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IMMINENT COMMERCIALIZATION OF SEXED BOVINE SPERM

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Introduction

Cattle breeders demand products and services that improve efficiency and maximize profits. Research and development for these products, once done primarily in academic settings, now is occurring more within the private corporate sector who now fund research that was previously funded from public sources. These products often are patented and licensed to individual companies. Product availability is hastened, but sometimes at higher costs. Sex-specific sperm soon will be available to the cattle industry for use in high profile, genetically elite herds. Widespread availability of sex-specific sperm for commercial herds should follow within 2 years.

Sex-specific sperm for use with artificial insemination (AI) will enable producers to pre-determine the sex of calves from specific genetic matings, resulting in faster genetic gain within herds. This technology will reduce numbers of unwanted dairy bull calves that currently end up as dairy beef. Efficacious use of sex-specific sperm depends upon increased genetic merit and/or phenotypic gain for production traits, and increased economic efficiency of genetically superior females by the production of specific sex offspring. Managerial gains also will be realized with accurately predicted calf sex.

Hair color, milk production and rate of gain are examples of many traits determined by genetic code contained in the DNA (deoxyribonucleic acid). DNA, which is organized in packets called chromosomes, specifies what each cell does and when. Bovine sperm contain 30 chromosomes, one of which is the sex chromosome. Sex is determined by X- (female) or Y- (male) chromosome-bearing sperm. In mammalians, more DNA is contained within the X-chromosome (Table1); for bulls, X-chromosome-bearing sperm have 3.8% more DNA content than Y-chromosome-bearing sperm. This forms the basis for separating sperm via flow cytometry/sperm sorting. DNA content is the only reliably proven difference between X- and Y-chromosome-bearing sperm (Johnson and Welch, 1999).

Table 1. Difference in DNA content (%) of X- and Y-chromosome bearing sperm[a].

Species	%	Species	%
Turkey	0	Bull	3.8
Man	2.8	Dog	3.9
Boar	3.6	ram	4.2
Stallion	3.7	Chinchilla	7.5

[a]Johnson and Welch, 1999.

Flow Cytometry/Sperm Sorting

Sperm stained with Hoechst 33342, a DNA-specific binding dye, fluoresce when excited by ultraviolet laser light of ~360 nm wavelength. During sorting, a fine stream carrying the stained sperm is illuminated by a laser light beam. Since X-chromosome-bearing sperm contain more DNA, they bind more Hoechst 33342 than Y-chromosome-bearing sperm, and thereby glow brighter when exposed to laser light. Properly positioned detectors on the sperm sorter quantify the fluorescence emitted from the sperm DNA and transfer that information to a computer for processing. The highly sophisticated sperm sorter discriminates between the brightness differences of the X- and Y-chromosome-bearing sperm. As sperm flow through the sorter, a vibrating crystal breaks the stream into small droplets, of which 30% contain sperm. A positive or negative electrical charge is assigned to droplets according to the sperm's fluorescence intensity. Droplets then pass an electric field where positive charged droplets are deflected towards the negatively charged field, and negative charged droplets are deflected towards the positively charged field. Streams of droplets containing selected sperm are collected into a test tube for further processing (Figure 1, Johnson, et al., 1999). Only live, membrane intact sperm are sorted; dead sperm are removed from the sperm population during flow-sorting.

