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Evaluating the Success of Female Selected Sex-Sorted Semen at Western Kentucky University's Dairy Farm

Briley Loggan
Western Kentucky University, briley.loggan565@topper.wku.edu

EVALUATING THE SUCCESS OF FEMALE SELECTED SEX-SORTED SEMEN AT WESTERN KENTUCKY UNIVERSITY'S DAIRY FARM

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Date Recommended 12 April 2019

Fred Degraves

Fred Degraves, Director of Thesis

Elmer Gray

Elmer Gray

Hunter Galloway

Hunter Galloway

Cheryl D. Davis 4/16/19

Dean, Graduate School Date

I dedicate this thesis to my parents, Ted and Gena Loggan, who inspired and encouraged me throughout this journey. Also, I dedicate this work to my dear friends Brooke Cooper and Ben Benton, who helped greatly in encouraging throughout this project and editing the manuscript numerous times.

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Department of Agriculture and Food Science Western Kentucky University

The purpose of this study was to evaluate the use of female selected sex-sorted semen and to determine the association of variables on the success of Western Kentucky University's Dairy Farm. Official breeding and calving records (n=144) were used to determine the relation of lactation number, breeding season, breeding number, breeding year and semen type on pregnancy results, sex of offspring, and the mortality of the offspring. Previous research has shown pregnancy results can be influenced by lactation number, breeding season, number of breedings and semen type. Results from this study show that pregnancy results were not associated with lactation number (P=0.21), breeding year (P=0.22), breeding number (P=0.52) or semen type (P=0.99). Breeding season was associated with pregnancy results (P=0.04). Lactation number (P=0.40), breeding season (P=0.20) or breeding number (P=0.12) did not influence the sex of the offspring. The year of breeding and semen type (conventional or sexed) had a significant or close to significant effect on the sex of the offspring (P=0.01) and (P=0.06). The mortality of offspring was not associated with lactation number (P=0.46), breeding season (P=0.94), breeding year (P=0.76), breeding number (P=0.40) or semen type (P=0.49).

Introduction

Productive herd life (the length of time a cow remains in the herd after her first calving) continues to fall on US dairy farms. The mean number of lactations fell from 3.2 in 1980 to 2.8 by 1994 (Hare, Norman & Wright, 2006). With high cull rates and declining productive years, dairy producers were looking for a solution to increase the number of replacement heifers. Sex-sorted cattle semen was the solution for many dairy producers. De Vries and Nebel (2009) stated that 12.4% more heifers would be born in 2009, because of the use of female sex-sorted semen than if conventional semen had been used. The use of female selected sex-sorted cattle semen allows a dairy producer to maintain or expand his herd without buying additional females; therefore, herd size can be maintained or expanded without a large upfront cost or, exposing the herd to new pathogens. The objective of this study was to evaluate the value of using female sex-sorted semen and to determine factors affecting the success of using sex-sorted semen at Western Kentucky University's dairy farm. Factors being examined were lactation number, breeding season, breeding number, and semen type.

History

Often in livestock production, one sex is more desirable to a producer. The desired sex can change between operations or an individual mating. The more desirable sex depends on the producer and the goals of the operation. The desired sex has a greater value for the producer, with as much as a $300 value difference between the two sexes (De Vries, 2015). Determining the sex of an offspring at the point of conception has long been desired by livestock producers with dairy producers preferring heifer calves to raise as replacements, while beef producers selling animals to the meat industry desire bull

calves. The ability to sex animal semen was made possible by the work of numerous researchers, companies, and government agencies. An accurate method to determine the sex of sperm cells did not exist, prior to the 1980's.

In 1981, Mr. William Goddard, an investor and entrepreneur, approached the faculty at Colorado State University asking the university to fund research methods to sex sperm cells. Colorado State University declined to fund the research proposal because no promising leads had been discovered in sex-sorting technologies at the time. The proposal got the attention of Drs. Rupert Amann and George Seidel, Jr. Dr. Amann and Dr. Seidel, Jr. organized a symposium, sponsored by Warwick Land Company, to learn about technologies and research being done on sperm sexing technologies. From the symposium, the duo learned that Dr. Daniel Pinkel developed the first flow cytometer, which orients the head of the sperm cell allowing a measurement of the DNA content in a semen sample. Researchers at the Lawrence Livermore National Laboratory and Oklahoma State University demonstrated that flow cytometry could identify X- and Y-sperm by the DNA content differences. Mammalian offspring carry at least one X-chromosome, female offspring carry two X-chromosomes, while male offspring have a single X- and Y-chromosome. The Y-chromosome determines the sex of an offspring (Genetics Home Reference).

