The effects of handling and thawing bovine semen on viability post-thaw

J. D. Akers, * C. D. Parker, and B.M. Terry *[2]

*Cisco College, Agriculture Department, 717 E. Industrial Blvd, Abilene, TX, 79602

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[2] Brandi Terry, M.S.: brandi.terry@cisco.edu

Abstract: In the past fifty years, the process and technology of artificial insemination has improved tremendously; as well as conception rates. The technique is intensely used in the dairy industry but few producers use AI (7.6%) in beef production. Artificial insemination is heavily preferred due to the prospect of increasing performance of the herd by selecting superior sires, as well as decreasing the transmission of sexually transmitted diseases. Not only has the type of straw changed, glass ampule to polyvinyl chloride (PVC) French straw, but also the size of straw has transformed from 0.25 cc straw to 0.50 cc straw for faster insemination. Mistakes can occur from handling issues due to human error, thawing procedure errors, and mistakes in AI technique. In addition to handling errors, different thawing protocols (Table 1) with similar packaging exist depending upon the company where the semen was purchased. This can lead to additional mistakes during thawing. The success of AI is dependent upon semen viability pre and post-thaw. Further research indicates a need for less variation in thawing protocols and an increase in education with a goal of decreasing mishandling and improving AI technique. The objective of this study is to determine the effects of handling and thawing bovine semen on viability post-thaw.

Key words

Artificial insemination, Handling semen, semen, thawing

INTRODUCTION

Artificial insemination (AI) is the process of manually depositing sperm that has been collected from a genetically superior donor sire into the reproductive tract of a recipient female. The technique is intensely used in the dairy industry but few producers use AI (7.6%) in beef production (USDA, 2009). Artificial insemination is heavily preferred due to the prospect of increasing performance of the herd by selecting superior sires, as well as decreasing the transmission of sexually transmitted diseases (Hagevoort and Garcia, 2013). The industry standard for semen concentration is 20 million sperm per straw but Champion Genetics recommends 40 million sperm per straw for higher conception rates. Concentration is important due to the correlation between higher concentration and increased fertility rate (Nebel and Marshall, 2017). However, it is not promised that the fertility level will rise, or that the producer will get a positive pregnancy (Nebel and Marshall, 2017). Increased semen concentration does not mean guaranteed higher pregnancy rates (Nebel and Marshall, 2017). Although AI requires attention to detail in all areas of cattle management to be successful, a common error in the AI protocol is the mishandling of bovine semen post-thaw. The mishandling of bovine semen post-thaw affects motility and morphology of the semen. The goal of every semen collection is to aim for the highest quantity and best quality sperm cells. Checking the quality of semen used in an AI is crucial to the success of the program. During semen collection quality is checked for viability as well as diseases that could be transmitted to the herd. Handling the semen can also affect the viability of the cells. Sperm can die from exposure to light, coming into contact with water, rapid temperature changes (Rouge, 2002). Viability is determining the number of cells alive in a given sample. Motility is the percent of how many cells move in a linear fashion. When straws contain dead sperm it is an expensive loss of product and money. Further research indicates a need for

less variation in thawing protocols and an increase in education with a goal of decreasing mishandling and improving AI technique. The objective of this study is to determine the effects of handling and thawing bovine semen on viability post-thaw.

MATERIALS AND METHODS

Straw retrieval

The frozen semen straws are stored in a Worthington AI24 liquid nitrogen semen tank which is kept at a temperature of -320 degrees Fahrenheit (Select Sires, 2019). To remove the straws from the semen tank, use your hand to raise the canister up to just below the neck of the semen tank. When the canister is locked in place carefully, lift one goblet, with your free hand take the cane tab and flip it up. Take the plastic straw tweezers and pull a semen straw out of the goblet as you simultaneously place the goblet back in the canister. Then flick the straw with a quick but careful motion to remove any excess liquid nitrogen from the outside of the straw. Place the semen straw in the Walther 2L Laboratory water bath for 40 seconds at 98 degrees Fahrenheit. A droplet of thawed semen is placed on a clean slide with a coverslip (n = 40). Samples are placed slide warmer set at 98 degrees Fahrenheit until microscopic observation.

Assessing the Effects of Mishandling

The mishandling of frozen semen straws can harm or kill the sperm cells within the semen. Therefore, it is important to handle frozen semen straws carefully and properly. The effects of mishandling are assessed by thawing a semen straw in a cold water bath at 78 degrees Fahrenheit for 40 seconds, placing an AI gun in the refrigerator for 5 minutes prior to loading with the semen straw, and lastly adding a droplet of H2O to the sample. Thawing a straw of

frozen bovine semen at 90-95 degrees Fahrenheit in a water bath for 40 seconds serves as the control.

