Case Report

# Laparoscopic artificial insemination of white-tailed deer with fresh sex-sorted semen

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## Abstract

This case study describes the feasibility of using chilled, sex-sorted semen for laparoscopic artificial insemination (AI) in the captive white-tailed deer (*Odocoileus virginianus)* industry. Ejaculates from 3 bucks were collected, extended, and shipped overnight for flow cytometric sex-sorting. Sex-sorted semen was chilled and shipped back overnight. Synchronized does were bred using chilled, sex-sorted (n = 30, farm 1; n = 10, farm 2) or conventional frozen (n = 7, farm 2) semen by laparoscopic AI. Does were subsequently turned out with bucks. Percentage of bucks born via AI with sex-sorted semen was 85% (28/33) for farm 1 and 100% (16/16) for farm 2. Percentage of bucks born by natural cover was 50% (12/24) for farm 1 and percentage of bucks born by using either conventional semen or live cover was 56% (9/14) for farm 2. This case study demonstrated acceptable results using chilled, sex-sorted semen in white-tailed deer herds as a technique to manipulate the sex proportions per breeding season.

**Keywords:** Cervids, flow cytometry, sex-sorted semen, white-tailed deer

## Background

In North America, white-tailed deer (WTD) farming is an industry generating an estimated $7.9 billion annually as of 2017.[1] The breeding stock represents the major component of this industry (> $900 million) as it involves all the animals raised and sold.[1] However, since trophy hunting and venison products are the end market for the breeding industry, male trophies are valued more than females for having larger carcasses and impressive antlers.[2] Of interest, AI with frozen or fresh semen is a widely used tool to select for desired features (e.g. fast growth, well-branched and broad antlers) in deer herds.[2] The high demand for producing more terminal bucks per season leads to increased demands for implementing the development of more advanced reproductive technologies to alter the sex proportion in the offspring. More specifically, the use of sex-sorted semen with laparoscopic AI has been gaining popularity in the WTD industry as a way to increase buck production and improve profitability for farmers. Here, we describe a case study to evaluate the feasibility of using chilled, sex-sorted semen for laparoscopic AI in captive herds of WTD. Since data collected from routine assisted reproduction procedures from private farms for commercial purposes were used, institutional animal care and use committee approval is not required, and this case study is exempt.

## Feeding and vaccination

Animals used were from privately-owned herds located in central Illinois, ~ 50 miles apart from each other. Farm 1 housed 3 bucks (buck 1: 1 year; buck 2: 2 years; buck 3: 3 years); collected semen was used to breed 34 does (mean age: 2.9 ± 0.3 years, ranging from 1 to 5 years) housed in farm 1 and 17 does (mean age: 3.1 ± 0.3 years, ranging from 2 to 6 years) housed in farm 2. Animals were maintained in pastures with trees and shade cloths. Animals were fed 18% protein textured feed with vitamin and mineral mix in farm 1 and 14% protein textured feed with vitamin and mineral mix in farm 2. Animals were given free choice alfalfa hay and were on clover grass pasture.

The vaccination protocol for both farms included a custom-made epizootic hemorrhagic disease (EHD)/bluetongue virus (BTV)

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9-way combination vaccine (contains antigens for EHD 1,2,6, BTV 3, *Fusobacteria* sp., *Pasteurella multocida* A, *Escherichia coli*, *Trueperella pyogenes*, *Clostridium perfringens* type A, and *Bibersteinia* sp.) from Newport laboratory (Worthington, MN). Additionally, Red Ridge autogenous vaccine for *Mycoplasma* sp. were given (Red Ridge Whitetails, Williamsport, PA). Animals from both farms were dewormed with topical moxidectin (Cydectin® 0.5 mg/kg, Boehringer Ingelheim, St. Joseph, MO).

## Semen collection and processing

Ejaculates were obtained from 3 white-tailed deer bucks housed in farm 1. Anesthesia and animal handling were performed by a team of experienced veterinarians. Bucks were anesthetized using a combination of 0.88 mg/kg tiletamine-zolazepam (Telazol®, Zoetis, Florham Park, NJ) and 2.2 mg/kg xylazine (Cervicine®, ZooPharm, Windsor, CO) given intramuscularly via projector. A 2.2 mg/kg dose of ketamine (Ketaset®, Zoetis, Florham Park, NJ) was given intravenously if needed for additional anesthesia. This anesthesia protocol has been used extensively for semen collection of WTD in both clinical medicine and research.[3-6] Following semen collection, anesthesia was reversed with an intramuscular injection of 4.4 mg/kg tolazoline (Tolazine®, Lloyd, Shenandoa, IA).