In 1982, Dr. Duane Garner presented, a research proposal entitled "Flow cytometric verification of the relative proportions of X- and Y-chromosome-bearing sperm in bull and boar semen" to the United States Department of Agriculture Beltsville Agricultural Research Center. The proposal received funding and the research was completed upon an agreement between Oklahoma State University, the United States

Department of Agriculture, the Lawrence Livermore National Laboratory, and the United States Department of Energy. The collaborative research demonstrated flow cytometry could accurately identify differences in DNA content of X- and Y-sperm cells in four species of mammals; cattle, pigs, rabbits, and sheep. However, the sperm cells were killed in the process. With this improved technique, it was discovered that the weight difference between an X- and Y- sperm cell is between 3.73%-4.98% depending on the breed of cattle. *Bos indicus* cattle had less weight difference between the X- and Y-chromosomes of 3.73% while *Bos taurus* had a weight difference between the X- and Y-chromosome of 4.98% (Garner & Seidel, 2008).

The first sperm sorting technology was developed by Dr. Pinkel at the Lawrence Livermore National Laboratory. Although the sorting process was improved, the sperm cells were still killed by the dye during the staining process. The problem was solved when a team of researchers consisting of Johnson, Flook, Look and Pinkel discovered that a bisbenzimidazole fluorescent dye, Hoechst 33342, did not kill the sperm cells (Garner & Seidel, 2008).

In 1989, researchers at the United States Department of Agriculture (USDA) Beltsville Research Center reported a breakthrough, the birth of a rabbit as the first live offspring from sex-sorted mammalian sperm. Results from the first insemination of sex-sorted sperm resulted in 81% males with sorted Y-chromosome carrying sperm and 94% females with X-chromosome carrying sperm. The sperm sorting technology known as the Beltsville Sperm Sexing Technology was patented by the USDA in April of 1991. Dr. Lawrence Johnson was credited as the inventor of the machine (Garner & Seidel, 2008).

With an animal being successfully born from sex-sorted sperm cells, the researchers faced a new challenge; the speed at which semen sample could be sorted. Early in the development of flow cytometers approximately 400,000 sperm cells could be sorted per hour. With a typical dose of non-sexed semen containing 20 x 10^6 sperm per straw, the sorting process would take roughly 25 hours to produce a single straw of sex-sorted semen at a dose of half the normal dose, 10 x 10^6. The slow sorting process eliminated sex-sorted semen as a possibility for artificial insemination. Though sex-sorted semen could not be used for artificial insemination, the fewer number of sperm cells was still a viable option for In-vitro fertilization. In 1993, Mastercalf, Ltd (United Kingdom) reported the production of male beef calf embryos from sex-sorted semen. The team used a modified Becton Dickinson FACStar Plus flow cytometer/cell sorter with the capability of sorting 100 sperm per second and a purity of 70% Y-sperm cells and 79% X-sperm cells. An unstated number of embryos produced during the experiment were implanted into recipient females. The embryo transfer resulted in four pregnancies. The remaining embryos were cryopreserved to be used in a later experiment testing the survivability of embryos with sex-sorted semen. After the cryopreserved embryos were thawed, 106 recipient cows received two embryos each. Thirty-five cows successfully carried calves to term including; 4 females (10%) and 37 males (90%) (Garner & Seidel, 2008).

In 1994, Seidel, Colorado State University, submitted a proposal to study conception rates of heifers with reduced total number of sperm cells, with as few as 100,000 unfrozen unsexed sperm cells per dose to the National Association of Animal Breeders. Although, the proposal failed to receive funding from that source, Charles

Allen, a member of the National Association of Animal Breeders board, funded it through the Atlantic Breeders Cooperative. The research resulted in another breakthrough with sex-sorted semen. He collected bovine semen in early morning in Lancaster, Pennsylvania and transported the unsexed, unfrozen semen to a flow cytometer in Beltsville, Maryland. The sperm was separated by sex for about 7 hours, 15:00 local time, cooled to 5°C, and flown to Denver, Colorado. The sexed sample arrived at 20:00 local time and transported by car to the final destination in Fort Collins, Colorado. The sexed-semen was loaded into straws and deposited into the uterine horn at approximately 24:00. Twenty-nine heifers were inseminated, four weeks later 14 (48%) of the heifers were identified as pregnant. The heifers were rechecked 8 weeks after insemination and 12 (41%) were confirmed pregnant (Garner & Seidel, 2008).

