# CONCEPTION RATE DIFFERENCES IN SEXED VS. NON-SEXED SEMEN

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# Abstract

The objective of this study was to determine if sexed semen has similar conception rates as non-sexed semen. Eighty-four Angus females from the Angelo State University's Management, Instruction and Research Center were synchronized at the beginning of this study; however, only 54 showed signs of estrus and were artificially inseminated (10 Heifers and 44 multiparous cows). The 54 females were split randomly into three different treatment groups. Heifers and cows in treatment one were inseminated with Y-bearing sexed semen. Females in treatment two were inseminated with X-bearing sexed semen; while the last treatment was our control which received non-sexed semen. Results in heifers and cows were similar, and no differences were noted among the three treatment groups ($P>0.5$). Differences might be noted with a larger number of females. Date of parturition was estimated using ultrasound, and is a viable management tool because it is possible to predict, on average, within 9 days of parturition with a 36 day maximum and an exact prediction for the minimum.

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## Introduction

Artificial insemination is beneficial to many cattle producers because it allows for an increase of economic returns due to improved efficiency of genetic improvement. With artificial insemination, producers are able to shorten calving seasons, creating a more consistent, uniform calf crop, which increases monetary value when sold as a group. Through artificial insemination, producers have the capability of using sires of superior genetic merit without the liability of a bull on the premises, and rapidly improving traits with the elimination of natural mating. Without natural mating, a significant decrease of sexually transmitted diseases occurs (Foote, 2002).

Artificial insemination technologies have significantly impacted the dairy industry in a positive manner. Modern technologies now allow producers to hand select not only the sire of their herd, but also the sex of the offspring before a female is ever inseminated. Considering the cantankerous temperament of dairy bulls, producers are often placed in dangerous situations, and dairies potentially benefit from the elimination of bulls used for natural mating by implementing artificial insemination practices into their reproduction management. Now that sexed semen is also an available option, it has become widespread in the dairy industry and grown in popularity because producers can eliminate the hassle of reproducing and dealing with bull calves on the facilities and focus solely on producing and improving milking females.

Even though artificial insemination with non-sexed or sexed semen is not as

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frequently mentioned in the beef cattle industry, it has grown in popularity as well. With artificial insemination, beef cattle producers are able to increase economic returns due to improved production efficiency. Calving seasons will be shorter, creating a more uniform calf crop, and without natural mating the risk of sexually transmitted diseases decreases dramatically; with both, the producer will experience a significant monetary advantage (Foote, 2002). Although not seen as often, sexed semen has its own advantages in the beef industry. Gender selection in the beef industry is most commonly used to aid in dystocia issues in first-calf-females by using X-chromosomes (female) to reduce the calf size. Sex sorted semen can also be beneficial depending on the market the producer is interested in, whether it is bull production for sale, or replacement females for the herd.

However, some producers have experienced a reduction in conception rates when using sex sorted semen rather than conventional non-sexed semen (Zorn, 2006). This study was conducted to examine and determine if sexed semen has similar conception rates to non-sexed semen. Conception rates of X-bearing and Y-bearing sperm was analyzed as well during the research trial.

# Literature Review

## History

Artificial insemination has been around for several centuries; however, it did not become a common practice until the past few decades when technological advancements made the process more readily accessible, cost efficient and more successful.  In 1784, the first successful documented artificial insemination was performed by Spallanzani on a dog, which gave birth to three pups 62 days later (Foote, 2002).  Around a century later, reports showed that artificial insemination had been used across the globe in several studies.  Heape (1897), amongst others, frequently used rabbits, dogs and horses in isolated studies to learn about different insemination methods (Foote, 2002).

In the late 1930s, the first cows were inseminated in the United States, but reproductive processes of the cow and outstanding herd management were not well understood or established, so success was minimal (Pace, 1990).  The next advancement in insemination was the development of frozen semen.  When this process occurred in the 1950s, it gave management owners/operators an opportunity to inseminate their own herd if a management plan was accustomed to artificial insemination techniques already (Pace, 1990). Cattle operations which practiced only natural mating prior to frozen semen, first had to understand the intensive reproductive management planning related to artificial insemination, and secondly, managers had to acquire the skills to successfully inseminate by frozen methods before an operation experienced success.