Once bucks were anesthetized, the penis was manually exteriorized and held with gauze to keep extended during collection. Semen was obtained using an electroejaculator and 3-electrode ram probe (31.75 mm diameter) under manual pulse stimulation (Pulsator IV Lane Manufacturing, Denver, CO). Semen was collected in a 15 ml conical tube submerged in a 37°C water bath. Raw volume was estimated by visual examination of the conical tubes and measured to be 1.7 ml (buck 1), 2.8 ml (buck 2), and 1.9 ml (buck 3). An aliquot of raw semen from each buck was diluted in lysis buffer at a 1:201 ratio for measurement of concentration using a NucleoCounter® SP-100™ system (ChemoMetec, Somerville, MA). Concentrations of raw ejaculates were measured to be 800 x $10^6$/ml (buck 1), 545 x $10^6$/ml (buck 2), and 800 x $10^6$/ml (buck 3). An aliquot of each ejaculate was also evaluated via light microscopy on farm and determined to have a total motility of > 70% before proceeding with processing. A final aliquot of each sample was formalin-fixed for evaluation of morphological assessment as a wet mount at 1,000 x magnification using a phase-contrast microscope. Ejaculates were determined to have acceptable morphology with ≤ 15% head defects, ≤ 15% tail defects, and < 25% total morphological defects.

Ejaculates were extended at 1:1-1:2 ratio with warmed TRIS A+ extender containing 20% egg yolk (provided by ST Genetics) to a final volume of ~ 5.6 (buck 1), 4.8 (buck 2), and 4.1 ml (buck 3) and were shipped overnight on ice to ST genetics, previously known as Sexing Technologies (ST Genetics, Fond du Lac, WI). Data provided by ST Genetics indicated that initial motility on arrival was ~ 80% for all samples and total concentrations were 643 x $10^6$/ml (buck 1), 316 x $10^6$/ml (buck 2), and 390 x $10^6$/ml (buck 3) prior to sex-sorting.

Sex-sorting was performed by ST Technologies using proprietary methodologies. Sorted semen assessments provided by ST Technologies are summarized (Table 1). Semen was packaged into 0.25-ml straws and either kept chilled or cryopreserved. Only Y-sorted (male) sperm were maintained as chilled samples for use within this case report. The X-sorted (female) sperm from bucks 1 and 2 and excess Y-sorted (male) sperm from buck 1 were cryopreserved, producing a total of 18 frozen straws with X-sorted sperm and 20 straws with Y-sorted sperm from buck 1 and 17 frozen X-sorted sperm from buck 2. The frozen sex-sorted semen samples were not used in this case study and kept by the farmer. Chilled samples were stored in the dark at 18°C and transported overnight back to Illinois. These fresh, chilled straws were used for laparoscopic AI ~ 48 hours (farm 1) or 72 hours (farm 2) after semen collection.

## Doe synchronization and laparoscopic AI with sex-sorted semen

Does from both farms were synchronized to allow for timed laparoscopic AI. To begin synchronization (day 0), does on each farm were guided through a chute system to allow for placement of intravaginal CIDR inserts containing 0.3 g of progesterone (Eazi-breed™ CIDR® Sheep Insert, Zoetis, Parsippany-Troy Hills, NJ). At CIDR insertion, the does were vaccinated with EHD/BTV 9-Way-Combination vaccine (Newport labs) and dewormed with topical moxidectin (Cydectin). On day 14, CIDR inserts were removed and does each received 150-200 IU of eCG (equine chorionic gonadotropin, Folligon, Merck Animal Health, Intervet Canada, Kirkland, QC, Canada). Laparoscopic AI was scheduled for 55-60 hours after CIDR removal and eCG treatment.

