLUTIONS

3 New Semen Collection Technique / Container Improves Semen Quality

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PUBLICATION

Fertility and Sterility Vol. 80 Supplement, p.30. Published in issue: September 2003 - oral presentation.

OBJECTIVE

To date, the common thread in the use of semen collection/extension techniques, whether being used for intrauterine insemination, cryopreserved semen, or advanced reproductive techniques such as in vitro fertilization, is that the samples are collected into a dry, unprotected specimen container. This container, by its nature, allows for drastic shifts in temperature and pH resulting in damage to the spermatozoa. Previous studies in the canine and limited studies in humans have shown that collecting semen into a modified collection vessel containing a warmed, osmotically balanced media, semen parameters can be improved by lessening cold and pH shock upon collection. The objective of this study was to determine if the use of this new semen collection technique / container would yield similar improvements in semen parameter in the human as compared to the traditional collection container.

DESIGN

The study was designed as a Latin Square to account for the variations between collections within the same donor and subjects were randomized by first collection.

MATERIALS AND METHODS

Twelve donors provided three semen samples each to be used in three separate treatments. The control samples (Treatment I) were collected into a standard, dry specimen container.

Treatment II and III samples were collected into a modified specimen container that allowed the sample to be funneled into an insulated, reduced volume center well containing 1 mL of pH-buffered media. In the case of Treatment II, the media was at 37°C, while the media in Treatment III was held at room temperature (23°C). After collection, the samples were held at 37°C and allowed to liquefy before processing using a standard semen washing technique.

The samples were then placed into 5 mL of fresh media and maintained in a 37°C, 5% CO_2 incubator at 95% relative humidity. Standard semen parameters and biochemical markers were evaluated at 0, 1, 3, 6, 12, 18, and 24 hours post-processing. Data analysis was performed with SPSS using the general linear model and appropriate t-tests.

3 New Semen Collection Technique / Container Improves Semen Quality

RESULTS

A higher percentage of motile sperm were maintained in both treatment groups as compared to the control ($p < 0.001$), over the 24-hour period. At 24 hours, the motility in Treatment II was at least 40% higher than the control, while the motility in Treatment III was at least 55% higher than the control when examining the subjects with lowest motility. In addition, viability ($p < 0.001$), linearity ($p < 0.001$), and velocity ($p < 0.001$) were also higher in both treatments as compared to the control.
There was no difference in morphology between the treatments and the control. Measurements of biochemical parameters, including acrosome reaction, are ongoing.

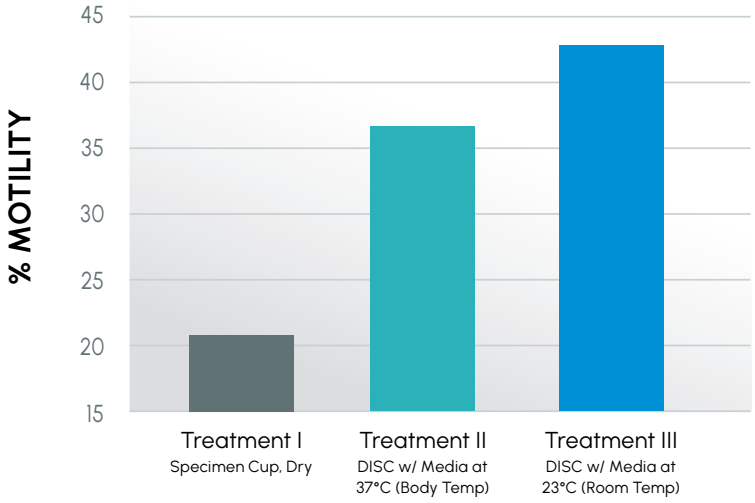
CONCLUSIONS

Modification of the semen collection / extension procedure resulted in improved semen parameters for extended time periods post-collection. The data suggests the new technique can yield significantly more motile sperm by placing the sample into a physiologically favorable environment (eliminating pH and cold shock), thus making more sperm available for use in a variety of infertility treatments. The improvement in semen parameters over time as seen in both treatments may lead to improved patient care by allowing the patient to collect at home while maintaining superior semen quality. Further studies, evaluating pregnancy rates, will be needed to confirm these observations.

FIGURE 1

MOTILITY AT 24 HOURS WITH CONTAINER AND MEDIA / TEMPERATURE VARIABLES

Twelve donors provided three samples each ($N = 36$) to be used in the three arms of the study. The motility in DISC with media at body temp was ~70% higher than the dry specimen cup. Disc with media at room temp was ~100% higher than the dry specimen cup.



INSIGHTS

In this study, it was confirmed that room temperature media improved semen parameters the most. By using room temperature media, the semen sample was allowed to drive the temperature change vs. the outside environment. Motility at 24 hours for these three treatments is different from the previous study (STUDY 2, FIGURE 1) due to differences in the human samples. However, the delta between Control and DISC(s) is similar between studies.

REPRODUCTIVE SOLUTIONS

4 Continued Evaluation of a New Semen Collection Technique / Container in Subfertile and Infertile Individuals Using a Cross-species Model

AUTHORS

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PUBLICATION

Fertility and Sterility Vol. 82 Supplement S178.
Published in issue: September 2004.

OBJECTIVE

To date, the common thread in the use of semen collection / extension techniques, whether being used for intrauterine insemination, cryopreserved semen, or advanced reproductive techniques such as in vitro fertilization, is that the samples are collected into a dry, unprotected specimen container. This container, by its nature, allows for drastic shifts in temperature and pH resulting in damage to the spermatozoa. Preliminary studies from this laboratory using a new collection container / technique to collect semen from canines and humans demonstrated an improvement in most semen parameters. The modified collection device optimizes semen parameters by controlling temperature, pH, and osmotic stress. The objective of the current study was to further investigate the usefulness of the modified device in improving semen parameters using a cross-species model focusing on subfertile and infertile individuals.