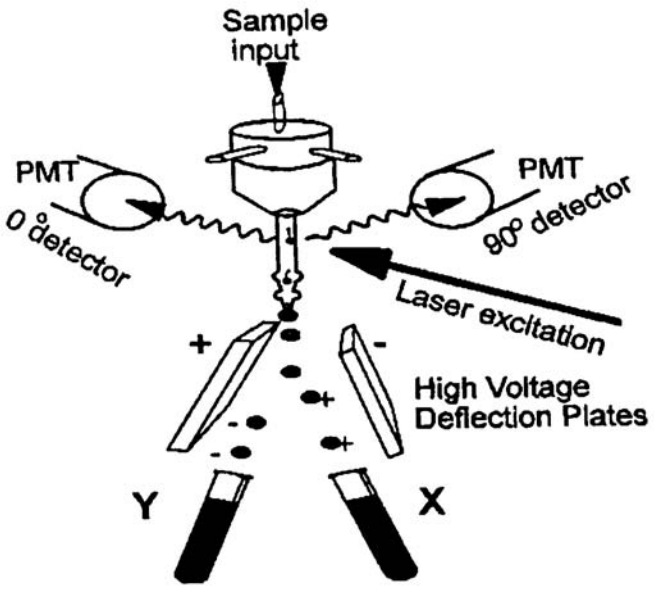

Figure 1. Diagram representing flow cytometric analysis of sperm based on DNA content and subsequent separation into X- and Y-chromosome-bearing populations. Fluorescence intensity is proportionate to DNA content (Figure from Johnson et al., 1999).

The sperm sorter (SX MoFlo®, Cytomation Inc., Fort Collins, CO) is capable of processing nearly 100,000 events/sec. Currently, sperm sorting is performed at ~25,000 events/sec. Because of the morphology of the sperm head, maximum fluorescence only is detected when the light beam intersects a properly positioned sperm. Droplets containing improperly positioned sperm are simply not charged and collected into a waste container. Accuracy of sexing can be virtually any purity between 50 and 95+% by adjusting sorting boundaries. Higher accuracy results in fewer sperm sorted per unit of time. For routine production, sperm are sorted at 90% purity of a specific sex. These requirements have resulted in current sorting rates for bull sperm exceeding 2,000 live sperm/sec of each sex. Improving sort rates through further instrument research and development is ongoing, and in due course will significantly impact the commercial cattle industry.

Artificial Insemination of Heifers with Sexed Sperm

Sorting sperm into X- and Y-chromosome-bearing populations is of little value unless sperm capable of fertilization are produced. Furthermore, sorting approximately 8 million sperm per hour of each sex is too slow to affect commercial cattle production. It, however, already could have limited application in genetically elite niche markets and/or emotionally driven industries. Maximizing sex-specific breeding units for AI from each sorting session is essential for efficient use of germ plasm, labor and time. Therefore, XY, Inc., a Fort Collins, Colorado company, and researchers at Colorado State University have focused on developing flow-sorting technology and novel methods to inseminate females with fewer sperm than used in conventional AI.

The primary goal of research thus far has been to compare pregnancy rates from using sorted versus non-sorted sperm by performing actual field trials. A second goal was to verify that flow-sorting does alter the sex ratio from 50:50. Most research has focused on sorted sperm to produce females; however, two studies have illustrated efficacious sperm sexing for males. The following section summarizes results from field trials (1997-1999), inseminating 1000 heifers with sexed sperm in 6 herds with different management practices. Twenty-two beef and dairy bulls of unknown fertility of various breeds were used.

Estrus was synchronized in all heifers used in these studies. Heifers were inspected for standing estrus mornings and evenings, but inseminated only in the evenings, ½ or 1 day after onset of estrus. Semen deposition was either into the uterine body as done conventionally, or half into each uterine horn using atraumatic embryo transfer sheaths (IMV, Minneapolis, MN). In the latter case, semen was deposited as far past the greater curvature of the uterine horn as possible without causing trauma, identically to nonsurgical embryo transfer. In most cases, semen was deposited at least halfway into each uterine horn.

Sorted, frozen-thawed inseminates contained 1-3 x 106 total sperm. Sorted sperm quality was determined by routine visual estimates of progressive motility and sperm morphological evaluation. Sorted, frozen-thawed inseminates contained at least 35% progressively motile sperm. Approximately 10% of sorted, cryopreserved sperm batches were discarded due to failure to meet minimum quality control standards. Based on laboratory evaluations of sperm quality,

sorted sperm were slightly compromised when compared to non-sorted sperm (Schenk et al., 1999). However, this compromise is minimal compared to damage that occurs due to freezing and thawing sperm, that in general kills half of the sperm.

Non-sorted control sperm served as a composite estimate of the intrinsic, normal fertility of the heifers within studies as well as for bull fertility and inseminator skill. Control inseminates contained 20-40 x 106 total sperm/dose.

