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

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### **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).



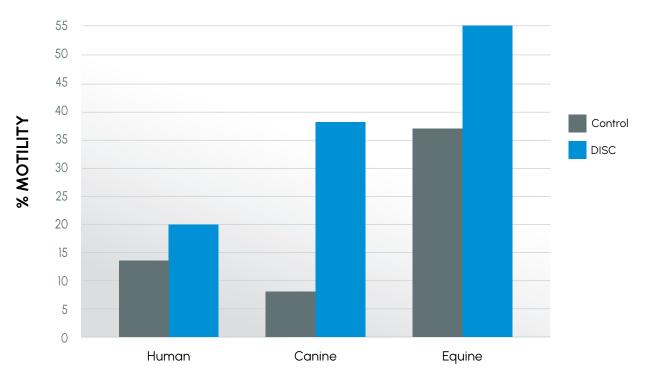
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Further, collection of the samples into the new device extended the time to last full insemination dose an average of 7-fold (7 hours vs. 50 hours) in the canine (p < 0.03) and 1.5-fold (148 hrs vs. 228 hrs) in the equine (p < 0.001) over their respective 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 15-fold (6 hours vs. 91 hours) in the canine and 2.5-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.

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



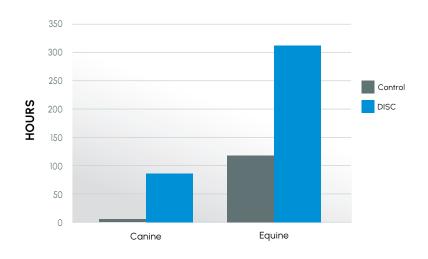


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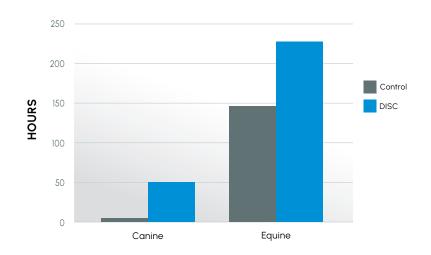
#### FIGURE 2

TIME TO LAST FULL
INSEMINATION DOSE SUBFERTILE CANINE
AND EQUINE



#### FIGURE 3

TIME TO LAST FULL
INSEMINATION DOSE ALL CANINE AND
EQUINE



**NSIGHT** 

This series of experiments examined how different 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, but the human subjects were considered normal by WHO. 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.