Assessment of Breeding Soundness in White-Tailed Deer in Alabama

By

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Abstract

The deer family (*Cervidae*) has a nearly world-wide distribution, and deer farming has, in recent years, been gradually accepted as an economically promising industry. It is demonstrated that ungulate body size and horn/antler size are honest indicators of male sexual dimorphism and reproductive success. It is believed that infertility is unusual in natural populations; however, previous research identified variation in the quality of semen from individual deer. This suggests that post copulatory fertility success might be as important as the male phenotype, when evaluating reproductive success. Sperm motility, concentration, and morphology are known indicators of male reproductive ability. In deer, the relationship between spermatozoal defects and fertilization success is not well established, and a deeper knowledge of deer reproduction and fertilization success is still lacking. There is a need to establish standard protocols to evaluate semen quality in different cervid species and to expand the knowledge regarding the fundamental aspects of cervid reproductive biology. The specific objectives of this study were to: 1) investigate the seroprevalences of infectious reproductive tract pathogens; 2) assess semen quality; 3) investigate correlations between semen traits, age, and male phenotype; 4) describe correlation between seroprevalence of pathogens and semen quality in an enclosed free-ranging population of white-tailed deer in Alabama.

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AGID	Agar gel immunodiffusion
AI	Artificial insemination
BCS	Boone and Crockett score
BHV	Bovine herpesvirus
BTV	Bluetongue virus
BHV	Bovine herpesvirus
BVD	Bovine viral diarrhea
CerHV	Cervid herpesvirus
CR	Calving Rate
DMR	Distal midpiece reflex
EHDV	Epizootic hemorrhagic disease virus
ELISA	Enzyme linked immunosorbent assay test
IB	Immunoblotting
IBR	Infectious bovine rhinotracheitis
IFAT	Immunofluorescence antibody test
IHC	Immunohistochemistry
IPV	Infectious pustular vulvovaginitis
MAT	Microscopic agglutination test
NAT	Nucleic acid amplification test
NR	Non return rate
PCA	Principal component analysis

PCR Polymerase chain reaction

QDM Quality deer management

RT-PCR Reverse transcription polymerase chain reaction

Chapter 1: Literature Review of Deer Management Practices and Reproductive Behavior

Introduction and History

The deer family (*Cervidae*) today has a nearly world-wide distribution, having been introduced into Australia, New Zealand, and New Guinea (Vos, 1983). Deer farming has been practiced for a century or more in Asia and has developed as a novel business elsewhere, and has been gradually accepted as an economically promising industry (Vos, 1983). Deer can adjust to a broad range of habitat types from woodlands to agricultural land, and they are considered generalists (Strickland & Demarais, 2020; Yoccoz et al., 2002).

Humans are a vital part of the ecology of deer (Mackie et al., 1998), and we have a great long-term impact on their habitat. Deer habitat is diverse and must include not only the basic elements for survival, but also support the social behavior of deer. In its early days, deer science had only a limited understanding of the complexities of natural ecological systems and deer-habitat interactions. A greater understanding of deer science was developed later (Mackie et al., 1998). In the future, deer farms could play an essential role in rural land planning as they can be economically viable units on land that is not fit for traditional livestock farming operations or other types of land utilization (Vos, 1983). This is one reason why deer farming could be appropriate, especially in developing countries (Vos, 1983).

Deer Management Practices

Deer farming was characterized as the husbandry of deer populations to produce profitable venison and other by-products including hides, velvet, antlers, and musk (Vos, 1983). People may influence the evolution of certain animal species by selective breeding

to adapt them to a specific habitat and husbandry, through the selection of desirable traits and culling of unsuitable ones (Vos, 1983). Herd management requires detailed knowledge of deer ecophysiology, as well as a knowledge of deer nutrition.

Deer managers use several methods to evaluate population density estimates (Mackie et al., 1998). Hoof print calculations, hunter observation data, spotlight data, and observational cameras are utilized to estimate deer count. The deer density in the population can be counted through these techniques. Although an exact number of deer cannot usually be obtained, the techniques can be beneficial in monitoring overall deer population changes. For the manager, it is crucial to know if a population is decreasing, growing, or remaining steady in an environment. Maintaining a deer population within the limits of a satisfactory carrying capacity should be the main objective of deer managers (Mackie et al., 1998). Evidence can be gathered for decisions regarding deer harvest management (Cook & Gray, 2003). Deer farmers must make husbandry decisions that include how many animals should be culled and what the male-female ratios should be for a reasonable herd growth. They must also assess fawn mortality and remove diseased individuals (Vos, 1983). A good economical profit depends on a well-managed herd composition (Vos, 1983).

Quality Deer Management, QDM

Managing deer herds in a socially and biologically healthy manner inside a deer habitat and area is the main goal of quality deer management (Pierce et al., 2015). The QDM cornerstone includes controlling several deer population parameters, including density, sex ratio, and male age structure. The first step in improving male age structure involves safeguarding young males from harvest. There is a dual benefit of this practice,

as there are more males alive within the population as well as an increased prevalence of older males later on (Strickland & Demarais, 2006). QDM enables managers to become supervisors of a deer herd by improving habitat quality, overall age structure, sex ratio, harvesting and by keeping comprehensive documentation on deer observations. This will lead to a successful QDM program (Pierce et al., 2015). Essentially, as concluded by Pierce et al. (2015), QDM is about allowing the deer herd in an area to reach its full potential, and are not about promoting only large trophy males. This promotes healthy deer management. Furthermore, Pierce et al. (2015) state that managing a deer herd is complicated and is affected by many factors, some of which even wildlife biologists are unaware of. Recognizing the biological and social factors that may be limiting management objectives, is the best approach to any deer management problem (Pierce et al., 2015).

Male Reproductive Success in Deer

Reproductive strategies amongst cervid species vary widely within and between areas (Asher, 2011). It would not be accurate to consider any deer species as 'typical' in terms of reproductive capability. Some deer display cyclical patterns of reproduction in cool temperate climates, while others have a aseasonal patterns of reproduction (Asher, 2011).

White-tailed deer (*Odocoileus virginianus*) use a tending-bond mating system (Demarais et al., 2012). The buck roams widely in search of a doe in heat; he may spend many hours tending, waiting for her sexual receptivity. On the other hand, other deer species, such as American elk (*Cervus canadensis*) and red-deer *(Cervus elaphus),* have a more competitive harem mating system (Demarais et al., 2012). This mating behavior in

deer allows easy access to females in heat and, the most dominant, older, and larger males gains exclusive breeding access to females and defends his harem (Demarais et al., 2012; Røed et al., 2002). Young males choose not to confront these mature males for breeding access, instead they use energy on skeletal growth and reaching physical maturity before investing energy in mating. A theory exists that in a more age-structured deer population, few dominant males perform most of the breeding, while behavioral interactions inhibit the breeding of subdominant males (Hirth, 1977; Miller, 1997). In white-tailed deer populations, subdominant and younger males were demonstrated to sire offspring as well (Demarais et al., 2012; Demarais et al., 2011; DeYoung et al., 2009; DeYoung et al., 2006; Newbolt et al., 2017; Turner et al., 2016).

Sexual size dimorphism and secondary sexual characteristics have reproductive advantages in mammals through evolution, which is the base of selection theory (Andersson & Iwasa, 1996). Important causes of sexually selected characteristics are thought to be generated through male rivalry (Andersson & Iwasa, 1996). A short breeding season and intense rivalry among males, are the hallmark of polygynous ungulate mating systems, and the sexual dimorphism and the secondary sexual characteristics in males are thought to be generated through selective pressures that form under these circumstances (Ciuti & Apollonio, 2016).