MATERIALS AND METHODS

Semen samples were collected from canine (n = 10), equine (n = 8) and human (n = 12) donors using standard techniques. In the case of the equine and canine donors, a splitter was placed in the artificial vagina to produce a true split sample into a standard collection vessel and the modified collection unit.

Human donors were collected into both devices in sequential samples at three-day intervals. Standard semen parameters (viability, motility, morphology, concentration) and biochemical markers were evaluated for all donors at 0, 1, 3, 6, 12, 18, and 24 hours post-processing. Canine and Equine samples were further evaluated at 24-hour intervals until the samples had reached 0% motility. All donors were then classified as either fertile or subfertile based upon species standard for count and / or motility. Data analysis within species were performed with SPSS using the general linear model and appropriate t-tests.

RESULTS

As in previous studies, semen quality at 24 hours (as reflected by motility) was significantly higher in samples collected in the new collection container as compared to the control regardless of species: human 20% vs. 14% ($p < 0.001$); canine 38% vs. 8% ($p < 0.001$); equine 55% vs. 37%, ($p < 0.001$).

REPRODUCTIVE SOLUTIONS

4 Continued Evaluation of a New Semen Collection Technique / Container in Subfertile and Infertile Individuals Using a Cross-species Model

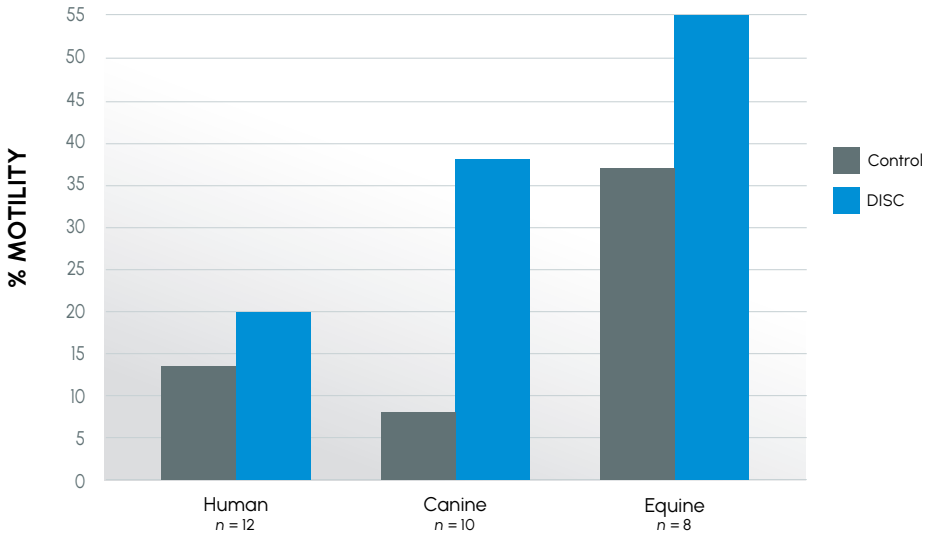
Further, collection of the samples into the new device extended the time to last full insemination dose an average of 6-fold (7 hours vs. 50 hours) in the canine ($p < 0.03$) and 0.5-fold (148 hrs vs. 228 hrs) in the equine ($p < 0.001$) over their respective control. This improvement was even more dramatic in animals classified as subfertile, where collection into the new device extended the time to last full insemination dose an average of 14-fold (6 hours vs. 91 hours) in the canine and 1.5-fold (120 hours vs. 312 hours) in the equine over their respective controls. Improvement was also seen in viability and biochemical parameters which will be discussed in detail at presentation.

CONCLUSIONS

Modification of the semen collection / extension procedure resulted in improved semen parameters for extended time periods post-collection. The data suggests the described technique can yield significantly more motile sperm by placing the sample into a physiologically favorable environment (eliminating pH and cold shock), thus making more sperm available for use in a variety of infertility treatments. Further studies, evaluating pregnancy rates, will be needed to confirm these observations.

FIGURE 1

MOTILITY USING THE DISC - CROSS-SPECIES VIEW AT 24 HOURS



INSIGHTS

In this study, the twelve human subjects were all diagnosed as subfertile with six subjects providing a unique sample in each arm. Note the human motility at 24 hours for both Control, DISC, and the resulting delta differs from a previous study (STUDY 2, FIGURE 1). This variance is due to the small sample size, starting motility, and the diagnosis of this group. Average starting motility for this sample was ~20%, and therefore nearly flat through the 24-hour mark. This coincides with previous trends that indicate a long-term stability of samples in the DISC.

REPRODUCTIVE SOLUTIONS

4 Continued Evaluation of a New Semen Collection Technique / Container in Subfertile and Infertile Individuals Using a Cross-species Model

