Effects of viability and motility on cold shocked bovine spermatozoa held at different temperatures

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Abstract: Artificial insemination is a common technique used worldwide to impregnate cattle. It is widely used within the dairy cattle industry to help produce heifers for milking purposes. While there have been many advances in AI technique, more research is needed for improvement. Based on current studies regarding AI, there is a lack of information regarding the effects of viability and motility on cold shocked bovine spermatozoa. In addition, limited information is available on the effects of cold shock bovine spermatozoa post thaw. Based on the lack of information, research is needed to determine the effects of cold shock on bovine spermatozoa during the thawing process. The objective of this study is to determine how cold shocked bovine spermatozoa will affect the viability and motility of sperm after post thaw.

Keywords

Viability, Mobility, Motility, Cold Shock, Bovine Spermatozoa, Artificial Insemination

INTRODUCTION

Technological advancements such as the ability to freeze semen has led to advantages in artificial insemination (AI) including the indefinite storage of sperm with easier transport, greater choice of sires, and elite genetic merit (Moore, Halser, 2017). Frozen semen is often mishandled post thaw prior to the animal being inseminated which leads to damaged sperm (Nur, Ileri, Ak, 2006). This is commonly known as cold shock. Cold shock is a byproduct of mishandling. Cold shock occurs when too much time is taken to get the frozen semen straw from the tank and to the thaw bath or from the thaw bath to the insemination gun. Cold shock is the permanent damage to sperm due to a decrease in post thaw temperature of the semen (Nur, Ileri, Ak, 2006). Straws suffering from cold shock is a crucial reason for a loss of viability and motility in the sperm (Blackshaw, A. W.,1957). With the introduction of frozen semen, the evaluation of post thaw survival became important (Foote, 2010). Initially protecting semen from cold shock leads to a better chance of fertility (Foote, 2010). A faster thawing process can prevent cold shock; which would eventually become a permanent injury resulting in lower conception rates (Walton,et al,1957). Cold shock affecting spermatozoa was first discovered in the U.S.S.R. when artificial-insemination was being developed in cold climates (Walton,et al,1957). The objective of the study is to determine if cold shocked bovine spermatozoa decreases viability and motility of sperm post thaw.

MATERIALS AND METHODS

Straw retrieval

One way of storing semen straws is in liquid nitrogen tanks at -320F to keep the semen from thawing or dying (Webb, 1992). First the straws will have to be removed from the

Worthington AI24 semen tank which is stored with liquid nitrogen and ½ milliliter conventional cattle semen straws. Open the tank and pull the canister handle up to the bottom of the neck . Take the plastic straw tweezers, pick a goblet and pull back the white cane tab pulling out a straw. Pick the straw straight up until it reaches the desired temperature and hold it for the amount of time needed. Then, flick the straw and place it in the Walther 2L Laboratory water bath to thaw (ABS, 2011). Repeat for samples collected from the middle of the neck and top of the neck in the liquid nitrogen tank.

Thawing

The semen straws need to be thawed at 98 degrees Fahrenheit (ABS, 2011). When transferring the straws from the tank to the thaw water, the thawing of frozen semen should be at maximum speed (Diskin, 2018). If kept exposed to air it will damage the sperm causing additional issues to the viability. After placing the straw in the thaw water, take the canister handle back down and close the tank so the temperature can be preserved. During the thawing time, take a paper towel and blow on it to keep it warm (ABS, 2011). Next, place a clean slide and coverslip on the slide warmer. After the semen is thawed, take it out of the thaw bath and dry the straw with the warm paper towel (Diskin, 2018). Cut the tip of the straw and place semen in a small holding tube. Use a dropper to place a drop of semen on the slide.

Assessing viability and motility of sperm

More accurate results can come from a well prepared slide on a warm stage using a good microscope (Foote, 1982). Therefore once each slide has semen on it and is covered by a coverslip, place it on the C&A Scientific Step-Up Slide Warmer 23 Slides 110 Volt: Medical Device until it is ready to be viewed under the microscope. This will help to keep the sperm on the slides at 98 degrees Fahrenheit and ensure that minimal damage to the sperm. Place the

specimen on the i4 12xDC Semen Evaluation Microscope, which is used for the viewing of live specimens and assess the viability and motility based on the movement and the amount of the live sperm. View the motility and viability of the semen through the mounted BioVID HD Camera for i4 Microscope w/ 11.6" 1080p color LCD screen for live presentation of the slide. It is said that sperm must be alive in order to be fertile and must be moving in order to be motile (Foote, 1982). Grade the motility based on how the sperm is moving using the motility rating. Classify the sperm movement as either P (Progressively motile), PS (Progressively motile slow) or NM (Nonmotile or dead). View the sperm closely and while observing them come up with percentages of the motile sperm for each grade. The normal recommendation is that bulls have greater than 30% of progressively motile sperm (Rouge, 2003). Record the data after each sequence.

RESULTS

Three methods were tested (n=90) 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=30) resulted in 80 % progressively motile or progressively motile slow and 20 % non-motile (Figure 1). Semen held in the middle of the tank resulted in higher motility (87%) than below the neck of the tank (80%) or top of the liquid nitrogen tank (57%) (Figure 1). Method 2 (n=30) resulted in 87% progressively motile or progressively motile slow and 13% non-motile (Figure 1). Method 3 (n=30) resulted in 57% progressively motile or progressively motile slow and 43% non-motile (Figure 1). The middle of the tank resulted in a higher motility compared to the other methods which could be due to decreased retrieval time when compared to samples from below the neck of the tank. The top of the neck is much warmer (+36 F to +54 F) than the tank and the middle of the neck (-108 F to -116 F), so when you pull them up the semen thaws too quickly.

Overall, Method 1 and Method 2 yielded the highest percent of progressively motile semen (Figure 1), but all methods exceeded normal recommendations of greater than 30% of progressively motile sperm (Rouge, 2003).

DISCUSSION

Cold shock in semen causes the semen to be damaged affecting the motility and viability post thaw (Nur, Ileri, Ak, 2006). Semen does best when thawed at a temperature of 98 F for 5, 10, or 15 seconds (Figure 1). The highest motility scores were seen at 5, 10, and 15 seconds when samples were held at the base of the tank compared to all samples collected (n=90) (Figure 1). Thus, the faster the straw retrieval process the less susceptible the semen from the straw is to cold shock (Walton,et al,1957). External factors could influence results such as variability of sample processing time, which is subject to human error, and variability in the temperature of the liquid nitrogen tank after each sample was collected. Given in the study the tank was closed after each straw retrieval in order to help ensure that the temperature would remain the same, but this is unknown due to lack of temperature measurements collected in this study. Faster thawing times from the liquid nitrogen tank to the thaw bath resulted in improved motility in sperm (Figure 1). Based on the results from this study, cold shock does affect the viability and motility of bovine semen post thaw (Figure 1)). Further research is needed to determine the extent of the effects of cold shock on bovine semen post thaw.

DISCLOSURES

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FIGURES

Figure 1: Experimental Design

Method	Thaw Procedure	Motility (n=90)
Below neck of the tank (-320F)	Thaw 98F for 40 sec	(n=30) 80%(*P,PS) 20%(*NM)
Middle of neck in tank (-108F to -116F)	Thaw 98F for 40 sec	(n=30) 87%(P,PS) 13%(NM)
Top of neck in tank (+36F to + 54F)	Thaw 98F for 40 sec	(n=30) 57%(P,PS) 43% (NM)

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