Male body size, reproductive success, and horn or antler size are indicators of sexual dimorphism and exist in several ungulate species (Festa-Bianchet et al., 2000; Kruuk et al., 1999; McElligott et al., 2001) These physical traits are linked to age in several ungulate species, male reproductive success therefore is generally greater for adult males than for young deer (Festa-Bianchet, 2012). Contrary to this belief, body size, age, and horn/antler size as determining factors of male breeding success has shown to be of

less importance in some ungulate species (Demarais et al., 2011; Hogg & Forbes, 1997; Neuman et al., 2016; Pelletier et al., 2006).

Genetic Paternity Studies in White Tailed Deer

The fundamental theory of deer ecology and management is based on the thought of a dominance-based breeding hierarchy of deer, and this theory impact both management policies and population estimates (DeYoung et al., 2006). Even though observational research on white-tailed deer has suggested a dominance-based breeding hierarchy, recent molecular research suggest a more complex system (Demarais et al., 2011; DeYoung et al., 2009; DeYoung et al., 2006; Sorin, 2004; Turner et al., 2016). The fact that all deer mating is not observed, and that all mating does not automatically result in fertilization, creates a discrepancy between observational versus genetic results in studies evaluating paternity in deer (DeYoung et al., 2006).

A pure dominance-based breeding system is not supported through genetic studies in white-tailed deer, in which paternity was assessed (Demarais et al., 2012; Demarais et al., 2011; DeYoung et al., 2009; DeYoung et al., 2006; Sorin, 2004; Turner et al., 2016). Previous studies demonstrated evidence for a high rate of multiple paternity in single litters (22-25%) of wild and captured white-tailed deer (DeYoung et al., 2006). The fact that breeding success is scattered among many males in a mixed population of free-ranging white-tailed deer is demonstrated by the patterns of genetic kinship. One adult male or a few larger males are therefore not monopolizing the breeding (DeYoung et al., 2006; Sorin, 2004). A microsatellite paternity analysis was conducted by Sorin (2004) in white-tailed deer, which demonstrated that the offspring was sired by males from all age

classes. The mating was not monopolized by the most dominant males in this study (Sorin, 2004).

A study by DeYoung et al. (2006) evaluated a captive population of white-tailed deer during the rutting season, and their dominance ranks were assessed through behavioral observations. While dominance of the deer was associated with mating success, monopolized breeding by a dominant male was not observed, as demonstrated by several multiple paternity litters and the siring of offspring by subordinate males (DeYoung et al., 2006). The explanation for the observed patterns of male breeding patterns in free-ranging white-tailed deer was likely due to behavioral and ecological factors, and that dominance is crucial to male breeding success (DeYoung et al., 2006). This agrees with the findings of Fiske et al. (1998), who obtained more thorough knowledge of deer lekking mating system and the sexual selection. A combined meta-analysis was conducted on a broad range of taxa, and the results indicated that male reproductive success was correlated with behavioral characteristics such as male aggression and lek presence (Fiske et al., 1998).

The antlers of white-tailed deer are a physical factor that has long been thought to play an essential role in establishing social dominance among males and equally securing mating success (Demarais et al., 2011). Another study evaluated the effects of body weight, while removing the effect of antlers (Demarais et al., 2011). Those results suggested that greater body weight was associated with a greater level of breeding success, while low relative body weight had a negative effect on mating success. However, the study also determined that greater body weight alone does not always guarantee greater breeding success. Just because dominant bucks with higher body weight

were able to mate more often did not mean that subordinate males were not frequently able to successfully breed as well (Demarais et al., 2011).

Newbolt et al. (2017) investigated factors influencing reproductive success in enclosed free ranging white-tailed deer bucks in Alabama. The study evaluated male breeding success within different age structures and sex-ratios. The male phenotype and secondary sexual characteristics were correlated with the paternity results of 143 fawns between the years 2007–2014. The study results indicated that individual breeding success was correlated with antler and body size (Newbolt et al., 2017). Antler size was correlated to differences in mean male age of the herd, and in older age structured groups the antler size had the biggest effect on male reproductive success. Younger males ≤1.5-year-old, bred more frequently in younger age groups and when there was a female-sex bias. A study of Demarais et al. (2011), demonstrated a clear association between dominance, age, and breeding success in a group with a younger male age ratio (1.5-2.5 years), where in older aged groups, the age did not necessarily correlated with mating success.

The findings of the studies by (Demarais et al., 2011; Newbolt et al., 2017) suggest that patterns of male reproductive success was determined by male age structure and sex ratio. These studies emphasize that antler size and body size are crucial to male reproductive success in white-tailed deer. The biggest males with the largest antler and body size have demonstrated not to monopolize breeding under any observed circumstances (Demarais et al., 2011; DeYoung et al., 2009; DeYoung et al., 2006; Sorin, 2004; Turner et al., 2016). One explanation for lower importance of dominance for breeding success of male white-tailed deer as compared to other deer species is that behavioral or habitat traits may limit opportunities to practice dominance (Sorin, 2004).

However, the display of alternative breeding tactics might influence breeding success (DeYoung et al., 2006). False conclusions can be drawn because physical qualities, assumed to express dominance, may not be properly evaluated in a field setting. Furthermore, a larger number of inferior males might gain access to mating opportunities, as dominance may not be steady throughout the entire rutting season in deer (DeYoung et al., 2006).

Turner et al. (2016) evaluated the genotype of 731 white-tailed deer housed on a farm with a balanced sex ratio and age structure. The study demonstrated that the younger <2,5-year-old males accounted for more than half of paternity (59%). According to Turner et al. (2016), a balanced sex ratio and age structure, enables mating by younger males. In a study of white-tailed deer breeding success was distributed among several populations, with no sign of breeding monopolization by any particular male (DeYoung et al., 2009). Remarkably, subordinate males (1.5-2.5 years of age), jointly sired 30–33% of fawns, even with older males present in populations (DeYoung et al., 2009). Monopolized breeding access to females is apparently hindered by ecological and behavioral variables (DeYoung et al., 2009). The primary influences of mating success of white-tailed deer would be the interaction among sex ratio, age structure, and dominance relationships (DeYoung et al., 2009). This conclusion is strengthened by findings of genetic studies in varied age class populations, in which breeding was distributed among all age classes (DeYoung et al., 2009; Sorin, 2004; Turner et al., 2016). Herd managers must be aware that white-tailed deer breeding systems are complex and the anticipated outcome is not always achieved by manipulating breeding systems through factors such as sex ratio and age (Turner et al., 2016).

Environmental Factors and Nutrition

Genetics ultimately control the restrictions of antler growth, but nutrition and age have the most significant association in most management programs (Demarais et al., 2012). Pierce et al. (2015) emphasized that the potential for male antler growth is passed on by both sexes, and antler development is influenced by environmental factors as well as proper nutrition. A habitat can only support a certain number of well-conditioned deer (Cook & Gray, 2003). Whenever the population density exceeds the limits, habitat quality and deer health will decline (Cook & Gray, 2003).

In a study conducted in Mississippi, other factors that influence the male phenotype, such as nutrition and age, were regulated (Michel et al., 2017). The study included pregnant females from a natural population and six-month-old fawns that were kept in a pen. The deer were fed ad libitum the same superior 20% crude protein deer pellet diet. The authors individually marked each neonate, recorded body measurements, and compared these values to a free-ranging control group. The deer morphometrics in the study group increased in size from the first to second generation. Antler size increased by ~40% and body size increased by ~25% in the second generation of the study group, as compared to the free-ranging control group. The results by Michel et al. (2017) indicated that differences in nutritional quality rather than an animals' genetic make-up have a significant impact on white-tailed deer phenotypic variation in a natural population.

