A COMPARISON OF SEMEN THAWING FOR ARTIFICIAL INSEMINATION IN CATTLE

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ABSTRACT

Beef cows (n=23) were used to compared conception rates of two different thawing methods used for artificial insemination (AI). Cows were divided into the treatment or control group based on time of estrus. In the treatment, frozen semen was placed directly into the AI gun and inserted into the female for deposition after a minimum of 30 seconds. The control females experienced the typical thawing process of semen straws placed in a water bath for a minimum of 30 seconds, then loaded into the AI gun and deposited into the uterus of the female. Conception rates were similar between semen thawing methods ($P > 0.51$). Additionally, the study analyzed semen motility using the two different thaw methods and found no difference ($P = 1.0$). In conclusion, thawing semen directly in the reproductive tract of the female provides the same conception rates as traditional thaw methods.

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INTRODUCTION

Artificial Insemination (AI) is one of the more useful technologies regarding reproduction in livestock species, and researchers continue to improve the efficiency and productivity of the techniques used in AI. Artificial insemination has been a well-established, practical procedure in livestock since the early 1900s. Through the years, protocols for AI have become standardized across the world. Because AI plays such a critical role in the reproduction and genetic improvement of farm animals, it is essential that studies continue to improve methods commonly used.

There has been significant research conducted on temperature variances and their effects on thawed semen. These studies have concluded that temperature variances can cause significant mortality rates of spermatozoa. Thawed semen may be exposed to temperature variances in transfer from the thaw bath to the female, thus alternative procedures to reduce this variation could increase the conception rates in cattle.

Additionally, time is of the essence when synchronization protocols have large numbers of females coming into estrus within a small window, and decreasing the amount of time for technicians to complete an AI would increase the efficiency of the protocol. Some facilities have limited space and electricity available for technicians to use as well.

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Though there is a great need to increase the efficiency of such procedures by decreasing the amount of time required to perform a successful AI per cow and decreasing exposure to temperature variances, little research has been performed to analyze an alternative AI procedure. Therefore, this study will compare conception rates of the standard AI protocol with a new protocol which calls for frozen semen straws to be placed directly into the AI gun and inserted directly into the cervix of the female for thawing. This study will also look at sperm motility rates for each thawing method. Information from this study will assist in determining alternative AI protocols to increase the efficiency of AI.

OBJECTIVE

To determine if thawing semen in the female's reproductive tract has a similar effect on conception rates when compared to traditional thawing procedures.

LITERATURE REVIEW

Artificial Insemination History

Spallanzini was the first scientist to report a successful artificial insemination, which was practiced on a dog, and the female whelped three puppies 62 days after the insemination (Foote, 2001). Heape (1897) and other scientists in the eastern hemisphere began reporting successful inseminations 100 years later on horses, rabbits and dogs. Russian scientist Ivanhoff (1907) truly saw the potential that AI could have on the livestock industries, and in the early 1800s he began working to make it a practical and common procedure. The United States did not report on AI until the 1930s when Cornell University begin practicing on dairy cattle. They felt that dairy cattle would most positively benefit from AI (Foote, 2001). Though AI is a common practice today, conception rates are typically lower than natural breeding, consequently, scientists continually work to improve those conception rates.

Utilizing estrous synchronization while using AI conserves labor and increases economic returns (DeJarnette, 2004). Beef cattle typically have an 18 to 24 day estrous cycle. Standing heat is a time in the reproductive cycles when cows are considered to be most receptive and will allow other cattle to mount for a few seconds, numerous times throughout estrus. Beef cattle may be in standing heat for just a few hours to over 30 hours. Once a cow is in a state of standing heat, she should be inseminated approximately 12 hours later, but there is typically a 10 to 16 hour window. This is commonly referred to as the AM-PM rule (Sorenson, 1979). If a cow is in standing heat in the morning, she should be inseminated that evening (Sorenson, 1979).

There are several practices used to aide in determining when a cow is in standing heat (Sorensen, 1979). There are heat patches, which may be attached atop the female's tail head and when she is mounted, the patch containing red ink will burst indicating she is in standing heat. Another possible practice is to make a scheduled chart based off the previously observed cycle of the female. This method is typically more time consuming considering that a previous cycle must be monitored and another full cycle must occur. Some scientists have found placing androgenized females or steers with females will cause them to show more signs of standing heat (Sorensen, 1979). One common practice is using electronic means to determine standing heat. In using electronic means, a Heat Watch® transmitter is placed in a pouch with the button up. The pouch is glued to the anterior side of the female's tail head. The transmitter numbers and female identification numbers are entered into a computer program. Once a female is mounted, the computer will receive the signal and will record the time and duration of the mount. This program considers a female in standing heat once she has been mounted at least 3 times for at least 3 seconds. Another common method of heat determination is smearing soft chalk on the tail head of the cow. When a cow is mounted some of the chalk will be removed thus indicating she is in standing heat.