Pregnancy was determined via ultrasound 28 to 37 d post-insemination and/or 56 to 92 d post-insemination, at which time fetal sex was determined. Accuracy of sexing resulted in almost 90% males or females, reflecting the validity of the routine sorting procedure.

Our first study compared pregnancy rates of sexed and unsexed non-frozen sperm at very low dosage inseminated in the uterine horn to the pregnancy rate from unsexed frozen-thawed control sperm deposited in the uterine body. Pregnancy rates for all treatments were similar, and accuracy of sexing was 95% heifers. No excess abortions were found between 2 months of gestation and term, and all calves (n=19) were normal and survived. Furthermore, 18/19 of these heifers derived from sexed sperm were subsequently bred with sexed sperm and are due to calve in March of 2000.

Ambitiously, the following year, sexed, frozen-thawed sperm was included in all further experimentation. Initial success with sexed, frozen-thawed sperm was such that use of sexed, non-frozen sperm was eventually halted. Furthermore, for global distribution, sexed, frozen sperm will be mandatory for most applications. Sexed, non-frozen sperm may be best in terms of maximizing a sire's reproductive efficiency and sorter production, but requires well-established, reliable distribution within finite distances from the sperm sorter.

To date, 11 field trials have been completed, each with limited numbers of heifers. Recent studies indicate use of sexed, frozen-thawed sperm will result in pregnancy rates that are >85% of frozen-thawed control sperm containing 7-20 times more sperm. In marginally managed herds or with lower fertility bulls, sexed sperm resulted in fewer pregnancies compared to controls. There was not much difference in pregnancy rates between 1 and 3 x 106 total sorted sperm. It is important to note maximum fertility for sorted sperm at these dosages may only be achieved with high fertility sires. Fertilizing potential of sorted sperm from average fertility bulls may approach maximum levels if increasing sperm dosage can compensate for lower fertility. Likewise, sperm from some bulls will have higher tolerances for sorting, freezing and thawing. Low dose insemination of sorted sperm from low fertility bulls likely will result in unacceptably low pregnancy rates.

Summary of Field Trials

Pregnancy rates with sexed, frozen sperm were 70-90% of those obtained with unsexed, frozen controls. Results of individual field trials are documented (Seidel et al., 1999). Limited data were obtained from individual trials because few heifers were bred per treatment per bull. Pooled pregnancy rates determined by ultrasound from all studies concerning sorted,

frozen-thawed sperm including frozen-thawed controls are given in Table 2. When data are pooled from different studies as in Table 2, comparison of treatment means are not quite accurate due to unequal numbers per group and possible interactions. Nonetheless, these data provide a useful overview of results.

Table 2. 30-76 d pregnancy rates from combined trials with sexed, frozen and control sperm.

Sperm No.	Site	No. Heifers	No. Pregnant	% Pregnant
1-1.5 x 10^6	body	298	155	52.0
1-1.5 x 10^6	horn	193	107	55.4
3 x 10^6	body	171	93	54.4
3 x 10^6	horn	72	43	59.7
20 x 10^6, Control	body	255	173	67.8

Pregnancy rates with sexed sperm pooled over all trials were 266/586 (45.4%) for inseminations 12 h and 203/414 (49%) for inseminations 24 h after estrus was detected. This non-significant higher trend is consistent to other research indicating preference to inseminate later than normally recommended with lower fertility bulls, or with compromised sperm. Pregnancy rate was not affected significantly by site of insemination.

In 6 of the 11 studies, fetal or calf sex was determined. The resultant predicted fetal or calf sex was 150/175 (86%) when pooled over these 6 trials. In the remaining trials, sex was not determined because of timing of pregnancy diagnosis, unavailability of persons skilled in sexing fetuses, and/or because calves are yet to be born.

Embryonic death, an indication of genetic damage to sperm during sorting was minimal. Pregnancy losses between 1 and 2 months after insemination were similar (P>.1) for both sexed (23/261; 8.8%) and non-sexed control sperm (9/145; 6.2%).