Importance of Knowledge in Deer Reproduction

There is a general belief that natural selection would make male infertility an unusual finding in natural populations (Jennions & Petrie, 2007). Sterile males would not be able to sire descendants and would therefore reach an evolutionary end point. Infertility in deer may be hereditary (Gomendio et al., 2000; Roldan et al., 1998), and animals may also have decreased fertility due to environmental reasons such as insufficient nutrition, stress, or infections with pathogens (Ahirwar et al., 2018; Boakari et al., 2022; Hopkins, 2007; Kastelic, 2014; Michel et al., 2017; Samsudewa et al., 2018).

As mentioned earlier, genetic studies in white-tailed deer demonstrated that breeding in white-tailed deer was distributed among various age classes in a more balanced age class population and not only dominant large males (DeYoung et al., 2009; Sorin, 2004; Turner et al., 2016). Deer managers must be aware that deer breeding systems are complex and influencing breeding systems through factors such as sex ratio and age structure may not generate the desired result (Turner et al., 2016). Not all observed deer mating necessarily result in fertilization (DeYoung et al., 2006), and a deeper understanding in cervid reproduction is therefore valuable.

Chapter 2: Literature Review of Male Reproduction in White-Tailed Deer

The Reproductive Tract of the Male Deer

The male reproductive tract structures in deer consist of the testicles, prepuce, penis, ductuli deferentia, epididymides and the accessory glands (Haigh, 2007a; Lincoln, 1971). The testicles are in the scrotum between the pelvic limbs. The penis has a simple rod-shape and does not increase considerably in circumference during erection, but its length increases by around 40%. In contrast to other ruminants, the sigmoid flexure is not present in deer (Haigh, 2007a). The urethra is located on the ventral surface of the penis, and it curves and thereby allows an ascending urination and ejection of spermatozoa, also called “thrash-urination” in the period of sexual excitement and activity. Males are able to spray urine upward almost at right angles onto themselves (Haigh, 2007a).

A very rapid forward and backward movement of the prepuce is possible due to the well-developed preputial musculature (Haigh, 2007a). At the onset of the breeding (rutting) season, the scrotal circumference increases significantly in deer to approximately double or triple in size, before the breeding seasons in some deer species (Haigh, 2007a). The circumference declines steadily after the rut and reaches its smallest size in spring. The scrotal circumference, the concentrations of serum testosterone, and the percentage of normal sperm in semen undergo synchronous annual changes (Haigh, 2007a). Activity within the testicular parenchyma increases in correlation with the rise in testosterone levels, as does the development of the seminiferous tubules and the epididymis. The testicular size therefore increase in size during the rut and the number of testicular interstitial cells peaks (Haigh, 2007a). The interstitial cells were demonstrated to be small and inactive in spring after the rut is over, and their lumen does not contain any mature sperm (Lincoln, 1971).

Spermatogenesis and Deer Spermatozoa

The study of sperm morphology connects closely with study of testicular histology. One needs to understand abnormal spermatogenesis and sperm morphology when engaging in this discussion (Barth, 1989). Sperm cells are formed inside the seminiferous tubule epithelium, which comprise 90% of the mass of the testicles. The testosterone producing Leydig cells, vessels, and lymphatics are scattered between the tubules. Cellular differentiation, in which spermatogonia stem cells undergo several mitotic divisions, occurs during spermatogenesis, where meiosis and cytological transformations finally produce a mature spermatid (Barth, 1989). The spermatids, which begin as haploid round cells produced by meiosis, undergo a complex series of transformation to form a final layer of elongated spermatids, each witch head and tail, which are delivered into the lumen of the seminiferous tubule. The final cellular transformation is referred to as spermiogenesis. The Sertoli cell, whose cytoplasm incorporates the germ cells, helps in the development of these cells by providing nutrition and hormonal support (Barth, 1989).

The means of transportation, maturation, and storage of spermatozoa is provided by the epididymis. Spermatozoal abnormalities can be traced back to the epididymis or the spermatogenesis (Barth, 1989). The spermatozoa of domesticated mammals consist of a flattened head, midpiece, and tail (Barth, 1989; Garner & Hafez, 2000). The spermatozoa of deer species and domestic ruminants appear to have the same overall appearance (Haigh et al., 1984). Sperm motility derives from energy supplied mainly by oxidative mechanisms within mitochondria in the sperm midpiece (Chenoweth & Kastelic, 2007). Spermatozoa gain the ability to be progressively motile when they are

transported within the epididymis and the cytoplasmic droplet travels from the proximal midpiece distally, the epididymis hereby prepares the spermatozoa for fertilization of the female (Amann & Schanbacher, 1983). The accessibility of testosterone is an important factor in sperm maturation (Chenoweth & Kastelic, 2007).

Semen Collection in Deer

Male infertility in natural populations has seldom been explored because it has been believed that a strong natural selection would automatically work against it and therefore result in a naturally high mating success associated with fertilization success (Gomendio et al., 2007). Domestic livestock have been studied more closely as the agricultural industry has been governed by finances with the goal of increasing overall productivity. In domestic livestock, strong artificial selection has been employed to maximize fertility (Gomendio et al., 2007). Male deer were demonstrated to differ markedly in their fertility in natural populations and variation in semen traits have been discovered, both among and within males (Gomendio et al., 2007).

Secretions from the accessory sex glands contain whitish fluid suspended with sperm cells called semen (Hussain, 1995). Wapiti and red deer appear to have the same sperm morphology as bovine bulls and therefore the same sperm classifications can be applied for those species (Haigh et al., 1984). Morphological traits of spermatozoa can be influenced by the intensity of their production in the testes as well as the number of spermatozoa stored in epididymides and excreted in an ejaculate (Wysokińska et al., 2009). The environment plays an important role in cervid sperm morphology (Haigh et al., 1984). In the northern hemisphere, semen samples from elk bulls contain a great

percentage of abnormal spermatozoa in the summer, but by the beginning of fall the concentration of normal sperm increases (Haigh et al., 1984).

Morphological Sperm Abnormalities

Abnormal spermatozoa have been historically associated with male subfertility and sterility in the bovine bulls (Saacke, 2004). Evaluation of sperm morphology is a crucial component of a thorough male breeding soundness examinations (BSE) in all species (Koziol, 2021). Assessment of semen is expected to provide an indication of fertility of the male or semen used in artificial insemination. The goal of this effort is to predict fertility (Saacke, 2004). No specific test of a semen sample can perfectly predict fertility (Ax et al., 2000). Through the development of spermiograms (description of sperm morphology during evaluation), veterinarians can develop a diagnosis for the visualized disruption in spermatogenesis and the likely probability for recovery (Koziol, 2021). When an abnormal spermiogram is found, the types and number of abnormalities combined with history regarding environment, nutrition and health status can be used to reach an explanation for an abnormal spermiogram. The veterinarian can then use that information to make a diagnosis and prognosis for recovery in an animal (Koziol, 2021).

The most common causes of abnormal spermatogenesis in males include: abnormal testicular thermoregulation; hormonal imbalances, stress, toxins, and expression of harmful genes (Barth & Bowman, 1994). Stress typically elevates systemic cortisol concentrations (Boakari et al., 2022), profoundly decreasing release of luteinizing hormone and testosterone (Knight et al., 2017). Stress has many origins, including environment, illness, or injury, causing changes in the spermiogram like those induced by disruption of thermoregulation. The primary spermatocyte is extremely susceptible to

changes in the hormonal milieu secondary to stress or illness. In the cases of testicular degeneration, the changes occur first in the cytoplasm, centrosomes, and spindles at the level of the primary spermatocyte which predispose to disturbances in the developing spermatid (Barth, 1989).