AI conception rates are commonly between 50% and 60% (Sprott, 2007). Conception rates can vary based on semen quality, temperatures and other environmental conditions. The health and productivity status of females also plays a role in conception rates.

Estrus Synchronization

Scientists have discovered ways to manipulate the estrous cycle of females to provide smaller windows of time for which the females are in estrus. Narrowing estrus time within a herd can be extremely helpful when using artificial insemination (DeJarnette, 2004). Additionally, even if AI is not utilized it provides producers with a smaller calving window and a more uniform calf crop in terms of size and age.

Synchronization protocols vary greatly in terms of costs, duration and labor. There is a large variety in number of shots to administer, types of shots and facilities needed to complete this process. One of the most common hormones used is prostaglandins (PGF), which are naturally occurring in the estrous cycle of the female. Prostaglandin, when released from the uterus, triggers the female to returning estrus (DeJarnette, 2004). Prostaglandin can be used in numerous different methods, most commonly a 1 shot PGF, 2 shot PGF or in conjunction with another hormone.

There are some limitations to using PGF as a synchronization protocol. Prostaglandin is only effective on females that posses a corpus luteum (CL). Prostaglandin will not be effective on females within a 6 to 7 days of a previous heat, post partum anestrous cows and prepubertal heifers (DeJarnette, 2004).

Thawing Rates of Semen

The thaw rates of the semen used for artificial insemination and the climates the semen is exposed to after thaw continue to be a challenge for AI technicians (Senger, 1976). Semen is extremely sensitive to temperature shock, which can negatively affect the percent motility and percentage of spermatozoa with intact acrosomes (Senger, 1976). According to a study by Brown et al. (1982), spermatozoa thawed at 35 C for 12 seconds and held at 0 C before being warmed to 40 C and it sustained immediate acrosomal damage due to delayed time before complete thaw. Additionally, spermatozoa thawed at 35 C for 1 m, held at 0 C, then warmed to 40 C experienced immediate acrosomal damage as well because of cold shock. Brown et al. (1982) found that the cold shock caused more damage than the delay in thawing.

According to Gilbert (1978), thawing semen at bath temperatures of 65 C or 95 C acrosomal retention and percent motility were greater than when it was thawed at 35 C. This study found that it is necessary to obtain maximum spermatozoa after freezing in straws, rapid thawing was necessary. Additionally, the amount of glycerol in the straw determines just how rapidly a straw may be thawed.

MATERIALS AND METHODS

This study will use 23 non-lactating, open crossbred beef cows. There will be one control group and one treatment group. The cows will be randomly divided into one of the two groups. Randomization will be based on when they come into estrus. The first cow exhibiting estrus will be artificially inseminated using the new protocol and the second will be artificially inseminated using the standard method. This procedure will continue until all cows have been artificially inseminated. Semen from the same bull, collected and frozen at the same time will be used throughout the study.

On Day 1, all females were brought to the pen and taken through the chute. A transmitter number was assigned to the females as they came through the chute. The transmitter number and the female's identification numbers were recorded. Transmitters were placed in a pouch with the button side up and sealed for protection. The patches were glued to the center of the hip of the female. The placement of the patch is very important. The transmitter should run from her head to tail and be placed on the anterior of the female's tail head and centered on her pelvis. Once the patch was securely fastened on each female, they were released back into the pasture. The transmitter numbers and female identification numbers were entered into the Heat Watch® software.

Females were monitored for seven days for natural heat. Any cows that did not come into estrus by Day 7 were administered 5 mL of Lutalyse® intramuscularly. Cows were again monitored over the next five days.

The software was checked every morning and every evening in at least 12 hour increments. Twelve hours after a female was considered in standing heat, she was penned. Females were assigned to either the control or treatment according to the order by which they entered the chute. The first female was assigned to the treatment, the second female to control and continued in this alternating manner. When a treatment cow was ready to be artificially inseminated, she was placed in the chute. The technician removed the frozen semen from the semen tank and placed it directly in the AI rod. The sealed tip was cut from the straw, then a sheath was placed over the rod. Once the rod was completely prepared, the technician placed it directly into the reproductive tract of the female. Once the rod was inserted completely through the cervix of the female, the procedure lasted a minimum of 30 seconds, the semen was expelled from the straw. The gun was then removed and the straw and sheath disposed. While the female was in the chute, her transmitter was removed from the pouch and the female was promptly returned to the pasture.