The United States Department of Agriculture was encouraged by the results of the conception rate trial to grant a license to Colorado State University Research Foundation to commercialize the Beltsville Sperm Sexing Technology. XY, Incorporated, a company consisting of Colorado State University Research Foundation, Cytomation, Incorporated, (a company manufacturing flow cytometers), and a group of private investors, was formed (Garner & Seidel, 2008).

Four companies were involved in the initial development of flow cytometers to sort cattle semen; the American Breeders Service (now ABS Global), the Atlantic Breeders Cooperative (now a division of Genex), Mastercalf, Ltd. (no longer in existence), and XY, Inc. (now a division of Sexing Technologies). Select Sires and Advanced Dairy Genetics were both a part of the field trials in the US, with worldwide conception rate trial contributions from Cogent, Ltd. (UK), Livestock Improvement

Association of Japan, Goyaike, Ltd. (Argentina), and Hokkaido Genetics (Japan) (Garner & Seidel, 2008).

By 2008 several offspring from several different species, including: swine, sheep, horses, elk, rabbits, dolphins, dogs, and cattle, had been born and survived. Therefore, the research focus shifted to lessening the time it took to sex semen samples. Modifications were made to the needle that dispenses the individual sperm droplets to be measured. The new needle orientated the sperm cells, so the head was flattened, allowing a high-speed sensor and computer software to more accurately identify the sex of the sperm cell. The modification reduced the time to sort a semen sample. As of 2008, a single flow cytometer could produce a single straw of sexed semen, with a dose of 2×10^6 sperm, in approximately 9 minutes. Several factors influenced the pace at which bovine semen could be sorted by flow cytometry, including the sperm concentration, motility, and viability which can vary dramatically from individual bulls (Garner & Seidel, 2008).

The next obstacle for researchers was to cryopreserve sex-sorted semen. The sex-sorted semen used to produce offspring up until that time was non-frozen sex-sorted semen (Garner & Seidel, 2008). The issue was solved in 1999 when Schenk, Suh, Cran, and Seidel (1999) used egg-yolk-Tris buffer medium to successfully cryopreserve the sex-sorted semen for the first time.

In 2003, Sexing Technologies, Inc. (Navasota, TX) which was granted the licensing rights for the sex-sorting process for bovine semen, provided custom sorted semen for numerous AI companies (DeJarnette, Nebel, & Marshall, 2009).

The first major A.I. company was Select Sires, Inc (Plain City, OH). The initial release of sex-sorted semen was an experiment in a commercial market setting. The use

of sexed semen in a commercial setting had a conception rate of 80% of conventional semen. The sex ratios of heifers from the initial commercial setting experiment were slightly lower than originally expected. At 89% the results did not meet the desired 90% threshold (DeJarnette, Nebel, & Marshall, 2009).

Sexed cattle semen was released commercially to the dairy industry in 2004. Sales of sex-sorted semen did not flourish commercially until 2006. A study by the Department of Animal Sciences at the University of Florida predicted that 3.7 million units of sex-sorted semen would be produced in 2009 (De Vries & Nebel, 2009).

Monsanto developed plans to commercialize a flow cytometer with 16 sorter nozzles on a single machine by 2006. The conception rates from the multiple nozzle flow cytometry were decreased compared to the conception rates with single nozzle flow cytometer causing Monsanto to desert plans for the multi-nozzle flow cytometer. The equipment and intellectual property for the multi-nozzle flow cytometer was obtained by Genetic Resources International/Sex Technologies (Garner & Seidel, 2008).