The occurrence of certain types of abnormal sperm are more serious and can indicate that normal-appearing sperm in the same sample may also be abnormal or incompetent in fertilization or maintaining embryogenesis (Saacke, 2004). The impact on the fertilization rate of sperm not capable of participating in fertilization would be dependent upon the total numbers of sperm in the dose and the level of abnormal spermatozoa in the sample. Such a deficiency can theoretically be overcome by increasing the sperm dosage (Saacke, 2004). Such an abnormal trait might therefore be considered compensable if the minimum number of sperm necessary by the female can be met by the "normal" sperm population in the insemination dose. On the contrary, sperm capable of penetrating the ovum, and initiating fertilization and/or embryogenesis, but not capable of maintaining either or both events would be considered uncompensable. In such a case, an abnormal spermatozoon would prevent fertilization by normal sperm. This would result in subfertility or sterility despite increasing the sperm dosage (Saacke, 2004).

Fertilization Studies in Domestic Animals

The reproductive success among polygynous mammals differs greatly, and a focus has been directed to understand how natural selection have formed characteristics that increase male mating success but also sperm competition (ability to fertilize female after coitus). There has been a general notion that male infertility is uncommon in natural

populations and that there is a possibility that males may differ in their fertility due to natural selection and evolution (Malo. et al., 2005). Approximately 50–60% of the variation in fertility among males can be accounted for by evaluating semen characteristics, leaving the objective of predicting fertility from laboratory evaluation of semen a real challenge (Saacke, 2008). Male infertility is as complex as female infertility (Saacke et al., 2000).

Strong associations between male infertility and sperm abnormalities in animals have been reported (Alm et al., 2006; Attia et al., 2016; Casey et al., 1997; García-Vázquez et al., 2015; Malo. et al., 2005; Ramón et al., 2013; Sullivan & Elliott, 1968; Tesi et al., 2018). These abnormalities vary from subtle changes to morphological defects that are apparent upon clinical examination. Fertilization and pregnancy outcome can both be governed by sperm morphology (Chenoweth, 2005). Sullivan and Elliott (1968) evaluated bull fertility using the minimum number of motile sperm required for fertilization. These studies noted that highly fertile bulls required less sperm than subfertile bulls to achieve maximum fertility. The fact that more sperm was required by subfertile bulls was due to morphologically abnormal sperm that was unable to access to the ovum and fertilization site. This finding was confirmed by a study by Saacke et al. (1998), that demonstrated that sperm with head abnormalities did not access to the fertilization site. These studies encourage to view semen morphology defects as falling into two major categories (compensable and uncompensable) (Saacke, 2008).

Tesi et al. (2018) evaluated 251 semen samples from 140 dogs, from which the concentration, volume, total number, motility, and amount of abnormal sperm were evaluated. The number of abnormal spermatozoa was lower in young animals than in older ones. Ejaculate volume was lower in small dogs compared to larger dogs, which

demonstrated that the size of the dog affected the ejaculate volume and total sperm number. Younger dogs had a lower proportion of midpiece defects in comparison to old ones. The semen used in artificial inseminations (AI) resulting in a pregnancy had a sperm number of 627.6 million, motility of 83.9%, and a proportion of morphologically normal spermatozoa of 64.9%. This was greater than in the unsuccessful AI, in which average sperm numbers of 389.4 million, an average motility of 66.5%, and an average morphologically normal spermatozoa of 42% were detected. Male fertility after artificial insemination may therefore be affected by altered semen parameters as well as the age and size of the animal (Tesi et al., 2018).

Profitable artificial breeding of dairy cattle today requires high-quality semen (Attia et al., 2016). In the study of Attia et al. (2016), the author correlated sperm morphology of 695 bulls with calving rate (CR) of cows. CR was affected by the amount of normal sperm in the AI ejaculate, highlighting the need of morphological analysis in the evaluation of fertility (Attia et al., 2016).

To evaluate fertility in swine, Alm et al. (2006) evaluated 10,773 homospermic samples (semen from a single male animal) from 50 boars that were used for AI at a dose of two billion spermatozoa. Semen with three billion spermatozoa was used for 34,789 homospermic inseminations from 96 boars. A 60-day non-return rate (NR%) (percentage of sows that were not returned to service after the first inseminations) was used as a fertility determinant (Alm et al., 2006). The primiparous litter size and multiparous farrowing were counted. Litter size and NR% of both multiparous and primiparous farrowing were greater the greater dose of spermatozoa compared with the smaller dose. NR% in both insemination regimen, in both insemination doses, correlated clearly with

sperm morphology. These results confirm the quantity of sperm in the insemination dose can partially compensate for poor sperm morphology (Alm et al., 2006).

Casey et al. (1997) evaluated sperm head characteristics in ten fertile and ten subfertile stallions, and a minimum of 200 spermatozoa were evaluated for each stallion. In the subfertile stallions there was a larger mean measurement for length, area, and perimeter for the spermatozoal heads than in the fertile stallions. The breadth of sperm heads from males in the fertile group was smaller than those of subfertile stallions. Fertile and subfertile stallions might therefore differ in their sperm head morphology (Casey et al., 1997).

An experiment conducted by García-Vázquez et al. (2015) evaluated the impact of the sperm morphology and uterine environment in swine. The amount of morphologically abnormal sperm was greater in the vaginal backflow (mechanisms by which sperm are cleansed from the female genital tract); in contrast, a larger amount of morphologically normal sperm was present at the fertilization site (García-Vázquez et al., 2015).

Fertilization Studies in Deer

Sperm samples were evaluated from 71 Iberian red-deer (*Cervus elaphus hispanicus*) (Garde et al., 1998). A total of 142 sperm samples were obtained and the stags were grouped in age groups: 2-3y, 4-7y and >8 y. The spermatozoa were acquired from the cauda epididymis of deer carcasses. The number of motile spermatozoa and normal morphology was assessed immediately after collection. Younger individuals had a lower semen quality (morphology and motility) than the adults and the lowest cell quality was found in the group aged 2-3 years. The observations in that study suggested that both

the spermatogenesis and the spermatic maturity in the epididymis are suboptimal in young red deer individuals (Garde et al., 1998).

Malo. et al. (2005) examined 188 semen samples from Iberian red-deer (*Cervus elaphus hispanicus*) carcasses over the rutting season. Spermatozoa were recovered postmortem from the caudae epididymides. A value of 62% of motile sperm was observed (range, 0–90%) with an intermediate motility quality. Sperm motility was 90%, acrosome integrity was 86%, and the morphologically normal spermatozoa was at a high average of 77% but with a large range of 12–97% (Malo. et al., 2005). Significant associations were observed in the sperm amount, sperm velocity, sperm motility, and amount of morphologically normal spermatozoa (Malo. et al., 2005). In the same study, an AI trial was conducted to investigate if male reproductive success was correlated to any specific semen traits. 247 females were inseminated with semen from 11 male red deer from natural populations. Spermatozoa from one male were used to inseminate each female once. To assess the importance of semen variables, all females were inseminated with the same sperm dose. The proportion of normal sperm and the sperm velocity parameters showed significant associations with fertility (Malo. et al., 2005). This agrees with Ramón et al. (2013) who determined in their study that red deer (*Cervus elaphus)* males with slow spermatozoa demonstrated lower fertility. Conversely, males that had a greater percentage of spermatozoa with faster velocity were more fertile. The results from Malo. et al. (2005); (Ramón et al., 2013) demonstrated that semen traits and fertility differ among male red deer in natural populations.

One reason for a variation in male deer fertility might be that domestic species have been studied more closely and undergone genetic selection for improved fertility for many generations in the same environment, whereas a wide degree of variation in semen

characteristics is observed in males from natural populations (Malo. et al., 2005). The ability of males to fertilize females after copulation is as important as body traits and secondary sexual characteristics when evaluating male deer reproductive success. However, abnormalities of spermatozoa in relation to fertilization success have not been evaluated in deer (Fitri et al., 2017). Furthermore, various artificial insemination methods with frozen-thawed semen in white-tailed deer evaluating the minimal efficient breeding dose of sperm has not been established (Stewart et al., 2018).

