

Insemination of Mares with 18h Stored, Sex-Sorted Spermatozoa

AC Lindsey¹, JE Bruemmer², RL Sondergard¹, and EL Squires²

¹XY, Inc., 1108 N. Lemay Ave, Fort Collins, Colorado 80524, USA

²Colorado State University, Fort Collins, Colorado 80523, USA

Several offspring have been produced following the insemination of sex-sorted stallion spermatozoa. Each of these foals is a result of sperm that had been sorted immediately following collection. In the equine industry, access to a freshly collected ejaculate at a sorting facility cannot easily be achieved. Therefore, to apply sex-sorting technology to the industry, a method must be developed to sort and inseminate sperm transported from distant locations.

The objective of this study was to compare the fertility of sperm that were sex-sorted immediately following collection to that of sperm that were stored for 18h prior to sex-sorting. Semen was collected from 4 stallions every other day throughout the duration of the project. Mares were examined daily by ultrasound, and when a follicle ≥ 35 mm in diameter was detected, 3000 IU of hCG was administered intravenously to induce ovulation. Mares were randomly assigned to 1 of 2 treatment groups and inseminated 30h after hCG administration. Mares were inseminated videoendoscopically, whereby sperm were placed on and around the uterotubal junction under visual guidance. Treatment 1 (n=20) consisted of 20 million sperm that had been flow-cytometrically sorted into X and Y chromosome-bearing populations immediately following collection. Treatment 2 (n=20) consisted of 20 million sperm that had been stored for 18h and then sorted by flow-cytometry. Sperm for Treatment 2 were stored at a concentration of 25×10^6 sperm/mL in EZ Mixin CST at $20^\circ\text{C} \pm 2^\circ$. Pregnancy status was determined based on ultrasound examination at Day 16 post-ovulation. Chi square analysis of the results was used to detect differences in treatment means.

Pregnancy rates were similar for those mares inseminated with fresh, sex-sorted spermatozoa (6/20 = 30%) and those inseminated with stored, sex-sorted spermatozoa (7/20 = 35%; $P>0.1$). One mare inseminated with stored, sex-sorted spermatozoa ovulated two follicles, and twins were detected upon ultrasound examination. For these results, this was considered a single pregnancy. Pregnancy rates achieved by the 4 stallions used for this trial varied greatly (55, 38, 30, and 9%).

Based on these results, stallion spermatozoa can be collected and stored for 18h prior to sorting without a decrease in fertility compared to those inseminated with spermatozoa that are collected and immediately sorted. Therefore, for commercial application, sperm could be collected locally, shipped to a centralized sorting facility for sex-separation, and then immediately inseminated into mares housed at that facility. Additional studies are in progress to improve semen handling during and following storage, as well as to minimize sperm damage caused by flow-cytometric sorting. Advancements in these areas will likely improve pregnancy rates resulting from the insemination of stored, sex-sorted stallion spermatozoa.

Keywords: Equine, sex-sorted sperm, low-dose, shipped semen