

1 A Novel Collection Technique for Improved Semen Quality

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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.

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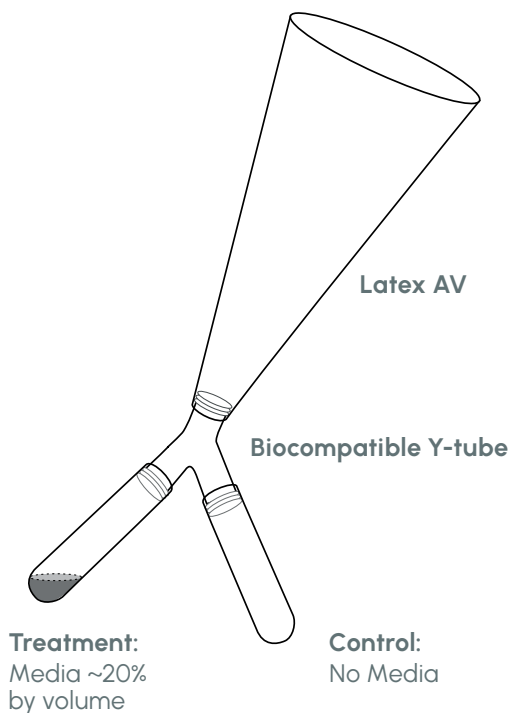
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INSIGHTS

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 device prior to collection should be ~20% by volume of the expected ejaculate. 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

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FIGURE 1

MOTILITY OVER TIME – ALL CANINES

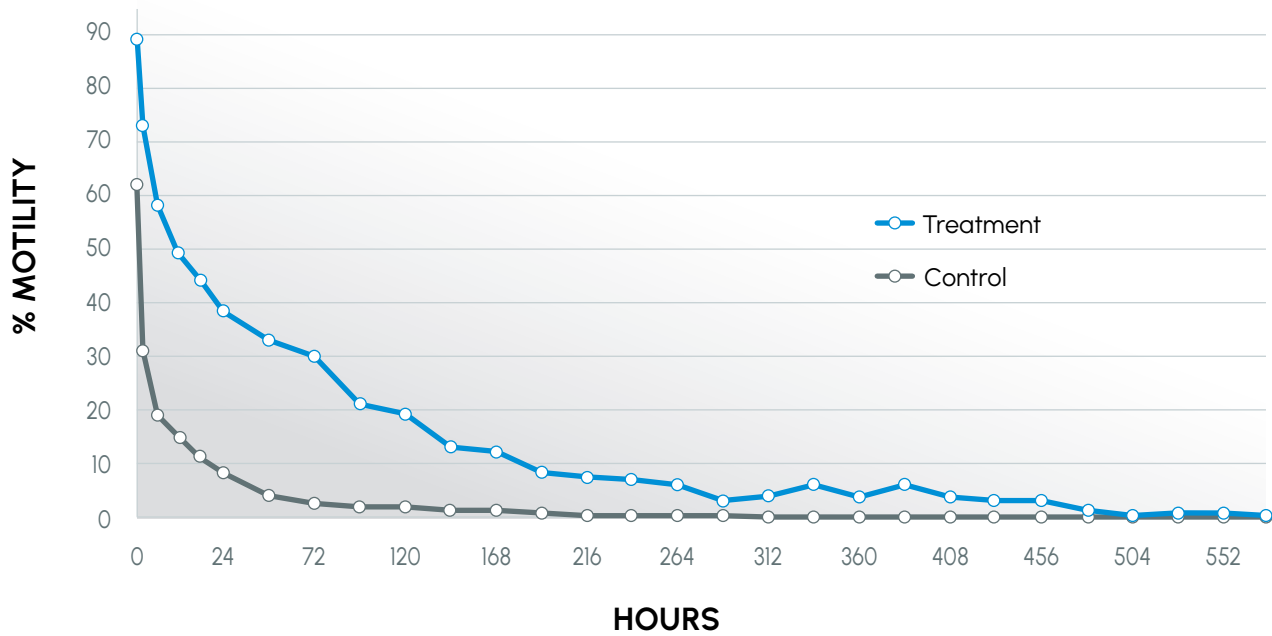
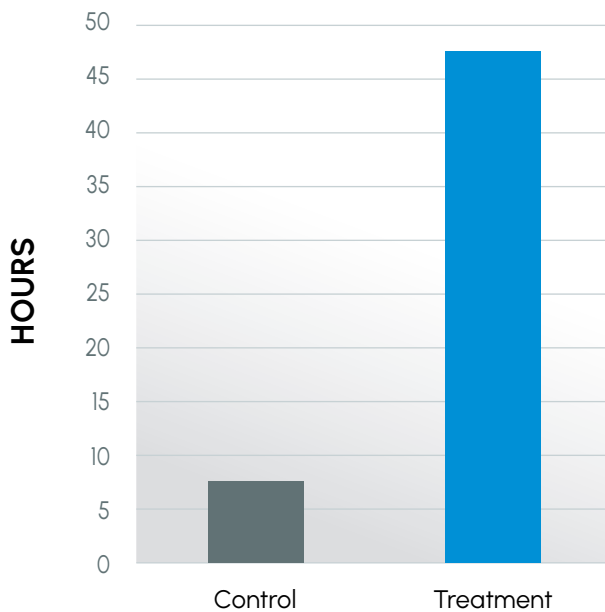


