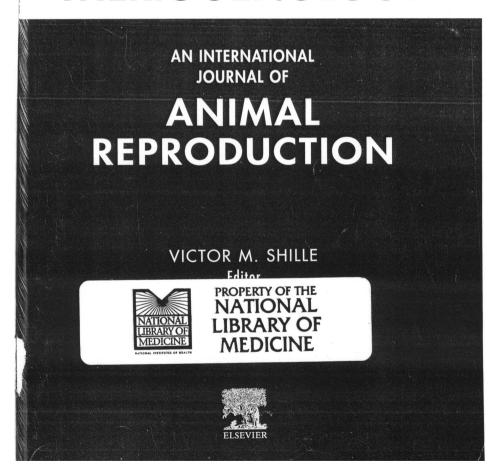
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INSEMINATION OF HEIFERS WITH SEXED FROZEN OR SEXED LIQUID SEMEN

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Angus heifers 13-15 mo of age in good body condition were synchronized by feeding MGA for 14 days followed by 25 mg of prostaglandin F-2-alpha 16 or 18 days later and inseminated 6-26 h after observed standing estrus. Freshly collected semen from four 14-26 mo old bulls was incubated in 224 µM Hoechst 33342 at 400 x 10⁶ sperm/ml in a TALP medium for 1 h at 34°C and then diluted to 100 x 10⁶ sperm/ml for sorting. Sperm were sorted by sex chromosomes on the basis of DNA content. An argon laser, emitting 150 mW at 351 and 364 nm, was used on a MoFlo® flow cytometer/cell sorter modified for sperm sorting operating at 50 psi with 2.9% Na citrate as sheath fluid. X chromosome-bearing sperm (~90% purity as verified by reanalyzing sonicated sperm aliquots for DNA) were collected at ~900 live sperm/sec into 14-ml tubes containing 2 ml egg yolk extender. Collected sperm were centrifuged and suspended to 10⁷ live sperm/ml in Cornell Universal Extender for liquid semen or 20% egg yolk in 2.9% Na citrate for frozen semen. Sexed semen to be frozen was cooled to 5°C over 90 min and loaded into 0.25-ml straws. After glycerolization straws were frozen by standard procedures. Straws of liquid semen were transported at 18°C in a temperature-controlled incubator 240 km for insemination 5 to 9 h after sorting. Sexed semen was inseminated using side-opening blue sheaths (IMV), one half of each straw into each uterine horn. As a standard control, semen from the same bulls had been frozen in 0.5-cc straws by standard procedures, thawed at 37°C for 30 sec, and inseminated into the uterine body. Treatments were balanced over the 4 bulls and 2 inseminators. Pregnancy was determined ultrasonically 62-65 days after insemination when fetuses also were sexed "blindly" (see table).

Treatment	No. heifers bred	No. sperm/dose	No. pregnant	No. ♀ fetuses
Sexed liquid	37	5 x 10 ⁵	11 (30%) ^a	11 (100%) ^a
Sexed frozen	35	1×10^{6}	11 (30%) ^a 18 (51%) ^{a,b}	17 (94%) ^a
Frozen control	37	40×10^6	27 (73%) ^b	16 (59%) ^b

a,b Values without common superscripts differ (P<0.02).

Pregnancy rates with liquid and frozen sexed semen were 41 and 70% of the control; this difference was statistically significant (P<0.05) for liquid sexed semen. The frozen semen control had 40 to 80 X more sperm than the sexed semen treatments. 28 of 29 fetuses (97%) were female when sexed semen was used.