The difference in conception rates between sex-sorted semen and conventional semen started to lessen (Lenz et. al., 2017). In April of 2017, Sexing Technologies (Navasota, Texas) released SexedULTRA™ 4M semen. The new product contains 4 x 10^6 living sex-sorted sperm cells compared to 2 x 10^6 sexed sperm cells, the standard dose with the XY method (Lenz et. al., 2017). SexedULTRA™ is the result of improvements in the equipment to sex bovine semen. The current method used by Sexing Technologies is the Gensis III sorting technology. The company claims the new method causes less damage on a cellular level to the sperm (Thomas, et. al., 2017). In a seperate study, SexedULTRA™ 4.0 had a conception rate of 66.73% compared to the previously

used XY 2.1 (55.89%). SexedULTRA™ 4.0 had an increased 56-day non-return rate (females did not come back into heat for up to 56 days after breeding) compared to conventional semen containing approximately 15 x 10^6 (66.73 v. 65.66, P<0.001) (Lenz et. al., 2017). A large trial using 6,000 commercial dairy heifers found similar results. The results showed an increase of 4.5 percentage points (41.6% vs. 46.1%) when comparing sex-sorted semen processed using the XY technology and SexedULTRA™ (Vishwanath, 2015).

The future of sexed semen is focusing on improving conception rates, specifically the optimal time for insemination and when embryo transfer is being utilized. Research on the optimal time to breed females with sex-sorted semen has shown that delaying insemination to 18-24 hours after the onset of estrus may increase pregnancy rates (Schenk et, al., 2009). However, more research needs to be done to identify the optimal time for artificial insemination with sexed semen (Hall, 2011).

Process

As of 2009, all major A.I companies in the United States use flow cytometry as the technique to sort semen. Several other techniques such as; sex-specific antibody binding, albumin gradient separation, fraction-action on a discontinuous Percoll gradient, free-flow electrophoresis and multitude swim-up, have all been suggested as techniques to sort semen. However, none of the techniques have been successful (Cerchiaro, et. al., 2007).

The first step in the semen sorting process is to dilute the sperm sample to a very low concentration level (De Vries & Nebel, 2009). Next, the sample is dyed with Hoechst 33342, a bisbenzimidazole fluorescent dye. Semen containing X-chromosomes have

approximately 4% more DNA content than a Y-chromosome. The increased weight of the additional DNA in the X-chromosome causes the sperm cell to absorb more dye. The dyed sperm illuminates blue, with X-chromosome sperm illuminating a brighter color than the Y-chromosome sperm cell (Garner & Seidel, 2008). The semen sample is also dyed with a red food dye (Galli & Balduzzi, 2009). The red dye quenches the fluorescence dye allowing only living sperm cells to be illuminated (Garner & Seidel, 2003). The dyed semen is then sent through a modified flow cytometer. The stained semen is aligned in a single-file stream by a crystal vibrator which breaks the stream of semen into droplets containing a single sperm with the head oriented toward the lasers, allowing the sperm cell to be identified (Garner & Seidel, 2008). A high processing computer evaluates the miniscule differences in the DNA content between the X- and Y-sperm cells. A photomultiplier tube (PMT) is used to rapidly measure the fluorescent differences in the sperm cells. The brightest 20-30% illuminating sperm cells are X-bearing sperm. The 20-30% of sperm cells illuminating less brightly are Y-bearing sperm cells. The remaining sperm cells cannot be accurately identified (Seidel & Schenk, 2006). Droplets with identified sperm cells are given opposite electric charges. The sperm droplets fall through an electric field. The cells are attracted to brass plates allowing the sperm to flow into different collection containers (Garner & Seidel, 2008) (Garner & Seidel, 2003). Only about 60-70% of the sperm cells are oriented allowing the sex of the sperm to be identified, with approximately half of that (30-35% of the original semen sample) being the desired sex (De Vries & Nebel, 2009). Sperm cells cannot be identified for various reasons including no sperm cells present, two or more sperm cells present in a single droplet, not enough difference in the illumination between the sperm cells,

damaged or dead sperm, or the sperm cells are not orientated to be identified. The unidentified sperm do not receive a charge and fall into a separate collection container (Garner & Seidel, 2008).