FIGURE 2

TIME TO LAST FULL INSEMINATION DOSE - **SUBFERTILE** CANINE AND EQUINE

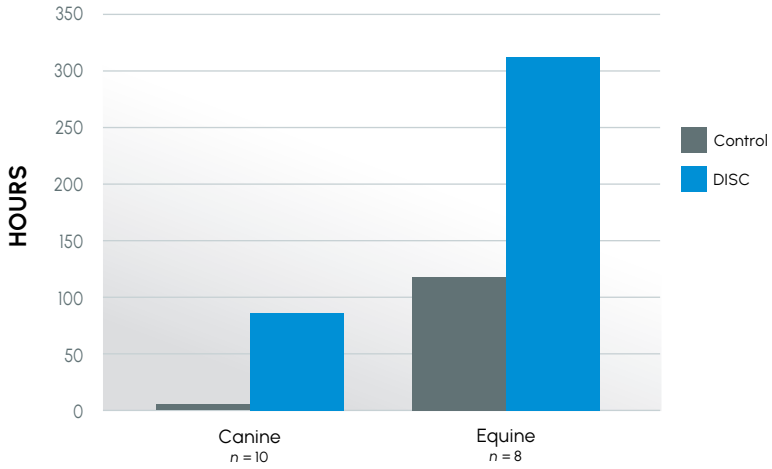
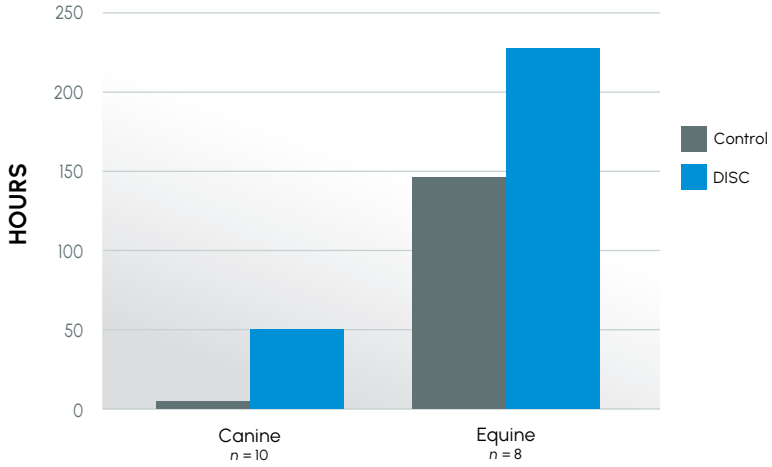


FIGURE 3

TIME TO LAST FULL INSEMINATION DOSE - **ALL** CANINE AND EQUINE



INSIGHTS

This series of experiments examined how semen from different species reacted in ProteX. The species evaluated included human, equine, and canine, all species that can have infertility. Both fertile and infertile canine and equine subjects were used, as well as subfertile human subjects. Motility was improved in all species, further, time to last full insemination dose was significantly improved in both canine and equine models. The greatest improvement was seen in subfertile animals. This indicates that protecting the sperm from damage at collection has the greatest impact on the infertile male.

REPRODUCTIVE SOLUTIONS

5 Improvement of the Semen Collection Environment Using a New Semen Collection Device

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PUBLICATION

Fertility and Sterility Vol. 86, Issue 3 Supplement S508–S509. Published in issue: September 2006 - poster presentation.

OBJECTIVE

It is well established that rapid changes in specimen temperature can be detrimental to semen quality. While specimen containers are often warmed to 37°C prior to collection, it is not uncommon for the collection to last long enough to allow significant cooling of the container and its specimen. Further, transport issues and / or delays in processing might allow for further temperature shifts. Recently, a new specimen collection device (SCD, ProteX) was introduced (ReproMax; Embryonic Technologies, Austin, TX). Previous work with experimental devices, suggests the SCD improves semen motility ($p < 0.001$) and forward progression ($p < 0.02$), by improving cooling rates over a standard specimen container. The objective of the present study was to verify the improved cooling curves of SCD and the resultant improvement in semen quality.

DESIGN

Laboratory testing of new collection device.

MATERIALS AND METHODS

Trials of temperature maintenance were performed using a standard specimen cup, a Corning 15 mL conical test tube and a new SCD pre-warmed to 37°C. Each container was loaded with saline, which was considered an approximation of semen culture media yet provided precise control for measuring temperature. Saline was warmed to 41°C so that the initial read within the standard specimen cup was approximately body temperature (37°C). An initial temperature was read for each device followed by temperature measurements being taken every minute intervals for 30 minutes. During this time, the container was left unprotected on a standard laboratory bench top while room temperatures and relative humidity remained constant at 21.4°C and 18% respectively. Resulting data were subjected to ANOVA with repeated measures.

RESULTS

In all cases, filling the different containers, placing the temperature probe, and collecting the initial reading took at least 15 seconds. Yet, in that time period, the fluid temperature had dropped approximately 3°C in the standard specimen cup and 2°C in the conical test tube. This finding drove the researchers' decision to initially warm all containers to 41°C, enabling a first reading at approximate body temperature (37°C). It took only 3 minutes for the temperature of the fluid in the standard specimen cup to drop 10°C from the initial 41°C reading.

5 Improvement of the Semen Collection Environment Using a New Semen Collection Device

The test tube, which improved the surface area-to-volume ratio, still lost 10°C in temperature in under 8 minutes. However, the temperature of the SCD changed only 0.1°C ($p < 0.001$) at the initial reading, and it took over 20 minutes for the SCD to show the same 10°C drop in temperature, over a 600% improvement in temperature maintenance ($p < 0.001$).