When a control female was ready to be artificially inseminated, she was placed into the chute. The AI technician recorded the female's identification number. The appropriate semen was taken from the semen tank and placed in a water bath. The water bath is required to be 34.4-36.7 degrees Celsius, and the semen was placed in the bath for a minimum of 30 seconds. Once the straw thawed, it was removed from the bath, dried and placed into the AI gun. The sealed end of the straw was removed and a sheath placed over the AI rod. The technician then placed the rod in a place that is safe from lighting, until ready to place the rod in the female. Once the technician was ready, the rod was placed in the reproductive tract of the female, and as soon as the technician passed the rod through the cervix, the semen was expelled from the straw. After insemination, the rod was removed and the sheath and straw were disposed. While the animal was in the chute, her transmitter was removed from her pouch. This method is described by Sorenson (1979) and commonly used for AI breeding. The female was then promptly returned to the pasture.

All females will be penned and pregnancy status will be determined through palpation at day 45 from the last insemination. Pregnancy status will be noted either bred or open, then females will be promptly returned to the pasture.

Semen from the same bull, collected and frozen at the same time as that used to inseminate the cows was used to evaluate semen motility to better evaluate the method. Two cows that were not part of the study were placed in a chute. Semen was them handled exactly as the insemination semen, but rather than being deposited in the cows reproductive tract, a drop was placed on a microscope slide and viewed at 100 times magnification. Semen was rated for motility on a scale of 1-5. A score of 1 indicates no movement of sperm cells and a score of 5 indicates a rapid swirl of sperm cells (Settle, 2008). This procedure was repeated for a total of three straws per method.

STATISTICAL ANALYSIS

The individual cow was considered the experimental unit. Single point data, including pregnancy status, was analyzed using the CATMOD Procedure of SAS (SAS Inst. Inc., Cary, NC, 1989). Chi square was used to analyze treatment differences considered significant at ($P \leq 0.05$).

RESULTS AND DISCUSSION

All of the open cows (n=23) were entered into the HeatWatch System and monitored for estrus. One of the cows never exhibited any signs of estrus and a second female exhibited physical signs of estrus but never registered in estrus through the Heat Watch System, thus both were eliminated from the trial. The females used in this project were purchased from a local sale barn, therefore there was no background history on any of the females in the trial, including reproductive history. The remainder of the cows (n=21) were registered in heat through the Heat Watch System and inseminated 8-16 hours after the program found them in estrus. Pregnancy status was determined 45 days after the last insemination by palpation.

Table 1 shows results from the data of inseminated females (n=21). The cows were categorized by treatment to compare the number of animals per treatment and the percent conception from AI. There was no difference in conception rates among cows *($P > .51$)*. The expected outcome of this study was that there would be no difference in the two thawing techniques, as was confirmed by this study. The overall conception rates of this study were lower than the average reported, most likely due to a lack of reproductive history on the females, body condition, weather, age and other environmental conditions. However, both treatments experienced the same environment and this should not have impacted the results.

Table 1. Number of cows, number of cows that conceived, and percentage of cows that conceived by treatment.

Treatment[a]	1	2
n	11	10
No. Conceived	5	6
% Conceived	45.5	60.0

[a]Treatment 1 = no thaw bath method, treatment 2 = thaw bath method. No differences between treatments (P=0.51).

In analyzing semen motility percentages of frozen samples, there was no difference *(P = 1)* in treatment. Both thaw methods experienced thawed motility rates of 2. Though this is considered very low, with both treatments experiencing the same low rate there is proof of no difference in thaw techniques.

Following semen evaluation or motility, it was concluded that motility was not different between methods (P = 1.0) since all evaluations resulted in motility scores of 2. This is considerably lower than that of good quality fresh semen (Turner, 2009). However, it is within the range of frozen and extended semen (Senger, 1997).

Due to the limited number of females in this trial as well as limited history on females used, more research needs to be performed with a greater number and some knowledge of reproductive history. During extremely cold weather conditions it becomes difficult to protect thawed semen from experiencing temperature shifts and possibly caused sperm cell death. Unfortunately, during this experiment, the ambient temperatures ranged from 84 degree Fahrenheit to 95 degrees Fahrenheit during the days and never dropped below 64 degrees Fahrenheit in the night. These temperatures are far from causing cold shock to semen and causing sperm cell death. Therefore, it would be beneficial to perform research such as this in different climates and seasons in which environmental conditions could provide a more negative effect on bath thawed semen. Additionally, placing females that show no sign of estrus with a bull to determine if they are cycling would be beneficial in determining their reproductive status. Females that do not breed with a bull could be removed from the project because they are likely anestrous.

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VITA

Brittni LaNay Kaczyk was born in Abilene, Texas but raised in Coleman, Texas where she graduated from Coleman High School in 2003. She received a B.S. in Animal Science from Texas A&M University in December of 2006, and worked in different positions on campus including the Sheep and Goat Center and for Dr. Shawn Ramsey. After graduation, she took an internship position for the San Antonio Livestock Show and Rodeo and then found employment as Livestock Director with the San Angelo Stock Show and Rodeo. She decided to continue her education at Angelo State University after a few years while still being employed by the San Angelo Stock Show and Rodeo.

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