Does from farm 1 (n = 34) were synchronized for laparoscopic AI to be performed on the day that sex-sorted semen arrived, ~ 48 hours after semen collection, using only the chilled, sex-sorted semen. There were 4 does from farm 1 that were excluded from AI due to losing CIDRs during synchronization but were turned out with bucks at the same time as the does that underwent AI for live cover. Does from farm 2 (n = 17) were synchronized for laparoscopic AI to be performed the following day, ~ 72 hours after semen collection. A total of 10 does from farm 2 were inseminated with chilled, sex-sorted semen, whereas the remaining 7 does received conventional, frozen-thawed semen provided by the owner.

**Table 1.** Semen quality and straw dosages in fresh and sex-sorted semen as provided by ST Technologies

| | Percent total motility day 0 | Percent progressive motility day 0 | Percent intact acrosome | Purity | Percent total motility day 1 | Sperm dose ($10^6$/straw) | Total fresh male straws |
|---|---|---|---|---|---|---|---|
| Buck 1 | 91.6 | 77.4 | 91 | 0.88 | 88 | 4.79 | 19 |
| Buck 2 | 89.6 | 61 | 88 | 0.87 | 86.2 | 4.99 | 24 |
| Buck 3 | 85.9 | 65.6 | 85 | 0.9 | 68.8 | 4.59 | 13 |


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**Table 2.** Fawning data from does bred by laparoscopic AI using sex-sorted semen, conventional semen, or live cover

| | | Farm 1 | Farm 2 |
|---|---|---|---|
| Sex-sorted semen | Pregnant/AI (%) | 20/30 (67) | 8/10 (80) |
| | Total fawns | 33 | 16 |
| | Fawns per doe | 1.7 | 2 |
| | Male fawns/total (%) | 28/33 (85) | 16/16 (100) |
| Conventional semen | Pregnant/AI (%) | | 5/7 (71) |
| | Total fawns | | 8 |
| | Fawns per doe | | 1.6 |
| | Male fawns/total (%) | | 4/8 (50) |
| Live cover | Pregnant/bred (%) | 14/14 (100) | 4/4 (100) |
| | Total fawns | 24 | 6 |
| | Fawns per doe | 1.7 | 1.6 |
| | Male fawns/total (%) | 12/24 (50) | 5/6 (83) |
| Live cover plus conventional semen | Male fawns/total (%) | | 9/14 (56) |

Does were anesthetized to allow for low stress handling throughout the laparoscopic AI procedure, as described.[7] Does were guided through the chute and hand injected with tiletamine-zolazepam (0.88 mg/kg each) and xylazine (2.2 mg/kg). Following injection with the anesthetics, does were released into a small pasture until anesthetized, after which farm personnel placed a face mask on the doe and used a gurney to bring the doe back to the barn for the procedure. Anesthetized does were placed into a cradle in dorsal recumbency, and the entire abdomen caudal to the umbilicus was clipped and aseptically prepared. The 2 laparoscopic port sites were identified (~ 6-8 cm cranial to the udder and ~ 6-8 cm lateral to midline) and desensitized with an infusion of 1 ml 2% lidocaine hydrochloride.

An experienced veterinarian performed the laparoscopic AI procedure. A small incision (~ 1 cm) was first made into each of the blocked sites. A teat cannula attached to a $CO_2$ regulator was inserted into the peritoneum to allow for manual insufflation with medical grade $CO_2$. A 15-mm trocar was used to place the first cannula that allowed for placement of the laparoscope. On visualization of the uterus (confirming correct placement), a 5-mm trocar was used to place the second cannula. A 0.25-ml semen straw was loaded into an aspic device (IMV Technologies, Maple Grove, MN) that was placed through the second cannula. Semen was injected directly into the greater curvature of the uterus, with half of the straw inserted into each of the 2 uterine horns. After manually deflating $CO_2$ from the peritoneum and removing the cannulas, the incision sites were closed using 1 or 2 staples per site. Does each received 50 µg of GnRH agonist (Cystorelin, Boehringer Ingelheim, Ridgefield, CT) and a combined subcutaneous injection containing 2 mg/kg flunixin meglumine and 30 mg/kg oxytetracycline dihydrate (Hexasol, Norbrook, Overland Park, KS) in the axillary region. Anesthesia was reversed with an intramuscular injection of 4.4 mg/kg tolazoline. Does from both farms were turned out with an intact buck at ~10 to 15 days after laparoscopic artificial insemination. All does fawned in the Spring of 2017. Fawning data were recorded by the owners of each farm and provided for use in this case study.