Conception Rates

Sex-sorted semen (while desirable by many producers for its ability to determine the sex of the offspring at the point of conception) has a major disadvantage, the conception rates are lower than conventional semen. Reports vary on the conception rates of sexed semen when compared to conventional semen, ranging from 60% to 90% (Galli & Balduzzi, 2009), (DeJarnette et al., 2011), (Norman, Hutchison, & VanRaden, 2011), (Cerchiaro, et.al., 2007), (DeJarnette et al., 2010) (Healy, House & Thomson, 2013). Hutchinson, Shalloo, and Butler (2013) found that pregnancy rates were increased when sexed semen was fresh and not frozen. Fresh sexed and frozen-thawed sexed semen had a pregnancy rate of 94% and 75%, respectively. A similar study from New Zealand found similar results, with conception rates of 90-95% of conventional frozen-thawed semen. The decreased conception rates in sexed semen are attributed to the stress associated with the sorting process. The stress put on sperm cells includes the diluting of the semen sample, dyeing the sperm cells with a DNA binding agent (Hoechst 33342), mechanical forces including being sent through the flow cytometer at 60 miles per hour at 40 pounds per square inch (De Vries & Nebel, 2009), light from the laser used to illuminate the DNA, pressure from the collection process, and finally, centrifugation to purify the sample (Cerchiaro, et. al.1, 2007). Sex-sorted semen does not survive cryopreservation as well as conventional semen (Garner & Seidel, 2003).

Conception rates in sex-sorted semen are also affected by the number of sperm cells per straw. The standard dose for a straw of sexed semen is approximately 2 x 10^6 sperm cells (Garner & Seidel, 2008) (Healy, House & Thomson, 2013). Conventional straws have approximately 15-20 x 10^6 sperm cells per standard dose (Garner & Seidel, 2003) (Healy, House & Thomson, 2013). The lower number of sperm cells in sexed straws is because of the cost of the equipment and expertise required for the sorting process, the time needed to create a dose of sexed semen, and the variability in bulls' semen viability to survive the sorting process. According to DeJarnette, Nebel & Marshall (2009), sex-sorted semen can enhance the differences in sire fertility rates. The reduced number of sperm cells in a dose exposes a sire's fertility which can be easily missed when more sperm cells are present. DeJarnette, McCleary, Leach, Moreno, Nebel & Marshall (2010) found that by increasing the number of sexed semen cells from 2.1 x 10^6 to 3.5 x 10^6 did not increase conception rates. Both dosages of sex-sorted semen had conception rates that were approximately 75% of conventional semen. When semen doses were doubled or tripled (4 x 10^6 or 6 x 10^6), pregnancy rates only increased slightly (5-7%). Increasing the number of sexed sperm cells present does not compensate for the damage that occurs during the sorting process (Hall, 2011).

Changes to the sorting process such as reduced sorting pressure and the use of a pulse laser instead of an unbroken beam, reduced the damage to the sperm cell and ultimately increased fertility (Hall, 2011). The gap in conception rates between conventional semen and sex-sorted semen started to recede when Sexing Technologies released SexedULTRA™ 4M semen in April of 2017. The sex-sorted sample contains approximately 4 x 10^6 live sperm cells compared to the original sexed semen that

contained approxiamately 2 x 10^6 live sperm cells. The results from a study on SexedULTRA™ 4.0 found that the new technology resulted in higher conception rates than sexed semen using the XY 2.1 (the traditional method) (66.73% v. 55.89% $P<0.01$), and the results showed that SexedULTRA™ resulted in higher conception rates than conventional semen (66.73 v 65.66, $P<0.001$) (Lenz et. al., 2017).

Sex-sorted semen has been recommended for use in first and second artificial insemination services in virgin heifers, because of increased fertility rates in heifers compared to lactating cows (De Vries & Nebel, 2009), (Seidel & Schenk, 2006), (Garner & Seidel, 2008) (DeJarnette, Nebel & Marshall, 2009). Heifers in a study conducted by Garner and Seidel (2008) had conception rates of 57% in heifers, and 39% in cows ($P<0.01$). Conception rates decreased as the number of services increased, with the conception rates ranging from 47% at first service to 32% with three or greater services in heifers, similar to non-sorted semen ($P<0.01$) (DeJarnette, Nebel & Marshall, 2009).

