

Efficacy of using sex-sorted semen technologies for laparoscopic artificial insemination in a commercial white-tailed deer farm (*Odocoileus virginianus*)

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Introduction

The objective of this project is to describe the efficacy of sex-sorting technologies in producing viable sperm for laparoscopic artificial insemination (LAI) in white-tailed deer (WTD). There is an increased demand for implementing advanced reproductive technologies in the WTD industry such as using sex-sorted semen. This could provide producers the ability to select for favorable gender ratios. Flow cytometry is the most effective cell-sorting method and uses DNA differences to sort X and Y chromosome spermatozoa. However, there is limited information available on the efficacy of this technique in cervid species.

Materials and Methods

Three bucks in a captive herd in Illinois were anesthetized via intramuscular darts containing tiletamine-zolazepam (0.4mg/lb each) and xylazine (1mg/lb) for semen collection by electroejaculation in November. Bucks 1, 2, and 3 produced a total of 1.7, 2.8, and 1.9 mL neat semen with concentrations of 800, 545, and 800 million/mL, respectively. Progressive motility was estimated to be ~90% in all bucks. Semen samples were pre-extended at 1:1 to 1:2 semen to extender ratio with TRIS A+ extender and shipped overnight to Sexing Technologies (Fond du Lac, WI). Processing began with selecting viable sperm via nylon mesh filtration of the semen. Sperm DNA were then coated with a fluorescent dye and run through a flow cytometer in a single cell stream that allows them to become electrically charged when struck by 2 laser beams. This electric charge allowed for sperm to be sorted into X (female), Y (male), and waste populations. Fresh and frozen sex-sorted semen samples were shipped overnight to be used for LAI the following day.

Does (n=59) were synchronized by placing a CIDR device (0.3 g progesterone) intravaginally for 14 days. Upon removal, 150 to 200 IU pregnant mare serum gonadotropin was administered IM to each doe, and LAI was performed ~60 h later. Does were anesthetized as above and given 50 mcg gonadorelin IM on the day of insemination. Fresh, sex-sorted semen (0.25 mL) was deposited into each uterine horn of each doe laparoscopically.

Results

Upon arrival to Sexing Technologies, concentrations of processed samples were measured to be 643, 316, 390 million sperm/mL in Bucks 1, 2, and 3, respectively, and motility was estimated to be ~80% in all samples (recommended $\geq 55\%$). Morphological assessment determined primary defects to be 4, 5, and 4% and secondary defects to be 10, 10, and 7% of total cells (recommended $\leq 15\%$ each). After sex-sorting, total/progressive sperm motilities were measured using computer-automated sperm analysis and found to be 91.6/77.4% in Buck 1, 89.6/61% in Buck 2, and 85.9/65.6% in Buck 3. At 1 day post-sex sorting, total motility declined only slightly to 88, 86.2, and 68.8%. Purity of sexed samples were measured to be 88, 87, and 90% in Bucks 1 through 3, with 0% agglutination present. The final sperm concentration per straw was 4.8, 5.0, and 4.6 million/mL for Bucks 1 through 3. Buck 1 produced 17 male straws, Buck 2 produced 22 male straws, and Buck 3 produced 11 male straws for fresh insemination. Female straws (n=15 for Buck 1; n=12 for Buck 2) and remaining male straws (n=15 for Buck 1) were frozen for use at another time. Results of fawning and gender proportions will be available when fawning occurs this summer.

Significance

This project determined that semen sex-sorting can be used successfully to produce an adequate number of good quality semen samples for LAI in WTD as indicated by in vitro sperm analyses. Pregnancy rates and sex ratios following this technology will be determined at fawning this summer and provide crucial information as to how cost-effective these technologies can be for use in the commercial WTD industry.

We would like to thank Sexing Technologies Genetics, as they were instrumental in providing our in vitro semen data and quick semen processing.