One study in Mississippi by Jacobson et al. (1989), used semen from eight white-tailed deer in 53 artificial inseminations. In the study, deer were artificially inseminated with doses of semen ranging from 12-60 million progressively motile spermatozoa and a pregnancy rate of 65-100% was reported; however, the semen morphology was not reported in that study (Jacobson et al., 1989). Twenty eld deer *(Cervus eldi thamin*) hinds were artificially inseminated in the uterine horns with semen containing 7.5 million motile spermatozoa per uterine horn, generating a pregnancy rate of 100% (Monfort. et al., 1993). A report in fallow deer (*Dama dama*) demonstrated pregnancy rates for does receiving laparoscopic intrauterine inseminations were 58.2% and 76.1% with 50 million spermatozoa in 79 does and 46 does respectively and 80.8% with 25 million spermatozoa in 26 does (Asher et al., 1990). It is noteworthy that in both AI research studies by Asher et al. (1990) and Monfort et al. (1993), the donor semen morphology evaluation was not reported, similar as to a study in white-tailed deer by Magyar et al. (1989). Similarly, Aller et al. (2009) compared pregnancy rates of red-deer does artificially inseminated with either an intrauterine deposition or an intracervical deposition of sperm. The donor semen was from cryopreserved straws and imported from New Zealand with a reported post-thaw motility of >30%, without mention of sperm morphology (Aller et al., 2009).

Does Semen Quality Matter in Natural Populations?

Malo. et al. (2005) discussed the variability in male fertility in natural populations should not be overlooked when evaluating factors which influence male breeding success. Inbreeding decreases the percentage of normal spermatozoa in wildlife (Gage et al., 2006; Opatová et al., 2016; Ruiz-Lopez et al., 2010), which could therefore be a defining factor of male fertility in natural populations. In contrast, there are studies that demonstrated that inbreeding does not increase abnormal sperm count, as in the study of Losdat et al. (2018), in which wild pedigreed song sparrow (*Melospiza melodia*) were evaluated. It is clear that a greater understanding is needed in how different fertility rates in natural populations correlates with male reproductive success, and how this can limit female reproduction (Gomendio et al., 2007).

Investigating reproductive factors like environmental and genetic stress would be helpful to understand how they affect a variety of semen characteristics. Providing more knowledge reproductive rates within deer populations and how they are affected by male fertility would be beneficial, especially when comparing them to different breeding systems (Gomendio et al., 2007).

Reproductive Tract Pathogens in Ruminants

Epizootic hemorrhagic disease virus (EHDV) and Bluetongue virus (BTV)

Epizootic hemorrhagic disease virus (EHDV) and Bluetongue virus (BTV) are classified into the genus Orbiviruses. Biting midges (*Culicoides)* are the transmitting vectors and the viruses affect both domestic and wild ruminants (King et al., 2012). EHD is a disease that often causes death losses in white-tailed deer and, less often, BT results

in a similar illness in cattle (Murcia et al., 2009). PCR testing is commonly used for detection of EHDV and BTV in blood or tissues, while antibodies against both viruses can be detected by ELISA or a virus neutralization test. When evaluating results from AGID assays, cross-reactivity between BTV and EHDV antibodies should be considered (Work et al., 1992).

Bluetongue virus, BTV

There are twenty-four different serotypes of BTV, and sheep are considered the most severely affected species, followed by goats, and white-tailed deer in the US (Murcia et al., 2009). A subclinical infection is usually seen in cattle, which act as reservoirs of BTV in endemic regions (Murcia et al., 2009). BTV has been reported in several outbreaks in Europe but also tropical and subtropical areas globally. Fever and hyperemia of oral and nasal mucosa are often noted in the beginning of an infection in animals, leading to significant salivation and nasal discharge. The tongue may become cyanosed in severe cases; thus, the disease is called bluetongue. Lameness and necrotic lesions of the oral tract and hooves can develop approximately one week following the beginning of clinical signs (Murcia et al., 2009). In pregnant sheep, weight loss is common, and abortions and death losses can occur. Protective immunity against other BTV serotypes may develop (Murcia et al., 2009).

Murcia et al. (2009) conducted a field study demonstrating that natural BTV-8 infection has a significant impact on semen quality in rams, leading to temporary infertility. 167 samples from 79 BTV-8 infected rams were assessed for changes in semen composition. After a natural BTV-8 infection, a significant change in all semen

characteristics (concentration, morphology, motility, percentage of living and dead spermatozoa), were observed. The most frequent morphological abnormalities were separated head, tails, and abnormally curled tails. Eighty-five days after clinical disease, a total recovery was observed (Murcia et al., 2009).

Rams infected with BTV were demonstrated to have testicular lesions without other clinical signs (Puggioni et al., 2018). Viral replication occurred in the endothelial cells of the testicular peritubules. This resulted in destruction of Sertoli cells and a reduction of testosterone biosynthesis by Leydig cells. In more severe cases, the blood-testis barrier was compromised. The study demonstrated that a BTV infection can cause testicular degeneration and momentarily affect male fertility (Puggioni et al., 2018).

BTV-8 infection in bulls was demonstrated to reduce sperm motility in post-thaw semen samples (Müller et al., 2010). Abnormal sperm morphology (>20%) was seen in both fresh and thawed semen from BTV-positive animals. Therefore, an infection with BTV-8 was demonstrated to temporarily affected semen quality in bulls (Müller et al., 2010). BTV virus has been evaluated in 1,500 Alabama cattle (Haynes et al., 1982). Of the serum samples collected, (16%) were seropositive. The western region of Alabama had a significantly greater prevalence than the eastern region. Positive animals were found in 52% of all herds tested (Haynes et al., 1982).

Vosdingh et al. (1968) conducted a study in which six sheep and nine white-tailed deer were experimentally exposed to the BTV-8 California strain. The infections were fatal for 7/9 deer. The incubation period, lesions and signs of BTV and EHDV in deer appear to be similar (Vosdingh et al., 1968). In another study, white-tailed deer were inoculated with BTV- 17 and sequentially euthanatized during infection (Howerth & Tyler, 1988). Remarkable changes of the microvasculature were noted by the fourth day

after inoculation as well as hemorrhage, thrombosis, and vessel rupture (Howerth & Tyler, 1988). Deer were demonstrated to have detectable BTV-4 RNA and BTV-1 RNA through RT-PCR in a study in Spain (Rodriguez-Sanchez et al., 2010).

Epizootic hemorrhagic disease virus, EHDV

EHDV has historically been linked with disease in white-tailed deer particularly, but also causes disease in other wild cervid species in the US (Savini et al., 2011). In white-tailed deer, infection with EHDV is characterized by dyspnea, weakness, facial edema, inappetence, profuse salivation, conjunctival- and mucous membrane hyperemia, lameness, stomatitis, and death (Roughton, 1975). Infertility was demonstrated during EHD recovery in white-tailed deer males (Haigh, 2007b). Peracute, acute, or chronic disease progressions can occur following infection with EHDV. In peracute cases, death often occurs within 36 hours during which the presence of clinical signs of EHD might be absent (Newcomer et al., 2021). Infection with EHDV has also been reported in cattle worldwide (Weir et al., 1997; Wilson et al., 2009). For example, 41 cattle were confirmed to be infected with EHDV in Turkey by an RT–PCR assay and sequence analysis (Temizel et al., 2009). The clinical signs in affected cattle included anorexia, fever, dysphagia, oral cavity necrosis, edema, hyperemia of the conjunctiva and udder, dehydration, hemorrhage and lameness (Temizel et al., 2009). qRT-PCR results from a study confirmed EHDV and BTV in the deer after evaluating bone marrow samples on white-tailed deer cadavers in the US (Becker et al., 2020).