## Outcomes

The fawning data are summarized (Table 2). Both farms had 100% total pregnancy rate for the herds (AI plus natural cover). AI pregnancy rates were determined using fawning dates. Any deer that underwent AI but surpassed a 201-day pregnancy length was assumed to be pregnant by the herd buck rather than by AI (n = 10 for farm 1; n = 4 for farm 2). The 4 deer from farm 1 that lost their CIDRs and did not undergo AI were also counted as pregnant by the herd buck, making a total of n = 14 counted as live cover for farm 1. The AI pregnancy rates for both sex-sorted and conventional semen ranged from 67 to 80%, which is consistent with industry standards. On average, there were ~ 1.6-2 fawns born per doe. The percentage of males born to sex-sorted semen was 85-100%, whereas the percentage of males born to live cover was 50% and born to conventional semen was 83%. The distribution of the specific bucks used for fresh, sex-sorted semen and pregnancy per AI rates between 2 farms are summarized (Table 3).

## Discussion

Semen sex-sorting has been used in domestic mammals (e.g. pigs, sheep, horses, cattle, and cervids) to obtain a high proportion of sperm carrying a chromosome of the desired sex.[8-10] To date, sex-sorted semen has gained the most commercial popularity in the dairy cattle industry that is attributed to ability of the bovine sperm to tolerate the sex-sorting process and subsequent cryopreservation.[8-10] In other species, more specifically pigs and horses, the high number of sperm needed to attain an optimal breeding dose and more fragile sperm have hindered the large-scale use of this technology.[8] In small ruminants, the use of sex-sorted semen is feasible due to the satisfactory results reported with low doses (1-5 x $10^6$ motile sperm) when laparoscopically inseminated directly into the uterus.[11,12] Sex-sorting semen could be an extremely beneficial tool for WTD producers to obtain a high proportion of male offspring. Sales of bucks to hunting farms across North America result in better prices, and slaughtered males yield more meat than females. White-tailed deer have an advantage over domestic animals, with excellent semen quality that has

**Table 3.** Does bred and pregnant per AI on each farm among 3 bucks collected for sex-sorted semen

| Buck | Farm | Does bred | Percent does bred (bred/total) | Percent pregnant (pregnant/AI) |
|---|---|---|---|---|
| 1 | 1 | 10 | 33 (10/30) | 60 (6/10) |
| | 2 | 5 | 50 (5/10) | 100 (5/5) |
| | **Combined** | **15** | | **73 (11/15)** |
| 2 | 1 | 13 | 43 (13/30) | 69 (9/13) |
| | 2 | 2 | 20 (2/10) | 50 (1/2) |
| | **Combined** | **15** | | **67 (10/15)** |
| 3 | 1 | 7 | 23 (7/30) | 71 (5/7) |
| | 2 | 3 | 30 (3/10) | 67 (2/3) |
| | **Combined** | **10** | | **70 (7/10)** |

a strong tolerance to cryopreservation when collected during rut.[4,5,6] Authors of an in vitro study reported that sex-sorting WTD semen using flow cytometry resulted in increased post-thaw motilities and decreased DNA fragmentation compared to conventional cryopreserved semen.[13] Despite this information on in vitro frozen-thawed sperm characteristics, there is limited information on field pregnancy rates in WTD using either fresh or frozen-thawed sex-sorted semen. The case study presented herein is the first to report the feasibility and usefulness of using fresh, sex-sorted semen in commercial WTD farms.

A recent review cited anecdotal data that reported pregnancy rates of 93-95% using cryopreserved sex-sorted cervine doses of 8-9 x $10^6$ sperm.[14] With fresh sperm, doses of 4 x $10^6$ sperm (used in the current report) have also reportedly produced better conception rates than conventional semen that is typically dosed at ~ 20 x $10^6$ sperm per straw.[14] In the current report, pregnancy per AI was numerically less for the conventional cryopreserved semen used (71%) compared to the chilled, sex-sorted semen (80%) for farm 2. For farm 1, we also report a lesser pregnancy rate (67%) than those reported anecdotally. In a large, controlled study using red deer (*Cervus elaphus*), pregnancy rates were greater for conventional frozen-thawed semen (~ 78%) versus Y sex sorted (50%) and control sorted semen (51%) when inseminated transcervically.[15] In dairy heifers, AI pregnancy rates did not differ between conventional frozen-thawed semen (60.9%) and fresh, sex-sorted semen (54.2-53.5%), but was greater than frozen, sex-sorted semen (52.8%).[16] On the contrary, cows inseminated with conventional frozen-thawed semen (48%) had greater AI pregnancy rates than those inseminated with sex-sorted semen, regardless if it was frozen or fresh (37.6-40.6%).[16] Based on these species differences, there is a distinct need to collect and publish data from a higher number of WTD to better determine if there are any relevant differences among conventional frozen-thawed, sex-sorted fresh, and sex-sorted frozen thawed semen that will allow us to make accurate recommendations to farmers on the best method to use to achieve acceptable AI pregnancy rates.

