

The Use of HeiferPLUS in Superovulated Heifers

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Introduction: The production of more heifers through [semen](#) sexing is of great [benefit](#) to individual breeders and to the beef and dairy industries as a whole. Semen sexing can also be an important [management tool](#) in most ET breeding programs. The purpose of this study was to determine if the bull semen sexing agent, HeiferPLUS could be used to predetermine the sex of embryos in superovulated heifers.

Methods: Day 7 embryos were collected from 15 super-ovulated beef heifers (AngusX) being run on Kentucky fescue/bluegrass pastures and supplemented with free choice mix of alfalfa/grass hay. Superovulation was done in mid-luteal phase of the estrus cycle with a 4-day regimen of FSH (Follitropin) and the luteolytic agent (Lutalyse) given on the third day. Heifers were visually heat-checked at 6 hr. intervals and bred with a single dose (0.5cc) of frozen semen (Angus) at 12 hrs. following onset of standing heat. Four heifers were also bred a second time, 12 hrs. following the first insemination. Treatment with HeiferPLUS was done by mixing the semen with HeiferPLUS and incubating for 20 minutes at 37°C (98.6°F) prior to insemination. Embryos were collected on the seventh day following estrus using standard non-surgical procedures. Embryos were washed in dPBS + 1mg/ml PVA and sexed using a standard PCR embryo sexing method for detection of a bovine male-specific DNA sequence found on the Y-chromosome (male).

Results: Average number of transferable embryos collected/donor was 3.3. Of the 50 transferable embryos collected, 50/50 (100%) were assigned a sex according to the PCR analysis. When the semen was treated with HeiferPLUS, 37/50 (74%) of embryos were female. This was a 26% shift in the sex ratio compared to an expected sex ratio of 52/48(M/F), i.e., 48% female. The shift in sex ratio is statistically significant ($p < 0.01$) according to a chi-square (X^2) analysis.

Table 1. Use of HeiferPLUS in Superovulated Heifers.

<u>Treatment</u>	<u>#donors</u>	<u>#embryos</u>	<u>ave# embryos</u>	<u>#male (%)</u>	<u>#female (%)</u>	<u>X²</u>	<u>significance^a</u>
HeiferPLUS	15	50	3.3	13 (26%)	37(74%)	7.04	p<0.01

^a p = probability that ratio is the same as the expected 52/48 sex ratio (M/F) as tested by chi-square (X^2) = (O-E)²/ E

Conclusion: Sexing of bull semen with HeiferPLUS resulted in 37/50 or 74% female embryos from superovulated donors. This was a 26% increase in the number of female embryos over what would be expected using unsexed semen.