Anaplasma

Anaplasma is a gram-negative, obligate intracellular bacterium with a global distribution (Myczka et al., 2021) *Anaplasma marginale* was demonstrated to infect red blood cells of cattle (Aubry & Geale, 2011) and *Anaplasma phagocytophilum* was shown to cause an infection of blood neutrophils in both humans and domestic animals (Myczka et al., 2021). The cELISA is an acceptable test for detecting infected cattle, but subclinical infections, vectors, or carrier animals are usually detected through a PCR (Aubry & Geale, 2011). Most wildlife studies conducted in the United States have used *A. marginale* isolates and tick species originating from the USA. Therefore, one must be very cautious in extrapolating the results from US studies to other countries.

A. marginale

A. marginale infects red blood cells of cattle and the red blood cells are removed by the spleen. Infected animals become weak, anemic, lethargic, anorexic, and febrile. Outbreaks of anaplasmosis in cattle are associated with abortion and acute death (Capucille, 2008). The parasites are transmitted through tabanid flies or *Dermacentor spp.* ticks, as well as using blood-contaminated equipment (Capucille, 2008) . The transmission of *Anaplasma* has been demonstrated to be highest during vector seasons. When naive animals are moved into an endemic area, severe outbreaks of the disease occur, or when carrier animals are moved to a nonendemic area. Herd mortality can reach 50% and adult cattle are the most susceptible to severe clinical signs, while cattle < 6 months of age usually display a subclinical infection (Capucille, 2008).

Both wild and domestic ruminants can become persistently infected to *A. marginale* (Aubry & Geale, 2011), but white-tailed deer are not likely reservoirs (Keel et al., 1995; Morley & Hugh-Jones, 1989). White-tailed deer can sustain low levels of *A. marginale* in their blood but are not easy to infect according to experimental studies (Keel et al., 1995). It is noteworthy that in Brazil, a RT-PCR detected seven (16.3%) positive pampas deer (*Ozotoceros bezoarticus leucogaster*) for *A. marginale* (Picoloto et al., 2010). Negative tests for *A. marginale* antibodies were demonstrated in 1376 white-tailed deer from 13 Southeastern US states, all with a high seroprevalence in cattle, which demonstrated that not many white-tailed deer appear to be infected with *A. marginale* in the US (Keel et al., 1995)

A. phagocytophilum

A. phagocytophilum is a generalist, infecting a wide range of hosts through *Ixodes* spp ticks (Aubry & Geale, 2011), but was also detected in (*Hyalomma marginatum, Rhipicephalus turanicus,* and *Boophilus kohlsi*) ticks from Spanish deer (Keysary et al., 2007) and (*Dermacenter marginatus, R. bursa,* and *Hemophysalis punctata)* ticks in Spain (Merino et al., 2005). *A. phagocytophilum* is present globally, and domestic and wild ruminants in Europe are usually affected clinically with the pathogen (Aubry & Geale, 2011). However*, A. phagocytophilum* has also been demonstrated in apparently healthy cervids (Sánchez Romano et al., 2019), and to cause a subclinical infection experimentally (Stuen et al., 2001). Clinical signs in mammals can include anorexia, lameness, lethargy, and ventral edema (Stuen et al., 2013). *A. phagocytophilum* was demonstrated to cause abortion in sheep, as well as reduced immunity and fertility in

rams (Stuen et al., 2013; Stuen et al., 2002).

The *A. phagocytophilum* variants seem to be prevalent in the white-tailed deer population of many areas of the US, where 24–64% of white-tailed deer were positive by an indirect fluorescent antibody (IFA) test (Dugan et al., 2006), 15–29% were positive by PCR (Dugan et al., 2006) and 9% were ELISA positive (Rainwater et al., 2006). *A. phagocytophilum* was demonstrated in Polish cervids and they are considered major reservoirs in Europe for this *Anapalsma* spp (Myczka et al., 2021).

Neospora caninum

The coccidian parasite *Neospora caninum* can affect several species but is primarily seen in cattle (its intermediate host), which acquire the parasite from feces of canid species, the definitive host of *N. caninum* (Dubey et al., 2007). Tachyzoites, tissue cysts, and oocysts are three known infectious stages in the life cycle. The intermediate hosts usually harbor intracellular tachyzoites and tissue cysts (Dubey et al., 2002). The central nervous system is the primary location where cysts are found, but muscles may also contain cysts (Dubey et al., 2004). Dogs (*Canis lupus familiaris*) and coyotes (*Canis latrans*) excrete the oocyst through in the feces as an unsporulated environmentally resistant form (Gondim et al., 2004). Parasite transmission is generated through bradyzoites, tachyzoites, and oocysts (Dubey et al., 2004). Herbivores become orally infected by sporulated oocysts in the environment and canid species become infected by oral ingestion of tissues containing bradyzoites (Dubey et al., 2004). Tachyzoites usually generate a transplacental infection and possibly an abortion in livestock (Dubey et al., 2007).

Neospora caninum was suggested to be the causative agent in an abortion outbreak of red deer (*Cervus elaphus*) (Soler et al., 2022). *N. caninum* was histologically demonstrated in clinically affected deer (Dubey et al., 2004). It is important to remember that finding DNA of the parasite in an animal does not prove an infection with the viable parasitic form of *N. caninum* (Dubey et al., 2007). Similarly, detection of *N. caninum* in serum does not reliably determine the presence of viable parasites in any animal (Dubey et al., 2007). Serologic cut-off values for *N. caninum* are therefore only presumptive (Dubey et al., 2007).

White-tailed deer were demonstrated to be serologically positive for *N. caninum* in the US. 400 deer were serologically tested through nucleic acid amplification testing (NAT) in Illinois, and a prevalence of 40.5% was determined (Dubey et al., 1999). Gondim. et al. (2004) identified a prevalence of 26% (50/193) in US white-tailed deer through a immunofluorescence antibody test. Anderson et al. (2007) evaluated *N. caninum* in 189 US deer and detected a prevalence of 20-48% through immunoblotting (IB). Seropositivity for *N. caninum* was confirmed in 14 different Southeastern states with a prevalence of (48%), with Alabama having a 50% prevalence (5/10) in white-tailed deer (Lindsay et al., 2002). Two out of 155 white-tailed deer fetuses in the US were positive for live *N. caninum* through isolation from brain tissue cells (Dubey et al., 2013), which demonstrated the occurrence of transplacental transmission in deer.

Bovine Viral Diarrhea Virus, BVDV

Bovine viral diarrhea virus (BVDV), is a single-stranded, enveloped, RNA virus of the Family *Flaviviridae,* and the genus Pestivirus (Simmonds et al., 2017). The first

description of pestivirus-induced disease was a report of classical swine fever from year 1833, long before viruses were recognized as pathogens of sub-bacterial size (Tautz et al., 2015). Even-toed animals (order *Artiodactyla*) are commonly infected with pestiviruses, which have a wide host range. BVDV infects domestic animals such as sheep, cattle and camelids, as well as wild ruminants such as deer and antelope (Peterhans et al., 2010). Infections may be cause abortions (Van Loo et al., 2021), hemorrhagic, enteric, or wasting diseases or can be entirely subclinical (Simmonds et al., 2017). When infection occurs during early gestation, BVDV may cause immunotolerant and persistently infected (PI) animals (Peterhans & Schweizer, 2013). A persistently infected (PI) animal develops in utero, before the development of immunocompetence. Infectious BVDV is shed from ocular and nasal secretions, semen, urine, colostrum/milk, and feces of PI animals. Therefore elimination of PI animals is the major control strategy in eliminating BVD (Walz et al., 2020).

In the 1960's, 200 serum samples in New York deer were evaluated for the presence of BVDV antibodies and a prevalence of 3% was observed. This was the first evidence of white-tailed deer being exposed to BVDV infection (Kahrs et al., 1964). A greater seroprevalence to BVDV and an endemic distribution was demonstrated in reindeer (*Rangifer tarandus*) in Norway (Lillehaug et al., 2003; Stuen et al., 1993) and mule deer (*Odocoileus hemionus*) and wapiti (*Cervus eluphus*) in the US (Aguirre et al., 1995). PI cervids were demonstrated to exist in US white tailed deer populations, through ELISA or IHC serologic antigen testing (Duncan et al., 2008; Passler et al., 2008). One white-tailed deer in Alabama was shown to be positive on IHC of a skin sample (Passler et al., 2008).