The next recommendation is to use sexed semen if the livestock producer has a successful artificial insemination program already in place (Seidel & Schenk, 2006), (DeJarnette, Nebel & Marshall, 2009). An additional recommendation is to use sex-sorted semen when estrus is detected with a primary sign of estrous, standing when mounted. Also, sex-sorted semen is not recommended in conjunction with fixed time artificial insemination because of the further reduced conception rates. The suggested time of artificial insemination with conventional semen (>12 hours after the onset of estrus) may not be compatible with sexed semen (Sales et. al, 2011). In a trial by Schenk et. al, (2009), a team of researchers found that delaying artificial insemination to 18-24 hours after the onset of estrus increased pregnancy rates per artificial insemination, when

compared to females that received artificial insemination 0-12 hours after the onset of estrus. Hall (2011) also recommended against using sexed semen when using embryo transfer, especially if super ovulated. One study found the number of transferable embryos was reduced by 20-35% when sexed semen was used. The reduction in transferable embryos is due to an increased number of unfertilized ova. Pregnancy rates after embryo transfer are similar whether produced using sexed or conventional semen. In-vitro fertilization reduces the number of sorted sperm needed to fertilize an oocyte. Artificial Insemination or Multiple Ovulation Embryo Transfer require millions of sperm cells for successful fertilization. In vitro fertilization requires only about 600-1500 sorted sperm cells to fertilize an oocyte (Hall, 2011).

The next recommendation is to handle sex-sorted semen with extreme care, including thawing at the proper temperature and to inseminate the female in a timely manner. Researchers recommend using a proven and successful inseminator to increase conception rates. An inseminator with below average conception rates with conventional semen will likely achieve less success with sex-sorted semen (Seidel & Schenk, 2006).

With lower conception rates with sex-sorted semen, a group of researchers suggest breeding heifers at a younger age. The earlier insemination allows a producer to increase the odds of the desired sex of the offspring, while overcoming the expected increase in the age at first calving. The study found the increased interval from first A.I. to calving cost the producer an additional $25 per head for sexed-semen (Chebel, Guagnini, Santos, Fetrow & Lima, 2010).

Synchronization

Estrus synchronization protocols have become popular in recent years because of the reduced labor and time commitment associated with fixed-time artificial insemination. Females brought into estrus by synchronization protocols are bred at a predetermined time, instead of breeding the female based on estrus detection. Fixed-time artificial insemination is desirable for many producers because of the pre-determined breeding time, the use of sex-sorted semen has been discouraged with fixed-time artificial insemination because of reduced conception rates. The reduced conception rates are a result of numerous factors. The first being the females are bred at a time that is less than optimal for conception. Conception rates can be further reduced because of the reduced number of sperm cells per straw, the reduced lifespan of sorted sperm cells in the female reproductive tract, and the possibility of pre-capacitation induced by the sorting process (Thomas, et. al, 2017).

Advantages

The increased use of sex-sorted semen will not only increase the number of replacement heifers available to address the high cull rates in the dairy industry, it will allow producers to expand their herd without risking the introduction of new diseases to the farm (De Vries, Overton, Fetrow, Leslie, Eicker & Rogers, 2008). DeJarnette, Nebel and Marshall (2009) found sexed cattle semen had 89% accuracy rate among breeds and parities. The researchers also looked at the accuracy of sexed semen when twins occurred. Of the twin births, 79% of the pregnancies resulted in female-female offspring, 15% were female-male, and 6% resulted in male-male offspring. When conventional semen was used, only 25% of twin births resulted in female-female offspring, 42% were female-male and 33% were male-male (DeJarnette, Nebel & Marshall, 2009). Also,

stillbirths were less frequent for twins when sexed semen was utilized (Norman, Hutchinson & Miller, 2010).

In addition to skewing the number of offspring to a more desirable sex, sex-sorted semen has numerous other benefits. Field trials were conducted to determine the normality of calves produced using sexed semen. The study found no differences in neonatal death, abortion rates, gestation length, calving difficulty, birth rates, birth weights, or weaning weights (Garner & Seidel, 2008). De Vries (2015) study found a heifer calving with a bull calf had a 10% higher risk of dystocia than a heifer calving with a female offspring (De Vries, 2015). Sexed semen reduced the percentage of births with dystocia by 28% for heifers and 64% for cows (Norman, Hutchinson & Miller, 2010).

