



Assessment of butorphanol-azaperone-medetomidine combination as anesthesia for semen collection and evaluation of semen quality in white-tailed deer (*Odocoileus virginianus*)



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ABSTRACT

The aim of this current study was to evaluate the level of anesthesia produced by a combination of butorphanol-azaperone-medetomidine (BAM) for semen collection by electroejaculation on captive white-tailed bucks (*Odocoileus virginianus*). Ten male white-tailed deer, weighing 68.2–115.9 kg, ranging in age from one to four years were randomly selected from housing pens and anesthetized with the BAM drug combination at a dose volume of 2.0 mL each. Semen was collected from each animal using a standard cervid electroejaculation protocol while under BAM anesthesia. Physiological data was recorded following induction of anesthesia and during semen collection. Collected ejaculates were prepared for analysis using a standard extender protocol for cryopreservation. Eleven sperm viability parameters were quantified for each sample using a Computerized Assisted Sperm Analysis system, including total seminal volume; sperm concentration and total sperm number. Kinematic parameters of motile spermatozoa were also assessed. Results demonstrated that BAM provided an effective plane of anesthesia for successful collection of viable sperm. Measured physiological variables of heart rate, respiration and body temperature all remained within safe, normal limits. Data recorded on semen characteristics from all collected ejaculates correlated well with key traits determined to be important for successful fertilization through measurement of total semen volume; sperm concentration; total sperm number; and kinematic parameters of motile spermatozoa. There were no serious adverse events. This field study indicates that BAM anesthesia is suitable for semen collection in white-tailed deer.

1. Introduction

Electroejaculation (EE) is the prevalent method of sperm collection in cervids and is used to repeatedly recover semen samples from selected males. (Garde et al., 2006) Historically the most common anesthesia protocol utilized for this procedure has been the drug combination of tiletamine/zolazepam and xylazine hydrochloride administered intramuscularly. (Monteith et al., 2012) This combination has been used in deer for over 30 years. Tolazoline hydrochloride or atipamezole hydrochloride is generally used to reverse this anesthesia after semen collection. Tiletamine/zolazepam and xylazine hydrochloride anesthesia has been known to induce such adverse side effects as acidemia, bloating, regurgitation, bradycardia, hyperthermia, hypoxemia, glycosuria, and anorexia. (Miller et al., 2009)

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Recently, a novel drug combination of butorphanol-azaperone-medetomidine has been developed for use as an anesthetic in non-domestic species. The site-specific alpha-2 – adrenergic agonist medetomidine has been administered as an alternative to xylazine for veterinary use for many years. It has proven to be a reliable, more potent alpha-2 agonist that can be quickly reversed by the alpha-2 antagonist atipamezole. Published studies combining medetomidine with butorphanol, a morphine-based schedule IV opioid, have reported effective immobilization of deer based on its synergistic central nervous system sedation (Miller et al., 2009). With addition of the neuroleptic sedative azaperone to this butorphanol/medetomidine combination, the formulation is abbreviated as the acronym BAM.

Since its introduction several published studies evaluating the efficacy of BAM have reported safe, rapid inductions and effective planes of anesthesia in several species (Miller et al., 2009; Siegal-Willott et al., 2009). These studies have also indicated a smoother, more rapid recovery when reversed with atipamezole and naltrexone antagonists versus traditional xylazine anesthetic combinations (Miller et al., 2009).

The BAM combination of butorphanol-azaperone-medetomidine administered in this study was a premixed solution containing 27.3 mg of butorphanol tartrate, 9.1 mg of azaperone tartrate, and 10.9 mg of medetomidine hydrochloride per 1.0 mL volume. This concentration of these pharmaceuticals enables convenient delivery of anesthesia using a single low-volume dart (Lance, 2008).

This study was the first assessment of semen collected from a single group of test bucks under BAM anesthesia.

2. Materials and methods

2.1. Animals

Ten white-tailed bucks were anesthetized using BAM, with one semen ejaculate per buck collected (on either December 8 or 9, 2015) at a captive breeding facility in Gillett, Wisconsin. All deer were identified by ear tags. Deer body weight ranged from 68.2–115.9 kg with age ranging from one to four years. Extensive studies have documented that the rutting period (the time each year when white-tailed deer are sexually active) typically occurs between October 1 and January 1 in Northern Wisconsin. During this breeding season, males are characterized by having hard antlers and maximal testis volume, proven to be associated with high levels of semen volume, sperm concentration, percentage of motile sperm, and serum testosterone concentrations (Mirarchi et al., 1975).

Division of Wildlife records have chronicled that white-tailed deer breeding begins in late October and peaks between November 3 and November 16 north of latitude 40 (Resources, 2001). Our selected study collection dates correlate positively with this optimal white-tailed breeding season in Gillett, Wisconsin (latitude 44.89° North).

Test group bucks were housed with individual breeding groups of does in separate multi-acre pens. However, since all mature does within the captive herd were bred via artificial insemination (through laparoscopic AI procedures) conducted November 5 and 6, 2015, males were not introduced into their assigned breeding group until November 20 or 21, 2015. With confirmed AI conception rates at this test facility documented to average 65% among each breeding group of 9–20 does, it could be estimated that test bucks had a limited opportunity to breed through natural cover with approximately 4–7 receptive does exhibiting estrus behavior. Published studies on male breeding success among both captive and wild white-tailed deer populations have reported the ability of individual bucks to successfully breed numerous does throughout the rut period (Ott et al., 2003; Sorin, 2004).

On the Wisconsin captive white-tailed deer facility where this study was performed (housing a herd of approximately 1500 head), a review of genetic breeding records compiled through use of modern paternity analyses, provided detailed offspring–sire relationships confirming their resident bucks have naturally bred up to 30 does in one season. In one published study conducted among wild white-tailed deer populations, Sorin analyzed genetic paternity assignments within a wild group of 47 females and 17 different males on a game reserve in southeastern Michigan. Tabulated results affirmed how some individual bucks mated with up to 7 females during one breeding season (Sorin, 2004). Reports from another study by Ott confirmed that individual male white-tailed deer were able to breed up to 64% of the females in two 200-ha enclosures populated with wild deer (Ott et al., 2003). Following review of database records from our captive deer test facility combined with previously-published studies of wild white-tailed deer populations, provided confidence that semen collections from animals in this study would provide acceptable ejaculate volumes and sperm quality. In addition, semen collections did not begin for a minimum of 30 days following final attempts for test bucks to naturally breed females, providing confidence that sufficient levels of testicular spermatozoa would be present for collection.

It was determined for this study to select bucks continuously housed with mature does based on previous trials reporting how continuous contact with females had enhanced male reproductive function in several species. While a recent study (Villagrán and Ungerfeld, 2013) confirmed that female presence within contained breeding groups resulted in increased testosterone levels and enhanced seminal parameters in pampas deer males (*Ozotoceros bezoarticus*), additional publications have reported this same correlation in other cervid species and non-domestic ruminants.

In 2004, Martin et al. discussed the socio-sexual signals and resulting stimulatory effects of “males on females, of females on males and of females on females” in many species (Martin, 2004). In addition to pampas deer males, this increase in testosterone was also observed in other cervids of differing social hierarchy including studies in wapiti (Haigh et al., 1984) Eld’ deer (Monfort et al., 1993) and red deer (Malo et al., 2009). Beyond cervids, a 1976 study by Illius et al. reported on the extreme social stimulus of housing male sheep in the presence of cyclic females and the resulting increased plasma testosterone levels in these rams (Illius et al., 1976).

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