Ruminant alphaherpesviruses

Ruminants have long been known to be reservoirs for many alphaherpesvirus species, and most of these viruses coevolve with their hosts (Azab et al., 2018). Bovine alphaherpesvirus BHV-1 is the most important of the eight herpesviruses so far known to naturally infect cattle. It is categorized within the varicellovirus genus of the alphaherpesvirus subfamily (Nettleton & Russell, 2017). Infectious pustular vulvovaginitis (IPV) and infectious bovine rhinotracheitis (IBR) are two main syndromes associated with BHV-1. Several clinical signs, such as encephalitis, abortions, and conjunctivitis have been reported (Nandi et al., 2009; Pastoret et al., 1982), balanoposthitis (Six et al., 2001), metritis and oophoritis (Graham, 2013), have also been demonstrated. Following an acute infection, animals can become lethargic, febrile, and anorexic (Nettleton & Russell, 2017).

Other ungulate species carry herpesviruses that are both antigenically and genetically related to BHV-1. Viruses isolated from water buffalo, reindeer, goats, elk and red deer form a group of related alphaherpesviruses that include BHV-1 and BHV-5 (Thiry et al., 2006). Common antigenic properties are shared with the viruses and the serological relationships between them makes a BHV-1 eradication program a challenge (Thiry et al., 2006). While it is generally understood that herpesviruses coevolved with their mammalian host species and are specialized with one definitive host, there is evidence that the herpesviruses are capable of infecting other related or unrelated host species (Azab et al., 2018). In Egypt, BHV-1 was isolated through PCR from goats and sheep (Mahmoud & Ahmed, 2009).

Alphaherpesviruses have been isolated from deer and were classified as cervid herpesvirus-1 (CerHV-1) (Inglis et al., 1983) and cervid herpesvirus-2 (CerHV-2) (Lillehaug et al., 2003; Sánchez Romano et al., 2020; Tryland et al., 2009; Tryland et al., 2017). In the US, a ruminant alphaherpesvirus was associated with infectious keratoconjunctivitis (IKC) in mule deer in Wyoming (Muñoz Gutiérrez et al., 2018). In Norwegian reindeer experimentally infected with CerHv-2, systemic infection was demonstrated, and respiratory and genital inoculations led to virus shedding, and fetal infection in utero (DasNeves et al., 2009).

Serological studies have investigated whether other ruminant species could be potential BHV-1 reservoirs. Serum samples from 24 Polish roe-deer, 59 red-deer, and 3 fallow deer were evaluated for alphaherpesvirus seroprevalence (Fabisiak et al., 2018). The seroprevalence of BHV-1 was 5.8% in roe deer. There is cross-reactivity by ELISA between BHV-1, CerHV-1, and CerHV-2, complicating the interpretation of the results (Fabisiak et al., 2018). 1194 serum samples of wild Polish ruminants were tested with an ELISA and virus neutralization test (VN) against BHV-1 and cervid CerHV-1 (Rola et al., 2017). The results demonstrated that free-ranging and farmed cervids in Poland were seropositive to BHV-1 or a related alphaherpesvirus infection. It was concluded that the dominant alphaherpesvirus was CerHV-1 based on the VN test results, therefore it is unlikely that deer in Poland are reservoirs for BHV-1 (Rola et al., 2017). Das Neves et al. (2009) evaluated a total of 3062 Norwegian reindeer serum samples for antibodies against alphaherpesvirus using an ELISA and serum neutralization test. The range of seropositivity was 7.6%-90.7%. In the ELISA-positive samples, greater titers of serum neutralizing antibodies were detected against CerHV-1 than BHV-1. Norwegian reindeer were therefore concluded to be endemically infected with CerHV-2 rather than BHV-1

(Das Neves et al., 2009). A high prevalence of antibodies to BHV-1 was demonstrated in Finnish reindeer, and the study suggested the occurrence of the BHV-1 virus or a closely related cross-reacting herpesvirus (saenEk-Kommonen et al., 1982).

Leptospirosis

The zoonotic and infectious bacteria of the genus *Leptospira* are the cause of leptospirosis (Loureiro & Lilenbaum, 2020). Pathogenic *Leptospira* colonize the renal tubules in a host and contaminate the environment through urine. Transmission of disease is via contact with contaminated environments or direct contact with infected urine (Haake & Levett, 2015). In the US, leptospirosis is a major economic concern in the cattle industry (Hanson, 1976). Losses are due to estrus repetition (Libonati et al., 2018), stillbirth, abortion, death, low milk yield, and slow weight gains in calves (Hanson, 1976). Clinical signs in cattle are typically caused by incidental strains from the *Icterohaemorrhagiae* and the *Pomona* serogroup (Libonati et al., 2018). The majority of leptospiral infections in ruminants are subclinical and silent, being associated to adapted strains such as those belonging to the serogroup *Sejroe* (Schneider et al., 2013).

Leptospirosis in Domestic Animals

Leptospiral DNA and the recovery of viable leptospires from vaginal fluid of a high number of cows without apparent clinical signs has been demonstrated. This suggests that vaginal carriers exist, indicating that venereal transmission could occur (Loureiro et al., 2017). The detection of renal carriers and urine testing have been the major focus for detection of *Leptospira*, but genital carriers are frequently overlooked

(Loureiro et al., 2017). The *Sejroe* serogroup, primarily serovar *Hardjo*, is maintained by cattle. Strains of this serogroup predominates in serological studies worldwide (Nally et al., 2018). Bulls are an important source of infection, and are generally subclinically infected, serving as a source of the infection for females trough semen (Masri et al., 1997). Pigs, rodents, and wildlife usually transmit serovars *Grippotyphosa*, *Pomona*, and *Icterohaemorrhagiae* to cattle, and contribute to incidental infections (Nally et al., 2018).

Serological diagnostic methods have several limitations for detecting chronic bovine leptospirosis in individual carrier animals. As a collective diagnostic tool, serology can be useful but not for detecting an infected individual (Libonati et al., 2017). The detection of renal carriers via urinary PCR is widely practiced, although it is understood that leptospirosis causes reproductive pathology as well. The testing of urine and/or kidney samples are usually the methods chosen for detecting carrier status in animals (Nally et al., 2018).

Leptospirosis in Deer

In one study, 1544 serum samples from white-tailed deer from 9 southeastern US states were examined for leptospiral antibodies, and 292 deer had significant titers of >1:100 (Shotts & Hayes, 1970). The state of Virginia had the highest prevalence with 108/351 deer with high titers. *Grippotyphosa, Interrogans, Canicola and Pomona* were the most commons serotypes detected. *Icterohaemorrhagiae*, *Australis*, *Pyrogenes*, *Georgia, Tarassovi (Hyos), Ballum*, *Bataviae,* Sejroe, and *Autumnalis* were further serotypes detected (Shotts & Hayes, 1970).

From 2011-2017, Pedersen et al. (2018) evaluated 13 US and Virgin Island wildlife species serum samples for the serovars *Canicola, Bratislava*, *Grippotyphosa, Icterohaemorrhagiae, Hardjo* and *Pomona*. 44.8% of 1,043 tested cervids were identified to have antibodies to all six serovars. The most identified serogroup was *Bratislava*. In the epidemiological cycle of *Leptospira*, wildlife species may be reservoirs and contribute to the persistence of the disease (Pedersen et al., 2018).