In the late 1970s management became less labor intensive for cattle operations due to development of synchronization of estrus (Pace, 1990).  With synchronization, herds were able to be inseminated within a week's time frame, rather than watching each individual female over the course of a full estrous cycle (21 days). Intensive manual labor was concentrated into a shorter period of time and was more extensive, but the final result benefits the operation more than daily observations for standing estrus (Pace, 1990).

A major technological advancement that has had a significant impact on the dairy industry is the capability of sexing sperm. In 1999, the first sex-selected calf by artificial insemination was born (Thomas, 2008). Semen is sexed by DNA quantification using flow cytometry instrumentation originally developed at Livermore Laboratories (Pinkel et al, 1985). This process did not become commercially available until 2004, and was only distributed through one company, Sexing Technologies, in four separate locations, Bryan, Texas, Ohio, Wisconsin and Brazil. Copious production for commercial application is still currently unavailable due to the amount of time it takes to sort through billions of sperm per each individual ejaculate. However, the flow cytometers are faster and sorting capabilities are improving, so with more time this process may become more commercially applied to industries other than dairy producers.

**Benefits**

Conventional artificial insemination methods alone are advantageous to many producers because it allows for a shorter calving season, which creates a more consistent, uniform calf crop. Seedstock producers especially appreciate the monetary return that is associated with uniformity. Since a bull is not necessarily required for an operation that utilizes artificial insemination, many sexually transmitted diseases are greatly reduced or eliminated amongst these herds. It is possible to run all cattle together as long as a decent record system is in place and separate breeding pastures can be eliminated with the absence of multiple sires on the facilities (Pace, 1990).

An operation's goals are always those which will optimize production. Strong genetics are the backbone to a successful operation year after year, whether it is the dairy industry, seedstock producers or a registered herd operation. Another advantage with artificial insemination is the capability to use sires of superior genetic merit. With a variety of available semen, producers can hand pick which cow/bull combination would create more superior offspring to improve the existing herd (Pace, 1990).

Artificial insemination with sexed semen has every advantage of conventional non-sexed semen, plus several others. Sexed semen is very beneficial to the dairy industry, because of the high demand for female offspring to serve as replacement females with better milking genetics than the elders. With sexed semen, dairy producers can optimize their herd by increasing their female calf crop from 50%-90% (Zorn, 2006). Sexed semen benefits are not limited only to the dairy industry. Any industry can benefit when selection is based on calving ease. When producers select female semen over male semen to use in virgin heifers, it is likely to decrease dystocia issues because heifer calves typically weigh less than bull calves and are easier to deliver at parturition (De Vries, 2009). Beef producers will prosper with sexed semen from both male and female offspring. An owner that sells registered bulls would prefer to have only enough heifer calves to serve as replacements, and the remaining bull calves to increase monetary value; sexed semen makes this scenario possible.

**Sexing Process**

Since the late 1980s, when the process of sorting semen by gender began, technology has allowed for many advancements of the procedure (Zorn, 2006). Flow cytometry is the complete process for gender sorting sperm; however, it is extremely slow and requires several steps. The process begins when semen is stained with a DNA-binding fluorescent dye (De Vries et al., 2008). Semen is then sent through the flow-cytometer at speeds up to 60 mph, and pressure around 40-60 psi (Zorn, 2006). With cattle, male and female sperm cells are differentiated by their size: the X-chromosomes bear 3.8% more DNA thanY-chromosomes therefore emitting more light (De Vries et al, 2008). The chromosomes are separated by a laser that detects gender by the amount of light emitted, and assigns either a negative charge or positive charge as they pass through the flow cytometer as single droplets of liquid. At the completion of this process there are three groups; one which contains positively charged sperm (which is one gender), another with negatively charged sperm (representing the opposite gender), and the final group is any undefined semen that passed straight through (Zorn, 2006). Flow cytometry separates sperm containing the desired gender with 85-90% accuracy (Garner and Seidel, 2003).

