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Retention of Liquid Within Insemination Equipment Using Various Equine Frozen Semen Insemination Methods and Two Semen Freezing Extenders

John J. Dascanio, VMD, Diplomate ACT, ABVP,[a] Ramanathan Kasimanickam, BVSc, DVSc, Diplomate ACT,[a] Stephanie Milburn, BS,[a] Andrea Canerossi, BS,[b] and Rebecca Splan, PhD[b]

ABSTRACT

The objective of this study was to examine the effect of different insemination techniques and extenders on the volume of liquid dispensed from insemination equipment. The method of insemination has a significant effect on the volume of semen deposited into the mare's uterus when low volumes are used. Insemination pipettes that allow for direct deposit of straw contents into the uterus are preferred. Aspiration of semen into a pipette is preferred over aspiration into a syringe with deposition through a pipette when direct deposit is not possible. Use of a pipette with a smaller lumen and less length of contact with liquid provides better results. Contact of semen with equipment may allow for residual liquid accumulation on the luminal surfaces and a decrease in overall semen dose. Extenders with differing amounts of egg yolk did not influence volume of liquid dispensed.

Key words: *frozen; semen; pipette; insemination; extender*

INTRODUCTION

A number of methods are used to inseminate frozen semen into a mare's uterus, depending on the number of straws, the size of the straws, addition of extender, instructions by the group freezing the semen, and the user's experience. Semen can be deposited by aspirating thawed semen into a warmed pipette to which a syringe is attached with air passed through the pipette to expel the semen into the uterus. Semen can also be aspirated or poured into a warmed syringe; the syringe attached to a warmed pipette for deposition, with air again being used to expel semen from the pipette's lumen. An alternative method allows for direct use of the straw(s), placing the straw within the distal end of the pipette and using a metal stylet to push the polyvinyl chloride (pvc) plug in the straw to expel semen. Single or multiple straws may be deposited with this last method. In addition to these techniques, frozen semen may be mixed with semen extender used for cooled semen shipment before insemination or extender may be dispensed through a pipette to clear the pipette of frozen-thawed semen.

Typical insemination dosages for equine frozen semen are approximately 800 million total spermatozoa.[1] These spermatozoa may be divided into one, two, or four straws each with a volume of 0.5 mL.[1-5] Other less common methods use eight straws with 0.5 mL volume each, a single 2.5-mL straw, a single 4- to 5-mL straw, or larger volume containers.[6,7]

This experiment was performed, using extender only, to determine the volume of liquid that may remain within a straw, within a pipette or within a syringe, thus reducing the overall volume dispensed. The volume of liquid dispensed was then converted into a theoretical sperm dose.

MATERIALS AND METHODS

Spermatozoa were not used in this experiment to avoid stallion and ejaculate effects. This project looked specifically at volume effects and retention of liquid within insemination equipment.

From the [a]Virginia-Maryland Regional College of Veterinary Medicine, Department of Large Animal Clinical Sciences, Center for Reproductive Excellence using Assisted Reproduction and Endocrinology (CREATE); and the [b]Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, VA.
Reprint requests: John J. Dascanio, Virginia-Maryland Regional College of Veterinary Medicine, Department of Large Animal Clinical Sciences, Center for Reproductive Excellence using Assisted Reproduction and Endocrinology (CREATE), Duck Pond Drive, Virginia Tech, Blacksburg, VA 24061.
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Two different commercially available equine semen freezing extenders were used. The first extender required 3.2 mL egg yolk and 4 mL glycerol per 100 mL distilled water (VP-EY equine freezing buffer with antibiotics, IMV International, Maple Grove, MN). The second extender required 7.5 mL egg yolk and 3.8 mL glycerol per 100 mL extender (Next Generation Universal Formula, Exodus Breeders Corp, York, PA). Extenders were mixed as per manufacturer's instructions. No spermatozoa were added to either extender.

An extender volume of 0.5 mL was aliquotted into separate micro centrifuge tubes using a calibrated single-channel manual pipette (Eppendorf Reference Series 100-1000 μL pipette, Eppendorf North America, Westbury, NY). (Note: The term "pipette" is used for both infusion pipettes and measuring pipettes.) A new pipette tip and micro centrifuge tube was used for each sample. Each straw was filled by applying manual vacuum using a 3-mL syringe with a flexible plastic teat cannula (J-12 teat infusion cannula, Jorgensen Laboratories, Inc., Loveland, CO) attached until the polyvinyl chloride (pvc) sealing powder became visibly wet. The entire contents of a micro centrifuge tube (0.5 mL) was aspirated into each straw. Each straw was then sealed using a stainless steel ball. All straws were frozen over liquid nitrogen vapor and then plunged into liquid nitrogen. Straws were stored in a conventional semen storage tank until used for the experiment. Straws were thawed in a 37°C water bath for 30 seconds. All disposable equipment was kept in a 37°C incubator.

Three different straw numbers: one straw, two straws, four straws; and four different pipettes: IMV (IMV Flexible Equine Catheter, IMV International), MiniTube (Universal Pipette, MiniTube of American, Verona, WI), MiniTube with inner catheter, In-Fu-Zee (In-Fu-Zee pipette, Barber Veterinary Supply, Richmond, VA) were used with three different insemination techniques. All procedures were performed in triplicate. A total of 144 measurements were assessed and 336 straws used.

Technique 1 (IMV, MiniTube, In-Fu-Zee pipettes)

Thawed extender was placed into a new micro centrifuge tube. Extender was then aspirated into each infusion pipette with a 0.5-mL air buffer at the end. The volume of each pipette was previously determined, and a volume equal to 2× the volume of the pipette was used to expel air through the pipette to clear the extender and any residual extender remaining in the pipette. The extender was dispensed into a new micro centrifuge tube each time. Measurement of the dispensed volume was done with a calibrated manual pipette by initially dialing the pipette to zero, placing the pipette tip into liquid, and then dialing upward on the control setting until all liquid was aspirated. Direct reading of the pipette was then taken to record the recovered volume.

Technique 2 (All Four Infusion Pipettes)

Thawed extender was placed into a new micro centrifuge tube. Extender was aspirated directly into a 12-mL syringe. The syringe was attached to the pipette and liquid expelled through the pipette into a new micro centrifuge tube. Enough air was then aspirated into the syringe to equal 2× the volume of the pipette used and was passed through the pipette. The liquid within the micro centrifuge tube was measured as described in Technique 1.

Technique 3 (Only MiniTube Pipette With Metal Stylet)

A thawed straw was placed into the pipette and advanced forward by use of an inner metal stylet (A.I. gun, MiniTube of America, Verona, WI). The straw was seated at the end of the pipette using the stylet and then the stylet pushed forward to expel the extender into a new micro centrifuge tube. Withdrawal of the stylet removed the dispensed straw. Additional straws were dispensed in the same way. The liquid within the micro centrifuge tube was measured as described in Technique 1.

Extender was also dispensed directly from one, two, or four straws into a micro centrifuge tube without the use of a pipette to measure how much liquid was retained within a straw(s).

The diameter and length of each pipette was measured and values calculated for curved inner surface area and volume in milliliters. The formula for inner surface area was =3.1415*diameter*length. The formula for volume was =3.1415*radius*radius*length.

The GLM procedure of the SAS System (SAS version 9.12, SAS Institute Inc., Cary, NC) was used to test for the effects of type of technique, straw number, type of pipette, and two-way and three-way interaction. Tukey's method was used to compare the least square means for equality. Results were considered significant if $P \leq .05$.

RESULTS

There were no statistically significant differences between the two semen extenders tested, so the data were merged for further analysis.