CONCLUSIONS

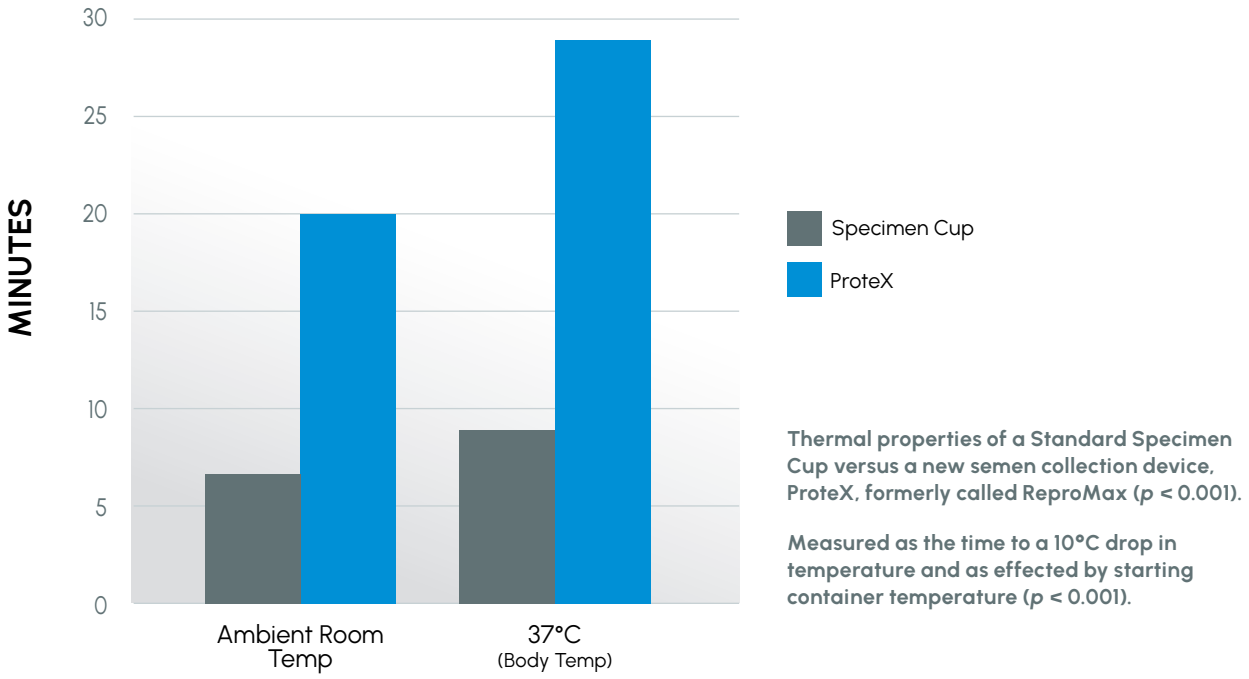
Results of the present study confirm rapid loss of specimen temperature in a standard specimen cup. The rapid decrease in temperature in a standard specimen cup would be expected to activate of cold shock proteins which might interfere with normal sperm cell function. Preliminary data from the SCD suggests it will improve semen parameters by maintaining temperature over the entire period of the collection, transport, and processing period.

SUPPORTED BY

Embryonic Technologies.

FIGURE 1

TIME TO A 10°C DECREASE IN FLUID TEMPERATURE



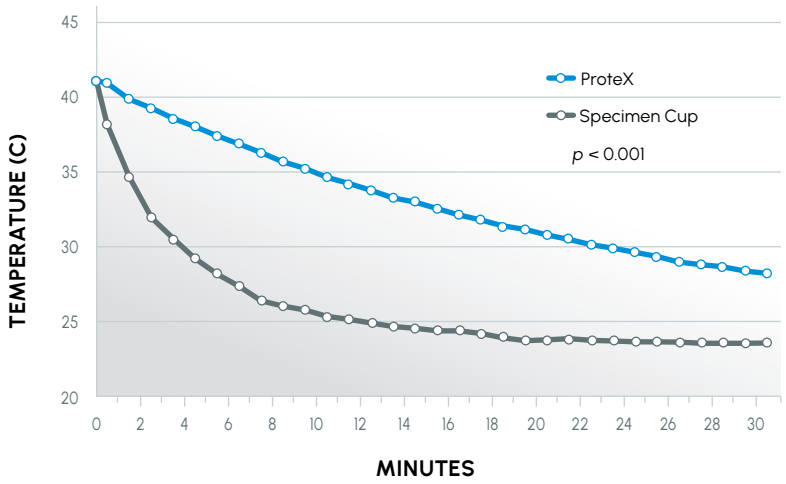
Thermal properties of a Standard Specimen Cup versus a new semen collection device, ProteX, formerly called ReproMax ($p < 0.001$).

Measured as the time to a 10°C drop in temperature and as effected by starting container temperature ($p < 0.001$).

5 Improvement of the Semen Collection Environment Using a New Semen Collection Device

FIGURE 2

TEMPERATURE LOSS OVER TIME FROM 5 ML OF FLUID STORED IN COLLECTION DEVICES



INSIGHTS

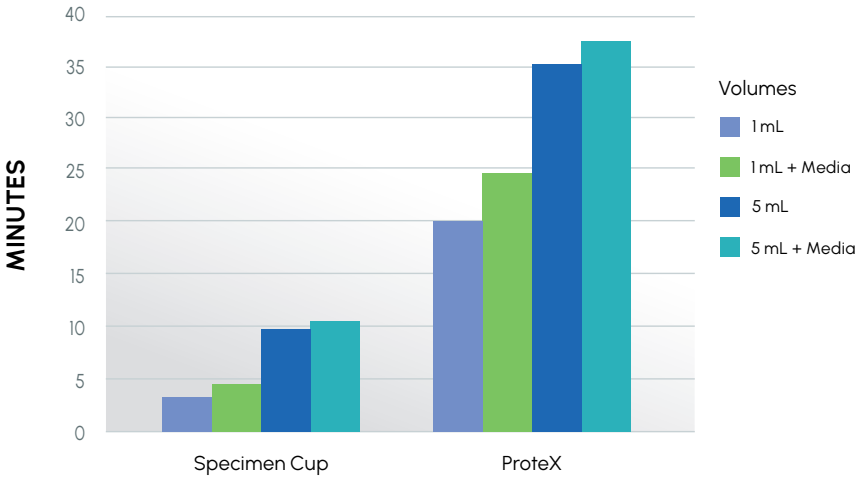
Heat shock proteins are known to trigger apoptosis in many types of cells, including sperm. Protecting the sperm from sharp decreases in temperature reduces shock to sperm maintaining their biochemistry and fertilizing capacity. In a standard specimen cup, 5 mL of fluid will drop by 10°C in 3 minutes, a cooling rate of over 3°C a minute. However, the cooling rate for 5 mL of fluid in ProteX was 0.3°C a minute, so that it takes over 20 minutes for 5 mL of fluid to drop 10°C.