Although sexed semen is advantageous to the producer in many ways, there are still several impediments when solely using this process. One impediment is the decreased conception rates some producers have experienced while using sexed semen that were not present when using conventional non-sexed semen. Reports have shown that sexed semen fertility rates average 75% of non-sexed semen (DeJarnette et al., 2007). A 25% decreased conception rate can dramatically influence whether a producer is willing to pay higher prices for sexed semen and be 90% certain of calf gender, or to use conventional semen and accept the 50/50 bull to heifer ratio rather than the decreased number of total calves. Sexed semen averages 30 dollars more per dose than non-sexed semen, which may not be economically feasible for all producers when conventional semen produces higher conception rates (Fetrow et al, 2007). Expenses are due to the extra handling and processing that sexed semen must undergo in flow cytometry. Additional handling is possibly one reason that producers have seen decreases in conception rate, as well as a decreased amount of sperm frozen per straw for sexed semen due to the lengthy sorting process. Sperm viability is yet another concern presented with sexed semen, also due to additional handling. But here the sorting process is helpful, because all dead or damaged sperm cells are culled out leaving more viable sperm in a lower dosage straw of semen (Thomas, 2008).

# Objective

1. To determine if sexed semen has similar conception rates to unsexed semen in cattle.
2. To determine if X-bearing and Y-bearing sexed semen produce similar conception rates in cattle.
3. To determine if ultrasound imaging is a viable source of predicting parturition dates in cattle.

## Materials and Methods

Eighty-four registered Black Angus females raised at the Angelo State University Management, Instruction and Research Center were assigned to two separate breeding groups for estrous synchronizing and estrus monitoring. The study was broken into two even groups, staggered one week apart, because facility space and equipment will not permit all eighty-four head to be managed simultaneously. All cattle were observed prior to the study for any health problems, and a forty-five day postpartum minimum was required from all mature multiparous females. Any females close to the 45 day postpartum minimum were placed in the second synchronized group, allowing more time for uterine involution prior to insemination. All females, heifers and multiparous alike, selected maintained a body condition score (BCS) of 6-7 coming from a pasture raised management situation (Ensminger, 1992).

Estrous synchronization was managed by the two shot Lutalyse® method (Upjohn Pharmaceuticals, Kalamazoo, MI). All 18 heifers were placed in the first group with 24 randomly assigned multiparous cows, and given 5 cc Lutalyse® by intramuscular injection at day 0. The second group, consisting of 42 multiparous cows, received their first shot of Lutalyse® at day 7. On day 11, the second 5 cc intramuscular injection of Lutalyse® was administered to the first group, transmitters were affixed to their tail heads and estrus monitoring began. Cattle were monitored by the HeatWatch® computer system via the transmitters, as well as visually to detect estrus/standing heat (Cow Chips, LLC, Manalapan, NJ). On day 18, the first group was concluded and transmitters were removed from all females that did not show signs of estrus. Also at this time the second group received a second 5 cc intramuscular injection of Lutalyse®, had transmitters affixed to tail heads and were turned out to begin estrus monitoring in the same manner as the previous group.

Artificial insemination occurred 10-18 hours following the HeatWatch® system's detection of standing heat. The inseminating method used in this trial was that which is described by Mitchell and Doak (2004). The sexed and non-sexed semen used for this research trial was purchased from a commercial producer and was sorted by gender prior to its arrival at the Management, Research and Instruction center at Angelo State University. Heifers were inseminated with separate semen than the multiparous cows, using bulls with lower birth rates to try and eliminate any dystocia complications. Mature cows and heifers alike were randomly selected as to which type of semen they received. The first mature cow through the chute for insemination received male sexed semen, the second received female sexed semen and the third was inseminated with conventional non-sexed semen. This pattern continued throughout the trial for all mature multiparous females. Random selection for heifers used the same technique described for the mature cows, but with semen from sires with an estimated progeny difference (EPD) of low offspring birth weights instead of the power bull semen. The first heifer in estrus was inseminated with male sexed semen, the second received female sexed semen, the third was inseminated using conventional non-sexed semen, and this pattern continued until all heifers were inseminated.

