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# Use of Sexed (Female) Sperm is Successful in Yearling Heifers

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Sperm can be sexed with 90% accuracy and the sexed sperm can produce AI pregnancy rates slightly lower than normal sperm. Sexed sperm may be available to beef producers next year.

## Summary

*A two-year study was conducted on 457 yearling replacement heifers to evaluate effects of sexed (female) sperm compared to normal (control) sperm on AI conception rates, fetal sex ratio and pregnancy rates of heifers. All heifers were estrous synchronized, heat detected and bred by AI. Semen from three bulls was collected and sexed by a sperm sorter located at Colorado State University. Results showed a 3% to 13% reduction in AI conception rates of heifers inseminated with low dose, sexed sperm compared to normal dose, control sperm; with overall 50-day pregnancy rates being similar. Fetal female sex ratio was 92% for sexed sperm compared to 49% for control sperm. Sperm can be successfully sexed resulting in slightly lower AI pregnancy rates, but yielding 90% of the preselected sex of calf.*

## Introduction

Many techniques to separate male and female sperm have been investigated during the past 30 years with little success. However, a new technology using flow cytometry/cell sorting for DNA content of sperm has been developed with promising results. In cattle, the X-chromosome-bearing sperm (female) have 3.8% more DNA than the Y-chromosome-bearing sperm (male).

The sperm sorting procedure involves staining the sperm with a dye that binds specifically to the DNA. The diluted mixture passes through a flow cytometer in a fine stream; and a vibrating crystal breaks the stream into droplets. The stained sperm are illuminated by a laser beam and fluoresce. The female sperm glow brighter than the male. A computer quantifies the fluorescence of the sperm and attaches a positive or negative electrical charge to the sperm droplet. The male and female sperm are then deflected in an electrical field and collected into separate test tubes. Currently, the sperm sorter (SXMoFlo, Cytomation Inc., Ft. Collins, Colo.) is capable of sorting bull sperm at up to 4,000 live sperm/sec. of each sex. The current sorting accuracy is about 90% for each sex (Schenk and Seidel, 2000).

Because of the expense involved with sorting sperm, research has focused on developing improved sorting technology and on methods to inseminate females with reduced sperm dosage per unit compared to conventional AI.

However, pregnancy rates with sexed sperm must be similar to unsexed sperm if it is to be used extensively in commercial livestock production.

Two years of research was conducted in cooperation with Colorado State University and XY, Inc. at Ft. Collins, Colo. to compare the effects of sexed (sorted) female sperm with normal (unsorted) sperm on AI conception rates, fetal sex ratio, and pregnancy rates of yearling heifers managed under ranch conditions. Other treatments evaluated effects of bulls, ranches, AI technicians, and the site of sperm deposition.

## Procedure

The two-year study was conducted at the West Central Research Center, North Platte, Neb., using yearling replacement heifers from one ranch (n=102) in 1999 and from three ranches (n=355) in 2000. Heifers were delivered to the center in February each year (except from one ranch in 2000), managed in drylots, and fed ground alfalfa hay, corn silage, and corn with Rumensin to reach prebreeding target weights of about 800 to 850 pounds. Heifers remaining on the third ranch were managed similarly in all aspects.

All heifers were fed MGA for 14 days and injected with PGF (Lutalyse) 19 days after the end of the MGA feeding period. Heifers were heat detected three times per day but bred by AI only once a day (evenings, 12 or 24 hours after onset of estrus) for five days after the PGF injection.

**Table 1. Results of sexed (female) sperm on heifers — 1999<sup>a</sup>**

Trait	Group <sup>b</sup>	
	Control	Sexed <sup>b</sup>
No. of heifers inseminated	31	62
AI conception <sup>c</sup> , %	71	68
Fetus female sex ratio <sup>c</sup> , %	38 <sup>d</sup>	81 <sup>e</sup>
Heifers pregnant in 50 days, %	87	87

<sup>a</sup>Heifers were estrous synchronized with the MGA/PGF 19-day program. They were heat detected and AI bred with semen from two Red Angus sires. Cleanup bulls were placed with heifers 10 days after AI period for a total 50-day breeding season.

<sup>b</sup>Semen from each bull was collected: Control-normal dilution and freezing, 7 million live sperm per straw; and sexed-semen sorted for female sperm, frozen, 0.5 - 1.0 million live sperm per straw.