All data were from the insemination of virgin heifers. We also have limited data from inseminating lactating beef (Doyle et al., 1999) and dairy cows with sexed sperm. Generally, the pregnancy rate with sexed sperm has been lower with cows than in heifers, but too few data are available for valid conclusions. Lower pregnancy rates in cows may be due in part to uterine involution, lactation, and higher nutritional needs, which occur during the time cows are inseminated. Furthermore, more bull-to-bull variation may exist in pregnancy rate with sexed sperm in cows than in heifers.

Discussion of Results

Commercial economics may justify a product containing 80% of the desired sex at lower cost than one containing greater purity. Costs associated with sexing sperm will be greatly influenced by sorter efficiency and throughput. Acquiring more sperm per unit of time will lower production costs. Ultimately, cattlemen will determine acceptable accuracy and costs

associated with sexing sperm.

Cattlemen producing or purchasing first calf heifers should benefit considerably from using sexed semen. A major advantage for beef producers using sperm selected to result in heifers is to reduce dystocia in replacement heifers. Some management and genetic factors associated with dystocia include calf birth weight, calf sex, dam's pelvic area, gestation length, age of dam, breed of dam, breed of sire, dam's sire, nutrition and condition of dam, and exercise. Being born alive is the most important factor affecting profits in the cow/calf business; without a live calf, all other economic factors are meaningless.

Calf birth weight is the most important variable affecting dystocia. Dystocia occurs in part to incompatibility at birth between the size of the calf and the pelvic opening of the mother. Heifer calves tend to be smaller at birth than bull calves giving them an advantage in minimizing dystocia. Reports indicate bull calves are 1.5-10 pounds heavier and require 10-40% higher assistance than heifers. In addition, researchers from the Meat Animal Research Center (MARC) at Clay Center, Nebraska reported that calf losses due to difficult births were 27% higher for bulls (22.4%) than heifers (16.3%). First-calf heifers experience more calving difficulty due to physical immaturity. Dystocia results in increased postpartum interval, increased days open, decreased conception rate, decreased milk production, and increased retained placentas, cow mortality, veterinary and labor costs; dystocia decreases profits. Sexed-pregnancies from replacement heifers could command $50-$100 premiums above that which is already paid for quality replacement heifers.

The USDA estimates for the number of beef cows and heifers that have ever calved was 34.8 million head in 1998. If 90% had a live calf within the past 12 months with a sex ratio of 50:50, approximately 15.7 million bulls and heifers, each, are produced annually. Replacement heifer estimates were 5.3 million head or 15% of the total cow inventory, indicating that approximately 34% of the heifer calf crop was used as replacement females. The remaining 66% or 10.4 million heifers produced could have been pre-selected as bulls. Importantly, steers gain weight more rapidly, convert feed more efficiently, and command higher prices on the market. Furthermore, fewer production cows would be needed to produce the same pounds of beef. Expenses associated with artificially inseminating sexed sperm could be offset by significant financial gains in terms of production. The economic advantage for steers over heifers by the time they are marketed at weaning is about $75 each. Presumably, cows rather than heifers would be bred to have male calves because dystocia due to males is minimal in parous cows.

Successful commercialization of sex-specific sperm will be highly coupled to pedigree information, bloodlines, production traits and phenotypic traits. Sexed sperm should have a huge impact on the efficiency of existing multiplication programs. It will allow producers to maximize sex related traits including maternal, rate of gain, and body composition. Seedstock producers can make the best use of the herd by producing pre-selected, curve bending herd sires and genetically high quality replacement heifers from specific dams and sires designed to target specific markets. Only genetically elite maternal cows will be required to produce replacements since the sex of the offspring will already be pre-selected.

Commercial producers will be able to make more efficient use of heterosis in crossbreeding programs. Two-way cross females with high maternal characteristics for fertility, milking ability and mothering ability could be mated to terminal cross sires with desirable traits for rapid, efficient and high growth rates to produce steers for beef production. Hybrid vigor will be at the highest possible level utilizing maternal, and growth traits to maximize calf weights.