FIGURE 2

TIME TO LAST FULL INSEMINATION DOSE - CANINES



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FIGURE 3
MOTILITY OVER TIME TOLERANT CANINES

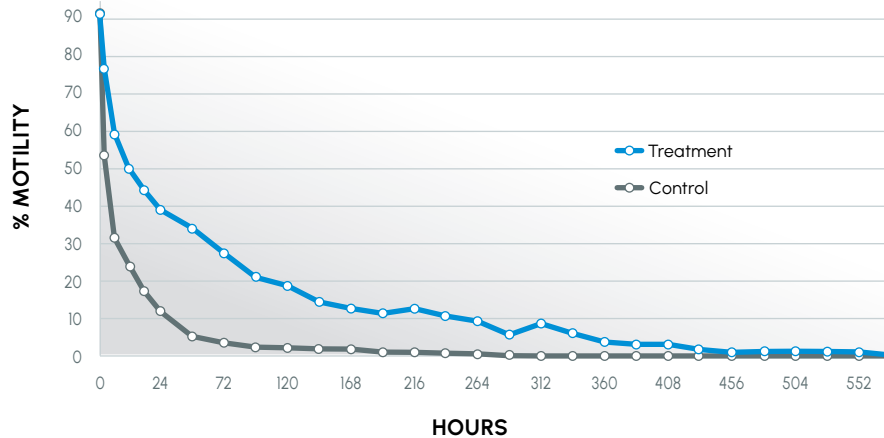
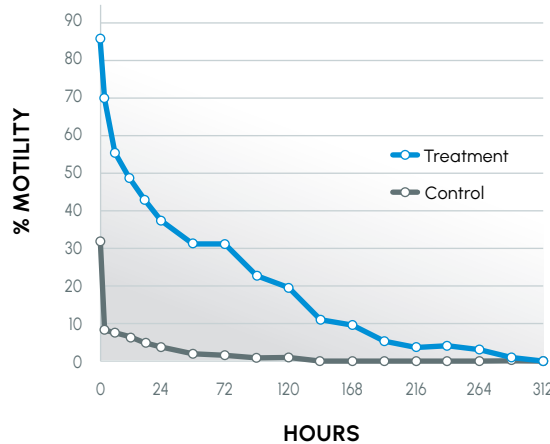


FIGURE 4
MOTILITY OVER TIME INTOLERANT CANINES

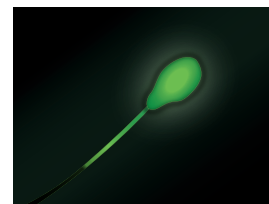


INSIGHTS

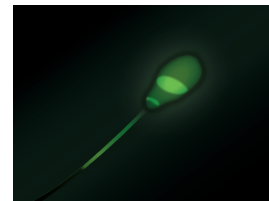
Method Tolerance and Acrosomes

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