FIGURE 3

TIME TO A 10°C DECREASE IN TEMPERATURE FOR FLUID VOLUMES

INSIGHTS

Volume of fluid in a container will influence how quickly that fluid changes temperature. This graph illustrates this phenomenon as well as the thermal protection offered by the ProteX device.



REPRODUCTIVE SOLUTIONS

6 Physiological and Biochemical Assessment of a New Semen Collection Device

AUTHORS

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PUBLICATION

Fertility and Sterility Vol. 96, Issue 3 Supplement S163-S164. Published in issue: September 2011 - poster presentation.

OBJECTIVE

Semen quality is a key factor in determining the outcome of most infertility treatments. Previously, this lab demonstrated that a modification of the collection container, known as the device for improved semen collection (DISC), resulted in significant improvement in semen quality. Studies in two animal species suggested improved semen parameters and increased pregnancy rates. The objective of the study was to assess the effectiveness of a clinical-grade version of the DISC on sperm cell function and biochemistry prior to clinical trials.

DESIGN

A laboratory-based, controlled trial.

MATERIALS AND METHODS

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 simple sperm washing technique and then placed in culture for 24 hours. At predetermined intervals, aliquots were taken for standard semen analysis using an IVOS and biochemical assessment, including intactness of acrosomal membranes, lipid peroxidation level, mitochondrial membrane potential, and DNA fragmentation. Resulting data were subjected to ANOVA with repeated measures.

RESULTS

All parameters from semen collected in the DISC were either equivalent or superior to semen collected in the SSC. Specifically, samples collected in the DISC and / or DISC+ had higher rates of cell viability ($p < 0.005$), progressive velocity ($p < 0.05$) and motility index ($p < 0.034$) compared to the SSC and trended toward higher motility rates ($p = 0.066$) and path velocities ($p = 0.061$). Further, cells collected in the DISC had more intact acrosomes ($p < 0.017$) and retained higher mitochondrial membrane potential ($p < 0.004$) over the 24-hour period compared to the SSC.

CONCLUSIONS

Semen collected in the clinical-grade DISC appears to have superior physiological activity and biochemical stability compared to semen collected in the SSC. Clinical trials are ongoing to assess the usefulness of the DISC in the clinical environment.

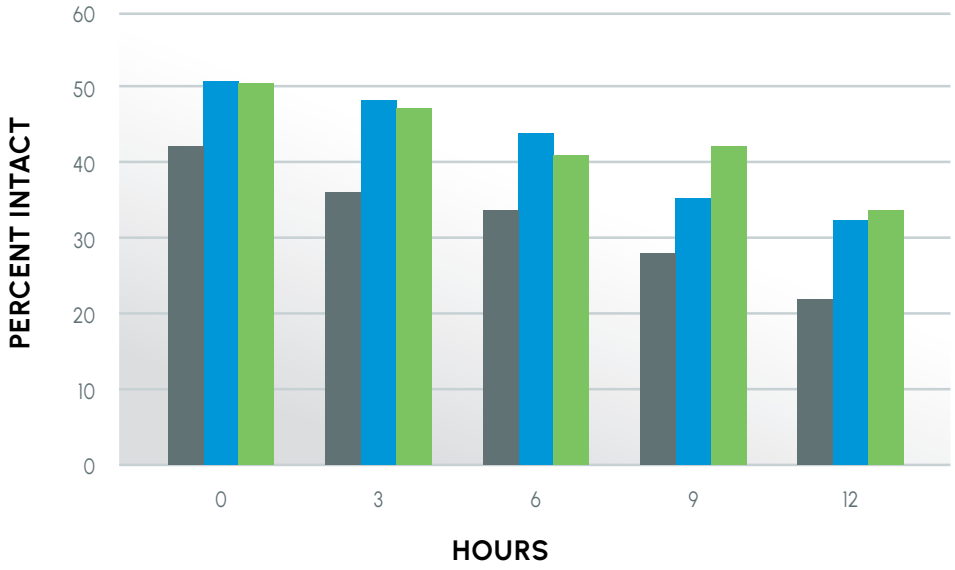
SUPPORTED BY

TTU Office of Commercialization.

6 Physiological and Biochemical Assessment of a New Semen Collection Device

FIGURE 1

PERCENTAGE OF INTACT ACROSOMES OVER TIME



Percentage of intact acrosomes for spermatozoa collected in either a Standard Specimen Container (SSC), the Device for Improved Semen Collection (DISC; $p < 0.017$) or the Device for Improved Semen Collection containing 1 mL Ham's F-10 media (DISC+; $p < 0.009$).

INSIGHTS

While standard semen analysis parameters like motility index are excellent indicators for sperm health, acrosome data is crucial for a final assessment. If the sample sperm are highly motile yet a low percentage of sperm have intact acrosome membranes, their likelihood to fertilize decreases greatly.