The use of sexed semen allows livestock producer to increase the genetic gain from female offspring of genetically superior dams much more rapidly than the use of conventional semen (Van Doormal). Sex-sorted semen allows a producer to breed genetically inferior cows to male selected semen with more rapid culling of the lesser genetics from the herd (Chebel, Guagnini, Santos, Fetrow, & Lima, 2010). Results of one study showed that the rate of genetic gain could increase up to 15%, the maximum rate of gain for sire selection (De Vires, Overton, Fetrow, Leslie, Eicker & Rogers, 2008). Sex-sorted semen use allows closed herds to increase the number of replacement heifers without buying females. The practice allows the farm to be more bio secure by not introducing new animals that could be carrying new diseases (Van Doormal). Also, the number of cows required to produce replacement heifers could be nearly cut in half with the use of sex-sorted semen (Van Arendonk, 2011).

A collaborative study between the University of Minnesota and the University of Florida found heifers born from sex-sorted semen were on average $400 less expensive to raise from birth until parturition because of the revenue from the sale of extra replacement heifers (Chebel, Guagnini, Santos, Fetrow & Lima, 2010)

Disadvantages

The biggest disadvantage of sex-sorted semen is decreased conception rates. Numerous studies have shown conception rates can vary from approximately 60-90% of conventional semen (Galli & Balduzzi, 2009), (DeJarnette et al., 2011), (Norman, Hutchison, & VanRaden, 2011), (Cerchiaro, Cassandro, Dal Zotto, Carnier, & Gallo, 2007), (DeJarnette et al., 2010) (Healy, House & Thomson, 2013).

A straw of sex-sorted sperm is approximately $15-$50 more expensive than a conventional straw of semen, depending on the bull (Garner & Seidel, 2003) (Seidel & Schenk, 2006). The additional cost for a straw of sexed semen is associated with the high cost of a single flow cytometer, approximately $340,000 and the expertise required of individuals working with the flow cytometers (Seidel & Schenk, 2006) (Garner & Seidel, 2008).

In addition to the added cost per straw of sexed semen compared to conventional semen, another disadvantage of sexed semen is the economic impact on the price of female offspring. The supply of dairy replacement heifers will exceed demand. This will reduce the price for replacement heifers. The reduced price for replacement heifers will cause the average price for a cow to decrease. As a result, cull and herd expansion rates are expected to increase. The milk supply will increase, decreasing the price of milk for producers. Also, because dairy producers selected for female calves, the price of dairy

beef has fallen, a result of using heifers in feed lots to produce beef for the meat industry, instead of more feed efficient steer calves (De Vries, Overton, Fetrow, Leslie, Eicker & Rogers, 2008).

Inbreeding percentages in dairy cattle continue to increase. The continued use of sex-sorted semen will only accelerate the inbreeding percentages because of the limited number of bulls available with sex-sorted semen (De Vries, Overton, Fetrow, Leslie, Eicker & Rogers, 2008).

Schenk, Suh, and Seidel (2006) found in two separate trials, fewer embryos were fertilized when sexed semen was used compared to conventional semen (Schenk, Suh & Seidel, 2006). Sex-sorted semen has been shown to have further reduced conception rates when utilized alongside fixed-time artificial insemination because females are being inseminated at times not optimal for conception (Thomas et. al, 2017). Embryos produced with sex-sorted semen are of a lower quality than embryos made with conventional semen (Mikkola, Andersson & Tapoenen, 2015).

Materials and Methods

Sources of Data

Official breeding records of 42 nulliparous and multiparous Holstein, Jersey and Holstein/Jersey crossbred females (n=144 breedings) were obtained from Western Kentucky University's dairy farm in Bowling Green, Kentucky. The information included identity (ear tag number), lactation number, breeding number, semen type (conventional, sexed semen or natural service), bull identity, breeding season, pregnancy status, sex of the offspring, and mortality of the offspring. The breeding's occurred between October 2010 and January 2018. Calving's occurring between July 2011 and

September 2018. Conventional semen used in the study had a standard dose of 20 x 10^6, sexed semen dose was 2 x 10^6 from various bull stud companies. Females were determined to be pregnant with blood test or rectal palpation. The calving date, sex of calf, and mortality of the offspring were recorded the day of parturition.

Statistics

The data was analyzed using Generalized Estimating Equations (SAS, Inc., Cary, NC) to statistically evaluate the association of lactation number, breeding number, breeding season and semen type with pregnancy rate, sex of calves, and calf mortality at birth (Stokes, Davis, and Koch, 1995).