Observing the Motility and Viability of Semen

Samples are placed slide warmer set at 98 degrees Fahrenheit until microscopic observation. An i4 semen microscope is used to assess semen viability post-thaw. The number of sperm per AI straw, semen concentration, is of great importance to a producers breeding program. Motility is used to see the swimming pattern of the cells. The sperm should swim forward. If the sperm aren't moving in a linear fashion, this could indicate problems with the head shape and tail formation. Motility can be measured using a microscope and divided up into three categories ("progressively motile," "non-progressively motile," or "non" motile) (Chapman and Rood, 2016). Each category is determined on sperm movement. Sperm should always be swimming so when they are inserted in the cow/heifer they would move through the uterus into the uterine horns and then to the ovaries to impregnate the cow. Progressively motile sperm move in a straight line, while non-progressively motile sperm might move but instead they move in a circle (Chapman and Rood, 2016). Therefore, non-progressively motile sperm do not make the needed forward progress. (Chapman and Rood, 2016) Lastly, the category of non-motile consists of sperm that do not move at all and may even be dead. (Chapman and Rood, 2016)

RESULTS

Four methods were tested (n=40) and post-thaw motility was evaluated and classified as P (Progressively motile), PS (Progressively motile slow) or NM (Non Motile or dead). Method 1 (n=10) resulted in 100% progressively motile (Figure 1). Method 2 (n=10) resulted in 15 %

progressively motile or progressively motile slow and 85 % non-motile (Figure 1). Method 3 (n=10) resulted in 10% progressively motile, 25% progressively motile, progressively motile-slow, 25% non-motile, and 20% progressively motile slow, 20% non-motile (Figure 1). Method 4 (n=10) resulted in 90% progressively motile-slow, non-motile and 10% progressively motile, non-motile (Figure 1). Semen thawed using the recommendations from ABS Global (ABS, 2011) resulted in a higher percentage of progressively motile semen (100%) when compared to cold thaw (15%), cold AI gun (55%), and the addition of water to the sample (10%). Overall, Method 1 yielded the highest percent of progressively motile semen (Figure 1), but both Method 1 and Method 3 met the normal recommendations of greater than 30% of progressively motile sperm (Rouge, 2003).

DISCUSSION

The study compared four methods; control, cold thaw, cold AI gun, and the addition of water to the sample. Semen was thawed at 98°F water bath for 40 seconds following recommendations from ABS Global (ABS, 2011). The current recommendation from ABS Global resulted in progressively motile semen for all samples (Figure 1). Semen was thawed at 78°F water bath for 40 seconds for cold thaw. This resulted in samples that were progressively motile (n=1), progressively motile-slow or non-motile (n=1), and non-motile (n=8). Semen was thawed and then put in a cold AI gun that cooled in the refrigerator for 5 minutes prior to loading. This resulted in samples that were progressively motile (n=1), progressively motile or non-motile (n=4), and progressively motile, progressively motile-slow, or non-motile (n=5). Semen was thawed and then 5 droplets of water were added to the sample. The sample was then mixed together using a vortex mixer. This resulted in samples that were progressively motile or non-motile (n=1) and progressively motile-slow or non-motile (n=9). The results of the study

show the importance of keeping all materials warm, clean, and free of water when processing, thawing, or loading the AI gun. Method 1 resulted in the highest rate of progressively motile semen compared to the cold thaw which had the highest death rate. The cold AI gun method and the addition of water to the sample resulted in lower motility. Although, all samples exhibited some percentage of non-motile sperm. The comparison of these methods exemplifies the importance of handling and thawing of bovine semen following recommended guidelines to protect the viability of the semen post-thaw.

DISCLOSURES

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TABLES AND FIGURES

Table 1: Genetics company protocols

Company	Protocol	Location
ABS Global	98 degree water for 30 seconds but less than 15 minutes	Global
Bovine Elite	90-95 degree water for minimum 40 seconds	College Station, TX
National Association of Animals Breeders	90-95 degree water bath for minimum 40 seconds	Global
Select Sires	95 degree water for at least 45 seconds	Plain City, OH
Semex Genetics	95 degree water minimum 45 seconds	Madison, WI
Total Livestock Genetics	98 degree water bath for 30 seconds	Australia
WestGen	98 degree water bath for less than 1 minutes	British Columbia

Figure 1: Experimental Design

Method	Procedure	Motility (n=40)
METHOD 1 - Control	Thaw 98 F for 40 sec	100% *P
METHOD 2 - Cold Thaw	Thaw 78 F for 40 sec	(n=10) 15% PS 85% NM
METHOD 3 - Cold AI gun	Place AI Gun in fridge for 5 min prior to loading	(n=10) 10% P 25% P, PS;25%NM 20% PS; 20% NM
METHOD 4 - Droplet of H20	Add a droplet of water to the sample	(n=10) 90% PS, NM 10% P, NM

*P = progressively motile; PS = progressively motile slow; NM = non motile