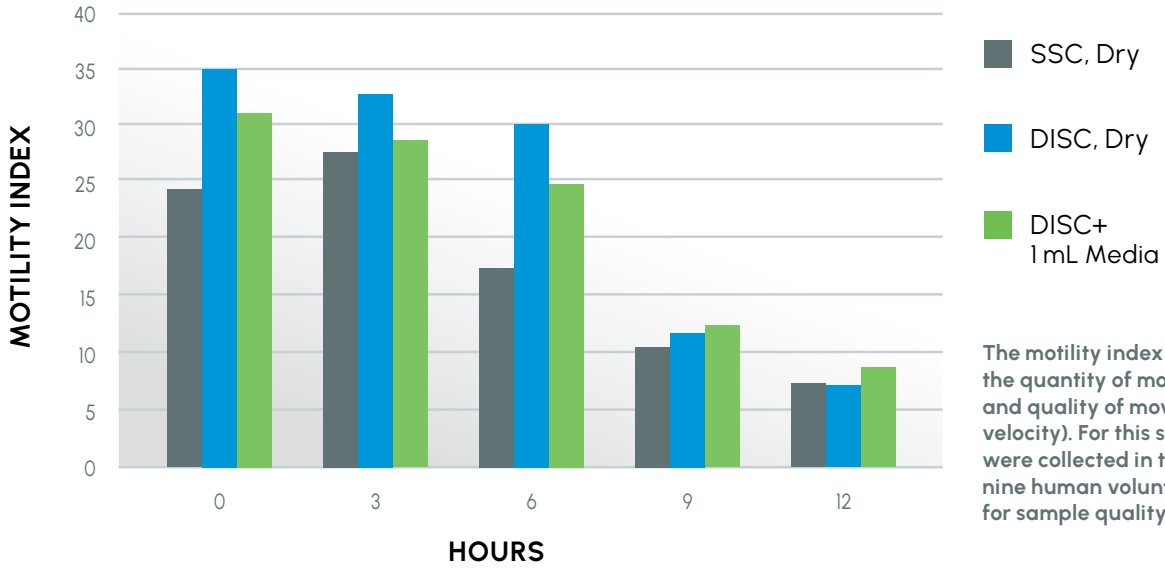
As in Study 2, Figure 2, the data here confirm samples within the DISC+ (with media) and even DISC dry, have a significantly increased number of intact acrosomes compared to the specimen cup across all time points. DISC treatments see an improvement over the specimen cup of 8% more intact acrosomes even at the initial reading.

REPRODUCTIVE SOLUTIONS

6 Physiological and Biochemical Assessment of a New Semen Collection Device

FIGURE 2

MOTILITY INDEX OVER TIME



The motility index is a combination of the quantity of movement (motility) and quality of movement (progressive velocity). For this study, sperm samples were collected in three treatments by nine human volunteers without control for sample quality / subject diagnosis.

INSIGHTS

Our researchers have questioned whether ProteX may be used dry and for what period of time does the sample stay viable in a dry container. In FIGURE 2 above, the results indicate the DISC dry has a higher motility index than both the SSC dry and the DISC with 1 mL media for up to 6 hours. However, coupled with the acrosome data in FIGURE 1, time points beyond 6 hours show the value of media in keeping the sample at a higher motility with more intact acrosomes.

Motile sperm, especially hyper-motile sperm, are already going through the biochemical processes needed for fertilization while also going down a parallel path to apoptosis. This process leads to the generation of cellular waste material that causes damage to the sperm over time. Research indicates the addition of media better controls production of these harmful metabolites and keeps more sperm in a quiescent state.

REPRODUCTIVE SOLUTIONS

7 Early Fertility Trials of Semen Collection Device Previously Demonstrated to Improve Semen Parameters and Pregnancy Rates in Animal Models

AUTHORS

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PUBLICATION

Fertility and Sterility Vol. 98, Issue 3 Supplement S249. Published in issue: September 2012 - poster presentation.

OBJECTIVE

It is well documented that sperm are susceptible to shock during processing which often induces biochemical pathways leading to cell death. Previous animal studies from this lab suggest a modification of the collection technique (Device for Improved Semen Collection; DISC) can prevent activation of these pathways, yielding larger, more motile pools of sperm for treatment and resulting in higher pregnancy rates. The objective of the present study was to evaluate the DISC in humans.

DESIGN

Lab-based trials in donors and infertility patients.

MATERIALS AND METHODS

Donors collected in a standard specimen cup (SSC) and the DISC. The samples were then processed and cultured for 24 hours. Aliquots were taken, over time, for semen analysis using CASA, and biochemical assessment, including: acrosomal status, lipid peroxidation, mitochondrial membrane potential (MMP) and DNA damage. A preliminary clinical trial was then conducted comparing the DISC to SSC. Couples undergoing IUI alternated semen collections between the DISC and SSC for up to 6 cycles.

RESULTS

Donor samples collected in the DISC exhibited improved semen parameters when compared to the SSC: viability ($p < 0.005$), motility rates ($p = 0.066$), path velocities ($p = 0.061$), progressive velocity ($P < 0.05$), and motility index ($p < 0.034$). Further, cells collected in the DISC had more intact acrosomes ($p < 0.017$) and retained higher MMP ($p < 0.004$). 24 couples completed 51 IUI cycles (26 SSC vs. 25 DISC). As in the donor trial, samples from the DISC trended to have higher motility ($p = 0.063$) and progressive velocity ($p = 0.057$). There were 9 pregnancies (17.6%) with equivalent results in the DISC vs. SSC, 4 vs. 5 ($p = 0.762$). However, while 100% of the pregnancies in the DISC delivered, only 40% in the SSC did ($p = 0.058$).

REPRODUCTIVE SOLUTIONS

7 Early Fertility Trials of Semen Collection Device Previously Demonstrated to Improve Semen Parameters and Pregnancy Rates in Animal Models

CONCLUSIONS

As in the animal studies, results suggest improved semen quality from the DISC. Larger numbers are needed to determine if improved semen quality will lead to the increased pregnancy rates seen in other species.

SUPPORTED BY

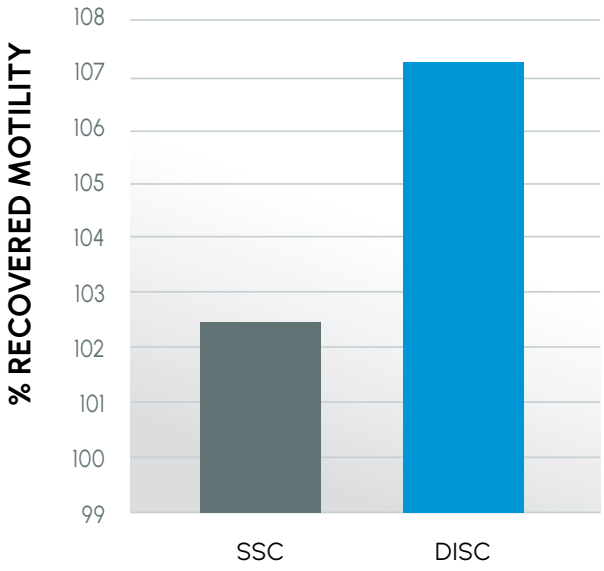
TTU Office of Commercialization.