<sup>c</sup>Heifers were ultrasounded at 60 days after AI for day of pregnancy and sex of fetus. Calf sex was confirmed at calving.

<sup>d,e</sup>Means differ (P<0.01)

**Table 2. Results of sexed (female) sperm on heifers from three ranches — 2000<sup>a</sup>**

Trait	Group <sup>b</sup>	
	Control	Sexed <sup>b</sup>
No. of heifers inseminated	112	211
AI conception <sup>c</sup> , %	67 <sup>e</sup>	54 <sup>f</sup>
Fetus female sex ratio <sup>c</sup> , %	49 <sup>g</sup>	92 <sup>h</sup>
Heifers pregnant in 45 days <sup>d</sup> , %	90	91

<sup>a</sup>Heifers were estrous synchronized with the MGA/PGF 19-day program. They were heat detected and AI bred with semen from two Red Angus sires. Cleanup bulls were placed with heifers seven days after AI period for a total 45-day breeding season.

<sup>b</sup>Semen from each bull was collected: Control-normal dilution and freezing, 7 million live sperm per straw; and sexed-semen sorted for female sperm, frozen, 0.5 million live sperm per straw.

<sup>c</sup>Heifers were ultrasounded at 60 days after AI for day of pregnancy and sex of fetus. Calf sex was confirmed at calving.

<sup>d</sup>Only two ranches bred heifers for 45 days total.

<sup>e,f</sup>Means differ (P<0.02), but ranch and sire differences exist.

<sup>g,h</sup>Means differ (P<0.01).

Each year semen from two Red Angus sires (one bull was used both years) was collected at Colorado State University by CSU and XY, Inc. scientists. The control (unsorted) sperm were diluted and frozen using conventional procedures and packaged to yield at least 7 million live motile sperm per straw after thawing. The sexed (female) sperm were collected from the sperm sorter (SX Mo Flo), as previously discussed, and were packaged and frozen in 0.25-

mL straws containing at least 1.5 to 3.0 million total sperm (0.5 to 1.0 million live motile sperm after thawing). Laboratory evaluations of sexed sperm quality showed some compromise of sperm, but this was minimal compared to the damage caused by freezing and thawing which can kill half of the sperm.

Each year, our study was a part of a larger number of field studies conducted by CSU researchers. In both years, each heifer was systematically assigned to a treatment group in the breeding chute according to the order of insemination. Two-thirds of the heifers were assigned to be inseminated with sexed sperm and one-third with control sperm. Equal numbers of heifers per treatment were inseminated within bull, site of semen deposition, AI technician and ownership of heifers. Sexed sperm were deposited either into the uterine body (as were all controls) or half into each uterine horn using embryo transfer sheaths. Usually semen was deposited at least half way into each uterine horn but not so far as to cause tissue damage.

After the AI period, heifers were returned to their respective ranches in mid-May and cleanup bulls were placed with the heifers about seven to 10 days later for a total 45 to 50-day breeding season. All heifers were given an ultrasound exam about 60 days after AI to determine AI conception and sex of fetus. Calf sex was confirmed at calving. Data were analyzed using least square analyses of SAS and chi-square analyses.

## Results

Since the secondary variables were equalized and blocked within the major treatments, the results of the treatments in 1999 are shown in Table 1. The AI conception rate for the heifers receiving the sexed sperm was similar (P>0.20) to the heifers receiving the control sperm (68% vs 71%). Conception rates were normal for both groups. Pregnancy rates in 50 days of breeding were identical for both groups. No differences were found between bulls, AI technicians, or site of semen deposition. The fetal female sex ratio was considerably higher

(P<0.01) for the sexed group compared to the controls (81% vs 38%). However, both percentages were lower than expected.

The overall treatment results for the heifers from the three ranches in 2000 are shown in Table 2. The AI conception rate was lower (P<0.02) for the heifers receiving the sexed sperm compared to the controls (54% vs 67%). This difference was disappointing, but appeared to be influenced by ranch effects. Heifers from one ranch had identical AI conception rates for the two treatment groups, and were from the same ranch as heifers in the 1999 study. However, heifers from another ranch had a 30% difference between groups. Heifers from the latter ranch were smaller in type and lighter in weight. Heifers from both ranches were managed similarly at breeding using the same AI sires and procedures. The overall 45-day pregnancy rate for the control and sexed groups were very similar and at normal levels. The fetal female sex ratio was 92% for the sexed group and 49% for the control group as expected.