Huge differences exist among bulls regarding pregnancy rates at low dosages of unsexed sperm (Figure 2, Den Daas et al., 1998). It is unclear what effect using sexed sperm will have on subsequent non-return rates of these different bull groups, but results will likely follow similar fertility trends as demonstrated with unsexed sperm. Laboratory tests alone cannot accurately predict the fertilizing potential of sperm with regard to minimum numbers per inseminate required for maximum fertility. Therefore, routine dose response curves representing sorted sperm numbers verses fertility level for each bull will likely become mandatory. Economic thresholds calculated by genetic centers would include costs associated with efficient sperm sorting, sperm numbers required to maintain maximum fertility, sire genetic merit, and what producers are willing to pay for sexed sperm from individual bulls.

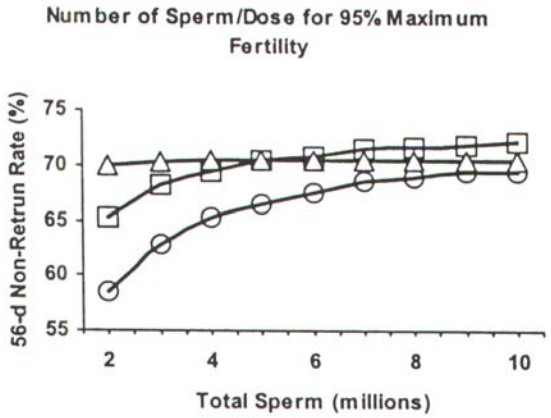

Figure 2. Relationship between non-return rate and the number of sperm inseminated. The rate at which maximum fertility is achieved varies by bull fertility level (△ high, n = 4 bulls; □ medium, n = 12 bulls; or ○ low, n = 4 bulls) and with increasing sperm dosage (Den Daas et al., 1998).

Pre-selected offspring using flow-sorting have been born to cattle, humans, swine, rabbits, sheep and horses. Some species represent lucrative niche applications driven by emotions, while others like the cattle and swine industries represent huge potential markets that would benefit and be positively influenced by commercial use of sexed sperm. Application of sexed sperm in some species may require costly unconventional methods to achieve fertilization. In vitro fertilization and intra-cytoplasmic sperm injection coupled with surgical intra-oviductal or intra-uterine insemination may be required to achieve reasonable pregnancy rates in species that normally require huge sperm numbers with conventional AI.

Conclusions

Sex-specific sperm will not be a panacea for all applications or all cattlemen, and will mainly be used with AI. Sex-specific sperm will cost more, may be packaged differently, and may require slightly different procedures for AI than conventional semen. As with many new technologies, effective commercial use of sex-specific sperm will require greater management

and labor skills, including well-trained inseminators and properly designed livestock handling facilities. Accurate estrus detection will be mandatory, because fewer sexed sperm are used than with conventional AI. The use of sex-specific sperm at low dosage from marginal fertility bulls probably will result in lowered conception rates. Furthermore, lower pregnancy rates associated with poorly managed herds, faulty field handling of frozen semen, or improper placement of the semen, will be magnified.

We have demonstrated that the sex ratio of calves can be altered to >85% based on DNA content of sperm using a flow cytometer/sperm sorter. Furthermore, AI of sexed, cryopreserved sperm has resulted in pregnancy rates in heifers that were 70-90% of unsexed, frozen controls. Extensive large-scale field trials are ongoing to demonstrate the effectiveness of inseminating low dose, sexed, frozen-thawed sperm in commercial herds. Sperm sexing technology is improving rapidly. Potential fertilizing ability of sexed, frozen sperm may be improved further with new methods of seminal processing. Highly fertile bulls whose sperm tolerate sorting and cryopreservation must be identified for optimal use of this technology. Pregnancy rates similar to unsexed sperm can be achieved in heifers with sexed sperm when highly fertile bulls are used at low sperm dosages. Herd management and semen-handling practices must be optimized for this to be achieved. Commercial sexed, frozen sperm for AI of heifers should be available to cattle producers within 2 years.

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