It is interesting to note that the pregnancy per AI was greater in farm 2 (~ 80%) from fresh semen that was 72 hours old compared to farm 1 (67%) where the semen was only 48 hours old, despite using similar synchronization protocols. There are few studies available that have investigated different dispatch-to-AI intervals and the potential effects on pregnancy per AI when using fresh, sex-sorted semen. In dairy cows, the dispatch-to-AI interval of fresh, sex-sorted semen did not affect pregnancy per AI, but did have an interaction with the bull.[16] Although pregnancy per AI was constant for most bulls, it actually increased with greater dispatch-to-AI intervals for 2 bulls.[16] This interaction highlighted the need to investigate variability in male semen characteristics and fertility when using fresh, sex-sorted semen. In an in vitro study using frozen-thawed semen from WTD, authors reported the sex-sorted semen had a decreased number of sperm containing fragmented DNA and improved DNA longevity over 72 hours compared to conventionally frozen sperm.[13] Sperm DNA fragmentation is apparently a useful predictor of male fertility, and future studies are needed to better evaluate its changes over time in fresh, sorted samples to better understand the in vivo results presented herein.

The purity of sex-sorted semen in cervids have been reported more recently to range from 92 to 95%.[14] The fawn crop produced from AI with sex-sorted semen herein reported 85% bucks born on farm 1 and 100% bucks born on farm 2. Interestingly, farm 2 also had a higher percentage of bucks born by live cover (83%) than farm 1 (50%). Though a low number of animals may likely have a role in this finding, combining those bred by conventional semen and live cover for farm 2 still produced a greater number of bucks born than expected (56%). It is worth noting that though diets and pasture were similar between these farms, farm 2 also used an experimental feed additive from ADM (ADM Animal Nutrition, Quincy, IL) that is formulated to increase the sex ratio for male fawns. Farm 2 used the feed additive until November 2016, encompassing 2 estrous cycles for the does on farm. Studies in both roe deer[17] and red deer[18] have linked higher energy diets and increased male offspring production, but additional studies on this management strategy are lacking. Further studies that compare the use of similar feed additives in conjunction with sex-sorted semen would be needed to better assess its usefulness in altering male offspring ratios in captive deer herds.

This case study demonstrated the feasibility of using fresh, sex-sorted semen for intrauterine laparoscopic artificial insemination in captive WTD farms. The semen from these 3 bucks survived transport and the sex-sorting processing. The sex-sorted semen was still viable for insemination at 48-72 hours after initial collection. Additionally, samples not needed for fresh insemination were cryopreserved and saved for use at a later date. This is the first published report for in vivo use of sex-sorted semen in WTD, though anecdotally, its use has

been gaining popularity throughout the past decade. Future controlled studies would be useful to help determine ideal dosages, timing of AI in synchronized does, and interactions with feed additives. In red deer, frozen, Y-sorted semen produced better AI rates when insemination occurred between 55 and 56 hours after eCG treatment (80-83%) compared to between 56 and 57 hours (< 33%).[15] Therefore, additional work at timing of insemination with frozen, sex-sorted semen in WTD is also needed. The use of sex-sorted semen with other advanced reproductive technologies, such as embryo flushing and in vitro fertilization, should also be critically analyzed to understand its usefulness in clinical practice.

## Learning points

- Semen from white-tailed deer can easily withstand sex-sorting for use in clinical practice with acceptable viability after sorting.
- The AI pregnancy rates using sex-sorted semen in white-tailed deer were consistent with industry standards.
- Selecting for Y-sorted sperm resulted in an increased percentage of males in captive white-tailed deer herds.


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## Conflict of interest

None to report

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