Day 25 of the trial, the second group was concluded, and transmitters were removed from all females that did not show signs of estrus. Also on day 25, a bull was placed with the first group of females for any that did not show signs of estrus or failed to conceive using artificial insemination. A bull was placed with the second group of cows on day 32.

Cattle were tested 45 days following bull removal via rectal palpation and ultrasound (ALOKA 500) to determine pregnancy.  Ultrasound images were saved and crown widths were measured to determine fetus size and predict calving dates.  Final pregnancy diagnosis was determined as parturition occurred.

# STATISTICAL ANALYSIS

Cows were randomly assigned to one of two synchronizing groups (n=42/group). Each individual cow or heifer served as an experimental unit. The model included synchronization group as well as the block (heifer or multiparous cow) and pregnancy rates were analyzed using $Chi^2$ and categorical models in SAS (SAS institute, Cary, North Carolina). Treatment differences would have been considered different at an alpha level of 0.05 ($P \leq 0.05$).

# Results and Discussion

## Conception Rates

All of the registered Black Angus females from the Angelo State University Management, Instruction and Research Center (n=84), were estrus synchronized at the beginning of the research trial.  Of those eighty-four females (18 heifers and 66 multiparous cows), only fifty-four showed signs of estrus and were artificially inseminated (10 heifers and 44 multiparous cows) and included in the analysis.  No age by treatment interaction was present; therefore only main effect means will be discussed.

As shown in Table 1, the first treatment, which was the male sexed-semen (Y-chromosomes only), a total of nineteen females were inseminated.  The second treatment, female sexed-semen (X-chromosomes only), eighteen total females were inseminated, and in the final treatment, which was the control consisting of non-sexed semen, seventeen females were inseminated.  In Treatment one, seven females conceived while the remaining twelve were not pregnant.  The percent conception for treatment one was 36.8%.  Treatment two had seven females conceive while the remaining eleven were not pregnant.  Percent conception for group two was 38.9%.  The third treatment (control) resulted in eleven females conceiving and six which remained not pregnant.  The conception percentage resulted in 64.7%.  Numerically it appears that there is a difference between conception rate in control and treatment groups, but with limited total numbers and variation being high, there were no differences (P=0.18) among treatments.  Upon completion of this study, it was determined following calving that gender of the sexed semen was 100% accurate.  All of the females

Table 1. Number of females, number of pregnant females, number of females open, and conception rate percentage in Angus females.

| Treatment[a] | Male Semen | Female Semen | Non-sexed Semen |
|---|---|---|---|
| n | 19 | 18 | 17 |
| Pregnant | 7 | 7 | 11 |
| Open | 12 | 11 | 6 |
| Conception, % | 36.8 | 38.9 | 64.7 |

[a] Treatments vary by type of semen used for insemination. Male sexed-semen was sorted via flow cytometry and stored prior to purchase and only Y-bearing sperm are present in these semen straws (1/4 ml). Female sexed-semen is sorted through the same process and only X-bearing sperm cells are stored in these straws (1/4 ml). Non-sexed semen, or the control, is the traditional semen. There is no sorting process it must complete; however it is stored in ½ ml straws. In this study, there were no differences among treatments.

who were inseminated with either X-bearing or Y-bearing semen and conceived gave birth to the gender of the dose they were inseminated.

If this trial was repeated with a larger group of females, it is likely the results may show a difference in conception rates of sexed vs. non-sexed semen. Adding more animal units should be done when reexamining this research. In this trial non-sexed semen conception percentage almost doubled that of the sexed semen. When compared to Mellado et al (2010), our conception rates were within the normal range for the control (non-sexed semen 64.7%); they reported their highest conception rate to be 62% and a low of 44%. In a trial done by Larson et al (2006), the conception rate for suckled beef cows averaged 66%. We also have to take into consideration that when semen companies freeze semen, there are different sizes of straws stored. Most typical non-sexed semen is frozen in ½ ml straws, while all sexed semen is frozen in ¼ ml straws (Bovine Elite, College Station, TX). With this in mind, it makes sense for sexed semen conception rates to decrease in comparison to non-sexed since only half the volume of semen is inseminated. On the other hand sexed semen may experience a decrease in viability and motility due to the sorting process and reduce fertility that is unrelated to volume of semen (De Vries, 2009).