No differences were found between technicians or site of insemination. However, one bull tended to have higher conception rates than the other. Bull fertility differences have been found in many research studies.

Other research results previously reported (Schenk and Seidel, 2000) were:

- sexed sperm conception rates were generally about 10% lower than those of control sperm.
- sexed sperm from lower fertility bulls resulted in significantly fewer pregnancies compared to controls.
- sperm from some bulls had higher tolerances for sorting, freezing and thawing than from other bulls.
- pregnancy rates were similar for sperm dosages of 1.5 and 3.0 million total sperm per straw.
- pregnancy rates from sexed sperm were not increased by depositing sperm into uterine horns compared to the uterine body.

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- f) fetal female sex ratio from sexed sperm were between 85% and 90% in most studies.
- g) results from using sexed male sperm were similar to female sperm.

In conclusion, sexed-frozen sperm have produced pregnancy rates that are slightly lower than control-frozen sperm, but fetal female sex ratio was close to 90% with sexed sperm. Maximum fertility from low dose sexed sperm may only be achieved with bulls of high fertility. Calf survival rate, calf birth weight and growth have been normal with sexed sperm.

Sex-specific sperm will not be used by all cattlemen, but could have a major impact on AI breeding programs. Dairy-men could produce more female calves; beef seedstock producers could perform more specialized matings; and, beef replacement heifer development producers could produce more female calves for less dystocia at calving. Sexed sperm will cost more and will require greater cattle management and AI breeding skills. More research is needed on sperm sorting efficiency and on large-scale field trials to improve pregnancy rates of low dose, sexed sperm. Commercial sexed, frozen sperm should be available within one to two years in the United States. A commercial product has been available in the United Kingdom since early 2001.

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<sup>2</sup>Appreciation is expressed to the Hansen 77 Ranch, North Platte, Neb., Schuler Red Angus Ranch, Bridgeport, Neb., and Jackson Ranch, Maxwell, Neb., for providing heifers (plus bulls for semen, Schuler) and excellent cooperation on this research. Also appreciation is extended to XY, Inc. for sexed semen and partial funding of research.

# Estrous Synchronization Programs for Lactating Cows

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The Select Synch program for synchronizing estrus in lactating cows produced better results in a small study than the one injection PGF program and similar results to the CO-Synch mass AI program.

## Summary

*Two estrous synchronization experiments were conducted on lactating cows to compare the Select Synch program with the one injection PGF-10-day program and the CO-Synch mass AI program. The Select Synch program in both experiments produced good results. Pregnancy rates during the synchronization period were 62% and 81% for the Select Synch program compared to 49% for the PGF and 61% for the CO-Synch programs. The Select Synch program induced estrus in some noncycling cows. However, the Select Synch program requires two injections (GnRH and PGF) and about seven days of heat detection and AI breeding.*

## Introduction

Methods of estrous synchronization are needed that will achieve high conception rates during a short AI period at low costs. A major challenge of synchronizing lactating cows is a high percentage of cows are anestrous before the breeding season.

The Select Synch program can induce cycling in cows that have not resumed cyclicity. Researchers also have found calf removal in combination with Select Synch increased pregnancy rates in anestrous cows. The CO-Synch program was developed to include mass breeding; therefore, labor for heat detection is not needed.

Experiments were conducted over two years to compare the Select Synch program with the one injection PGF-10-day program in 1999, and to compare Select Synch with the CO-Synch program in 2000, on estrous response, conception rates, and overall pregnancy rates of lactating cows.

## Procedure

### *Experiment 1*

In 1999, 83 red crossbred 3-year-old cows at the West Central Research and Extension Center, North Platte, Neb., were used. The cows calved in March and April and were fed brome grass and alfalfa hay after calving plus some corn silage to meet their nutrient requirements. The cows were body condition 6.0 before the breeding season in early June and were 25 to 77 days post partum.

Cows were allotted to two treatment groups according to calving date and cycling status (determined by ovary palpation). In addition, two blood samples were taken at 10-day intervals before treatments were imposed to determine serum progesterone levels and actual cycling status. Group A cows (Select Synch) were given a 2cc injection of GnRH (Cystorelin, Rhone Merieux, Inc., Athens, Ga.) on day zero