FIGURE 1

RATE OF POST-WASH SAMPLE RECOVERED MOTILITY

A total of 51 cycles were completed by 24 couples recruited from two fertility centers and randomized. 25 cycles were measured in the DISC and 26 in the SSC.

The rate of post-wash sample recovered motility (% of post-wash motility / % pre-wash motility) reflects how many motile cells per mL are present after washing.



INSIGHTS

Knowing that both the protective properties of ProteX and the addition of media yield biochemically and physiologically healthier sperm, researchers naturally questioned if healthier sperm result in healthier pregnancies. A very small trial of 24 patients was undertaken to evaluate if ProteX is equivalent to standard collection methods.

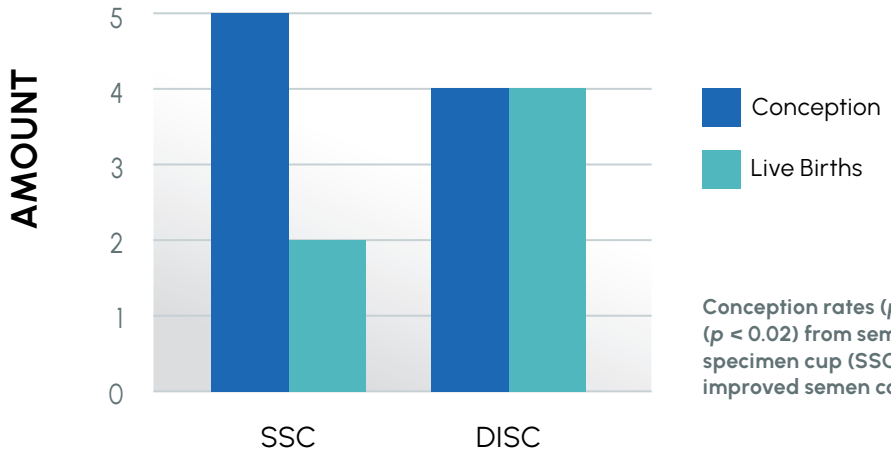
While there is often an expected lift in motility after washing sperm, there is no benchmark for recovered motility. It was found that across all patients, 5% more motile sperm were recovered post-wash when patients used ProteX. While this may seem like a small percentage, a 5% increase may be of relative significance as we look at healthy pregnancies and live births (FIGURE 2).

REPRODUCTIVE SOLUTIONS

7 Early Fertility Trials of Semen Collection Device Previously Demonstrated to Improve Semen Parameters and Pregnancy Rates in Animal Models

FIGURE 2

CONCEPTIONS AND LIVE BIRTHS



Conception rates ($p = 0.83$) and live births ($p < 0.02$) from semen collected in a standard specimen cup (SSC) versus a new device for improved semen collection (DISC)

INSIGHTS

As expected with this sample size, there was no statistical difference in conception rate. What was not expected by the team was that while over half of the patients who conceived using the standard cup experienced a miscarriage, all patients who conceived using ProteX had a baby come to full term, suggesting that healthier sperm result in more live births.

REPRODUCTIVE SOLUTIONS

8 Pregnancy Trials Using the Device for Improved Semen Collection

AUTHORS

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PUBLICATION

Fertility and Sterility Vol. 106, Issue 3 Supplement. Published in issue: September 2016 - poster presentation.

OBJECTIVE

It is well documented that sperm undergo significant physiological and biochemical processes, many of them brought on by changes in the environment at ejaculation. While the preponderance of the individual changes can be seen as positive and necessary for fertilization, collectively they set the cell on course for its eventual death. Previous research from this laboratory has demonstrated that modification of the collection environment using the Device for Improved Semen Collection (DISC), can lead to a delay in certain activation pathways and help provide a better quality sample for treatment procedures. A small human trial demonstrated superior semen parameters and equivalent pregnancy rates. The present study presents pregnancy data in two controlled trials in domestic animal species.

DESIGN

Controlled prospective trial.

MATERIALS AND METHODS

Two large scale pregnancy trials were conducted with the DISC in the equine and bovine. In both trials, semen was collected from the males in a real-time split collection where approximately half of the ejaculate was collected into the DISC or an appropriate control. Semen parameters were measured manually at the time of collection and time of insemination. In the equine trial mares were inseminated at ovulation with semen 24, 48, or 72 hours old to mimic industry practice (49 total inseminations). In the bovine, 43 females were divided for insemination with semen from either control or DISC collections. Inseminations were timed to occur 12 hours after semen collection using industry standard techniques. Pregnancy was determined by ultrasound.

RESULTS

Semen parameters were similar between controls and DISC samples at collection ($p = 0.832$). Further, as expected all parameters decrease with time ($p < 0.01$). However, semen collected in the DISC retained more motility at all other time points: Bull ($p < 0.002$) and Stallion ($p < 0.001$). Pregnancy rates in the mares were similar between treatments at 24 hours, but higher at both 48 and 72 hours ($p < 0.001$). Pregnancy rates in cattle trended higher in animals inseminated with DISC semen ($p = 0.06$).