**Ultrasound**

Ultrasound images (ALOKA 500) were taken 45 days after bulls were removed from the herd using a 7 mhz linear transducer. Only images with clear concise quality and ample definition were measured and analyzed in the study. Approximately 10% of the images were unclear and not able to be measured. Crown width was measured through a predefined table in ALOKA unless the fetus was too small and then fetus length was measured and observed through a separate table in ALOKA.

As shown in Table 2, there were forty-nine females with sufficient images. Among these females, parturition date predictions averaged within a nine day span, with the minimum being exact (parturition occurring on the predicted day) and the maximum parturition difference occurring thirty-six days from initial prediction. In 92% of the females observed, parturition occurred within one estrous cycle, while the remaining 8% resulted in more than one estrous cycle difference from the predicted parturition date.

From my observations, using ultrasound imaging may be a viable management technique for producers to use for parturition prediction. The average gestation length for bovine species is 283 days, but several different ranges have been documented (Ensminger, 1992). The most common gestation range for Angus females varies by author. Hafez (1962) noted a gestation range of 243-316 days; Bearden et al (2004) documented a range of 278-293 days, while Ensiminger (1992) stated gestation varied from 278-288 days. Obviously, accurate predictions of parturition dates are difficult. If my data are compared to that reported by Hafez (1962), all of the predictions were within the range and we accurately predicted all parturition dates. However, when compared to Ensminger (1992) and Bearden et al (2004) only 92% were accurately predicted. Considering these averages, ultrasound imaging may be a consideration for producers to implement into management practices to aid

Table 2. Number of females with defined ultrasound images, minimum number of days from prediction to parturition, maximum number of days from prediction to parturition, average number of days from prediction to parturition, percent of females that calved within one estrous cycle, percent of females that calved in more than one estrous cycle.

| Estimated Parturition | Date based on Ultrasound Predictions |
|---|---|
| n | 49 |
| Minimum[a] | 0 |
| Maximum[a] | 36 |
| Mean | 9 |
| 1 Cycle[b], % | 92 |
| + 1 Cycle[b], % | 8 |

[a]These numbers indicate the differences in the actual parturition date and the prediction date from the ultrasound images analyzed.

[b]Bovine estrous cycles on average are 21 days. Parturition occurred for the majority of the females, but a small percentage took more than one cycle.

in observation during parturition, since my results of parturition predictions averaged within nine days and all these ranges cover a nine day spread.

## Implications

This study found no differences among treatments between male sexed semen, female sexed semen and non-sexed semen when analyzing 54 artificially inseminated Angus females. However, the non-sexed semen conception rate percentage was twice the amount of either sexed semen treatment. More studies with larger numbers of females per treatment would be beneficial for further research in determining if there is a conception rate difference between sexed and non-sexed semen. The use of sexed semen could possibly be a viable management tool if the producer is willing to risk the lower conception rate and increased semen cost to have a 90% chance of predicted offspring. The accuracy of ultrasound imaging in this research trial proves that it could be a viable management tool for producers to use in management practices. Predicted calving dates could be beneficial to producers by allowing various options of management techniques near parturition time.

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## Vita

A native of Lampasas, Texas, Kayla Brooks is the daughter of David and Sheila Brooks. She is a graduate of Lampasas High School where she was active in basketball, soccer and golf. She was an active member in Future Farmers of America (FFA) exhibiting Brahman heifers. Kayla attended Angelo State University where she was a member of Delta Tau Alpha agriculture honor society, a member of Block and Bridle, and active with the Angus Show Team. She graduated with a Bachelor of Science degree in Animal Science from Angelo State University in May 2009 with a minor in Meat and Food Science. The following fall she returned to Angelo State to pursue a Master of Science degree with a research study in Animal Reproduction. She is married to a Ballinger native, Wes Earnshaw and she plans on pursuing a career in the agriculture field in south Texas.