Results and Discussion

The results from this study showed semen type did not significantly affect pregnancy results (P=0.99). This study contradicts results from previous studies that have shown pregnancy rates with sexed semen can be affected by semen type (De Vries & Nebel, 2009), (Seidel & Schenk, 2006), (Garner & Seidel, 2008) (DeJarnette, Nebel & Marshall, 2009). The reduced number of sperm cells in a standard dose of sexed semen is the main cause of the reduced pregnancy rates. Also, the reduced dose exposes a bull's true fertility, which can be overlooked with a larger number of sperm cells present. On farm factors such as semen handling, estrus detection and nutrition can influence pregnancy rates greatly among farms. Refer to tables 1 & 2 in text.

			Average Conception by Lactation Number			
Lactation Number	**1st**	**2nd**	**3rd**	**4th +**	**Overall**	**Stand Dev.**
Sexed	**52%**	**62%**	**50%**	**75%**	**60%**	**0.11541**
Conventional	**57.87%**	**55%**	**58%**	**56%**	**56.86%**	**0.0153**
Overall	**52%**	**52%**	**50%**	**58.33%**	**53%**	**0.03787**

Table 1. Average conception using sexed and conventional semen by lactation number.

			Percent Females born of Sexed and Conventional Semen			
Lactation Number	**1st**	**2nd**	**3rd**	**4th +**	**Overall**	**Stand Dev.**
Sexed	**54.67 %**	**52.94 %**	**56.25%**	**57.14 %**	**55.25 %**	**0.018479**
Conventional	**25.00 %**	**46.67 %**	**22.22%**	**28.57 %**	**30.62 %**	**0.110144**
Overall	**39.84 %**	**49.81 %**	**39.24%**	**42.86 %**	**42.93 %**	**0.048478**

Table 2. % female calves born using sexed and conventional semen by lactation number.

Sexed Semen Accuracy	
1st Lact.	**54.67%**
2nd Lact.	**52.94%**
3rd. Lact.	**56.25%**
4th+ Lact.	**57.14%**

Table 3. The accuracy percentages of the use of sexed semen and resulted in a calf.

Pregnancy rates were significantly associated with breeding season (P=0.04). The results agree with findings by Wolfenson et al,. (2000) who found high temperatures and humidity can adversely effect conception results. Breeding number was not significantly associated with pregnancy results (P=0.52) in this study contradicting results by DeJarnette, Nebel & Marshall (2009) who found as breeding number increased, pregnancy rates decreased. Lactation number was not significantly associated with pregnancy results (P=0.21). Since its commercial release, sex-sorted semen has been recommended for first and second use in virgin heifers. Virgin heifers have the highest fertility rates, while being genetically superior to lactating females (Cerchiaro et. al., 2007) (Galli, A. & Balduzzi, D. 2009). The year of breeding was not associated with pregnancy results (P=0.22).

The results from this study trended toward a significant association between the use of sexed semen and the sex of the offspring (P=0.06). Healy, House and Thomson (2013) and Cerchiaro, et. al., (2007) determined the use of sexed cattle semen had a significant effect on the sex of the offspring (P<0.001). The year of breeding had a

significant effect on the sex of the offspring (P=0.01). The number of breedings trended toward a significant effect (P=0.12). The results of the breeding number and breeding year might be the result of an unknown physiological effect. The lactation number and season of breeding did not have a significant effect on the sex of the offspring (P=0.40) and (P=0.20).

Stillbirth rates were not significantly associated with the type of semen (conventional or sexed) used (P=0.48). The results agree with a study by DeJarnette, Nebel & Marshall (2009) with sexed semen not having a significant association on stillbirth rates (P=0.46). Lactation number (P=0.46), season of breeding (P=0.94) and number of breedings (P=0.40) did not have a significant association on the calf being born alive or dead.

Conclusion

Female selected sex-sorted semen rapidly became popular with dairy producers upon its release with the demand greatly outweighing the supply. Sexed semen was seen as a solution to help combat the decreasing productive life of the US dairy cow and high cull rates. In this study the season of breeding had a significant effect on pregnancy results (P=0.04) agreeing with previous studies. Breeding year had a significant effect on the sex of the offspring (P=0.01). Semen type trended toward a significant effect (P=0.06) in this study. All other variables were not significantly associated with pregnancy results, sex of the offspring or the mortality of the calf. Additional research with a larger, more homogenized population is needed.

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