REPRODUCTIVE SOLUTIONS

8 Pregnancy Trials Using the Device for Improved Semen Collection

CONCLUSIONS

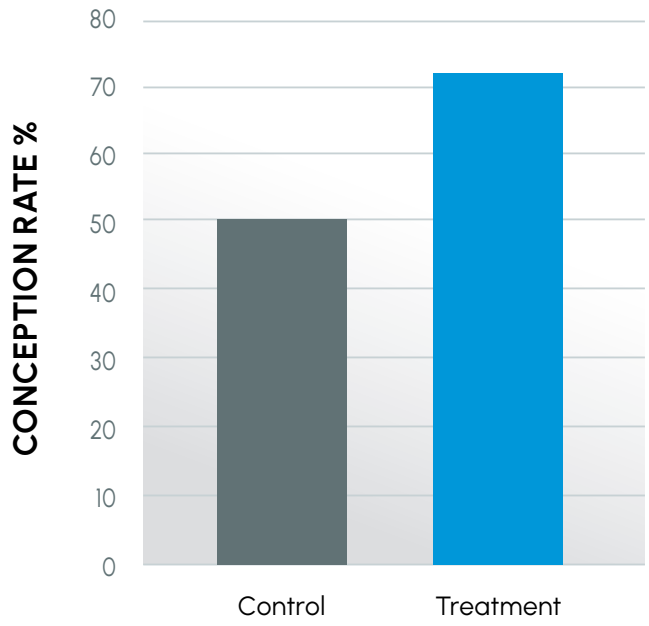
Data continue to indicate semen collected in the DISC provides higher quality cells for reproductive purposes. Further, pregnancy rates appear higher in animals bred with semen from the DISC. Additional research is warranted to confirm these findings.

SUPPORTED BY

TTU Office of Research Commercialization.

FIGURE 1

CONCEPTION RATES IN COMMERCIAL CATTLE



A commercial trial of conception rates in cattle using semen collected in the DISC (Treatment) vs. industry standard collection techniques (Control). Data suggests higher pregnancy rates from semen collected in DISC ($p < 0.02$).

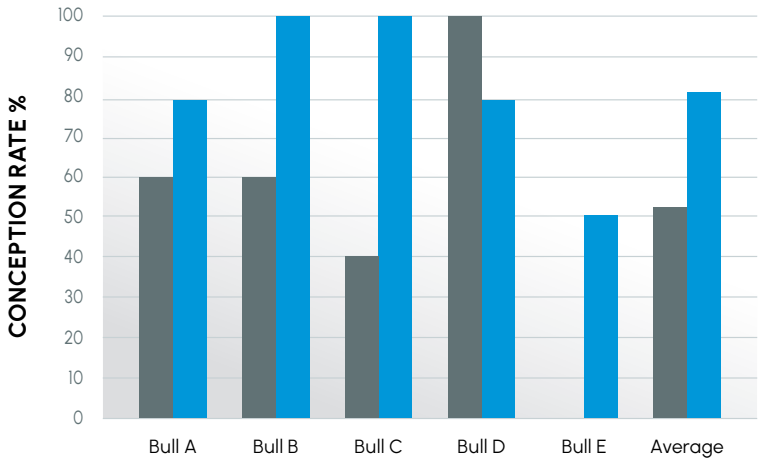
INSIGHTS

Large scale pregnancy trials were undertaken in both equine and bovine models using species-specific versions of ProteX (TruBreed). In a herd of 43 commercial cattle females, pregnancy rate increased from 50% with traditional methods to over 70% using ProteX. Across six proven bull sires, only one had higher conception rates using traditional methods.

8 Pregnancy Trials Using the Device for Improved Semen Collection

FIGURE 2

CONCEPTION RATES BY BULL IN A COMMERCIAL CATTLE HERD

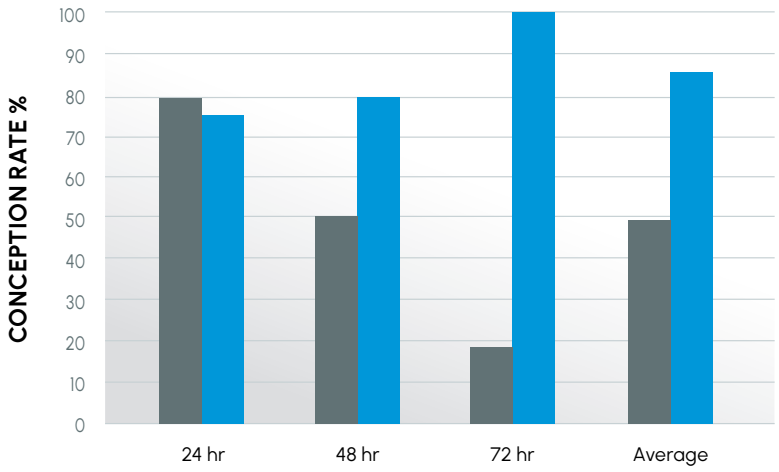


A controlled trial of conception rates of cattle using semen collected in the DISC (Treatment) vs. the industry standard collection techniques (Control).

Data for individual bulls demonstrated a trend toward higher conception rates with semen from the DISC ($p < 0.06$) and a higher conception rate among all animals bred ($p < 0.01$).

FIGURE 3

CONCEPTION RATES OVER TIME - EQUINE



A controlled trial of conception rates of horses using semen collected in the DISC (Treatment) vs. Industry Standard Collection Techniques (Control).

Data suggest both a higher pregnancy rate in all animals bred with semen after 24 hours. ($p < 0.001$) and a higher cumulative rate from all breedings ($p < 0.001$).

INSIGHTS

It is standard practice in the horse-breeding industry to not use semen that is over 24 hours old due to the rapid decline of sperm quality. This is observed in the steady drop of conception rate in the control arm. This is contrasted by the observation from semen collected in the DISC, which saw a steady increase in conception rate over time and a significantly higher conception rate compared to control.