White-tailed deer were sampled between 1984 -1989 in Minnesota by Goyal et al. (1992). Two hundred and four serum samples were evaluated through a microtiter agglutination test (MAT) for antibodies to *Bratislava, Interrogans*, *Grippotyphosa Canicola, Icterohemorrhagiae*, *Hardjo* and *Pomona*. A >1:100 titer was detected in 43% of the samples against the serovars *Bratislava* and/or *Pomona.* (Goyal et al., 1992).

Wisconsin deer were tested serologically from 2010-2013 for six serovars and the deer had evidence of exposure to *Pomona* 11.7%, *Bratislava* 1.0%, *Grippotyphosa* 2.5%, and *Hardjo* 0.3% (Dubay et al., 2015).

The serovar *Hardjo bovis* was demonstrated to be endemic in most farmed deer herds in New Zealand (Ayanegui-Alcèrreca et al., 2007; Wilson et al., 1998). In another New Zealand study by Subharat et al. (2010) one positive fetus was detected by RT-PCR. Culture negativity was demonstrated in the kidney of the dam, but the *Hardjo bovis* MAT was 1:192. The study highlights the possible role of *Leptospira* in fetal tissues and the reproductive tract of female deer, and the association with reproductive loss in captive deer (Subharat et al., 2010).

Chapter 3: Seroprevalence of Reproductive Tract Pathogens and Analysis of Semen Quality in White-tailed Deer (*Odocoileus virginianus*) of Alabama

Abstract

The deer family (*Cervidae*) has a nearly world-wide distribution, and deer farming has in recent years been gradually accepted as an economically promising industry. It is demonstrated that ungulate body size and horn/antler size are honest indicators of male sexual dimorphism and reproductive success. It is believed that infertility is unusual in natural populations; however, previous research identified variation in the quality of semen from individual deer (morphology, motility, and velocity). This suggests that success of post-copulatory fertilization might be as important as the male phenotype when evaluating reproductive success. Spermatozoal defects and their relation to fertilization success has not been evaluated in deer, and a deeper knowledge of deer reproduction and fertilization success is still lacking. Several ruminant reproductive pathogens were demonstrated to affect both male and female reproductive function. These pathogens have been described in deer, but their effect on fertility is not well documented.

A total of 41 deer, consisting of 28 adult male deer and 13 females were captured through chemical immobilization over two trapping seasons at Auburn University's Deer Research Laboratory. Blood was collected from all deer for the screening of ruminant reproductive tract pathogens (BTV, EHDV, bovine herpesvirus 1 (BHV-1), *Leptospira, Neospora caninum, Anaplasma*, and BVDV). From male deer, body and antler measurements were obtained. A PCA (principal component analysis) variable was generated from the body measurements (body length, hind foot length, and chest girth) and the Boone and Crockett score (BCS) was generated from the antler measurements.

Semen samples were collected from 22 males, and sperm concentration, motility, morphology, and volume were documented. Several spermatozoal defects were detected

in the collected semen samples. When using a threshold of >60% normal spermatozoa to pass a breeding soundness exam (BSE), as established for other ruminant species, 36% (8/22) of the deer would have successfully passed a BSE. When performing a correlation analysis to assess associations between semen parameters (concentration, motility, morphology, and volume), a very strong positive correlation was identified between the percentage of general spermatozoal motility and the percentage of progressive individual motility of spermatozoa ($r= 0.93$ $p= 0.000$ $df = 20$). A strong positive correlation also existed between the sperm concentration in a semen sample and the percentage of spermatozoal head defects ($r= 0.70$ $p= 0.000$ $df = 20$), as well as a strong positive correlation ($r= 0.50$ $p= 0.017$ $df = 20$) between spermatozoal progressive motility percentage and normal sperm percentage. The midpiece defect percentage had a strong negative correlation with the progressive motility percentage ($r= -0.55$ $p= 0.008$ $df = 20$), normal sperm percentage ($r= -0.86$ $p= 0.000$ $df = 20$), and a head defect percentage ($r= -0.51$ $p= 0.016$ $df =20$).

We compared deer-associated parameters including age, PCA variable, antler BCS, and scrotum circumference from deer with an “acceptable” semen quality, as defined as (deer with normal sperm morphology count of >60%), with those with “unacceptable” (deer with normal sperm morphology <60%) using the Student’s t-test. None of the evaluated outcome parameters were significantly different between deer with acceptable or unacceptable semen morphology ($p >0.05$).

Seropositivity was found for *Anaplasma*, *Neospora caninum*, bluetongue virus (BTV) or epizootic hemorrhagic disease virus (EHDV), and for *Leptospira*. All deer were seronegative for bovine viral diarrhea virus (BVDV) and bovine herpes virus-1 (BHV-1).

When comparing the percentage of normal spermatozoa (quantitative variable) between seropositive and seronegative deer for *Anaplasma* and *Neospora caninum,* using the Student's t-test, no statistically significant differences were identified ($p > 0.05$).

Introduction

White-tailed deer (*Odocoileus virginianus*) are a common species in Alabama (Cook & Gray, 2003). Management and restocking efforts contributed to the deer population in Alabama reaching a size of approximately 1.75 million animals in 2000 (Cook & Gray, 2003). Reproductive success of individuals is governed by natural selection, and the interaction between genetic and ecological factors has developed a complex range of phenotypic traits in animals. Heritable traits improve individual fertility through evolution and are passed down over many generations (Fiske et al., 1998; Gomendio et al., 2007). Sexual size dimorphism and secondary sexual characteristics have reproductive advantages in mammals through evolution, which is the basis of selection theory. Important causes of sexually selected characteristics are thought to be generated through male rivalry (Andersson & Iwasa, 1996). A short breeding season and intense rivalry among males are the hallmark for polygynous ungulate mating systems (Ciuti & Apollonio, 2016), and male body size, reproductive success, and horn or antler size, as indicators for sexual dimorphism, have been reported for several ungulate species (Festa-Bianchet et al., 2000; Kruuk et al., 1999; McElligott et al., 2001).

It is thought that infertility is uncommon in natural populations; however, individuals were demonstrated to vary in their ability to reproduce and survive in natural populations (Gomendio et al., 2007). For domestic animals and deer, sperm parameters such as concentration, motility, and morphology are documented indicators of

reproductive capability (Attia et al., 2016; Casey et al., 1997; García-Vázquez et al., 2015; Koziol, 2021; Malo. et al., 2005). Poor semen quality in different animal species (i.e., a low concentration, motility, or large proportion of abnormal sperm) can result in lower fertility or smaller-sized litters (Alm et al., 2006; Saacke, 2004; Sullivan & Elliott, 1968; Tesi et al., 2018). The fact that males in natural populations could have varying degrees of fertility has received little attention (Gomendio et al., 2000; Roldan et al., 1998), and there are no standard evaluation protocols for cervid semen to date.

The orbiviruses, bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV), were demonstrated to momentarily affect semen quality in domestic ruminants and deer respectively (Haigh, 2007b; Kirschvink et al., 2009; Müller et al., 2010). These viruses have also proven to cause abortion in domestic ruminants (Murcia et al., 2009). *Anaplasma* was demonstrated to cause abortion in domestic ruminants (Capucille, 2008; Stuen et al., 2013), as well as lower immunity and fertility in rams (Stuen et al., 2013). *Neospora caninum*, bovine herpesvirus 1, *Leptospira* and bovine viral diarrhea virus (BVDV) have been demonstrated to cause abortion in cattle (Dubey et al., 2007; Hanson, 1976; Nandi et al., 2009; Van Loo et al., 2021). *Neospora caninum* was also suggested to have caused an abortion outbreak in red deer (*Cervus elaphus*), in which *Neospora caninum* was detected in seropositive dams and their aborted fetuses (Soler et al., 2022). Leptospirosis was demonstrated to spread subclinically through bulls and their semen (Masri et al., 1997), and the spirochete has been demonstrated molecularly in one aborted deer fetus (Subharat et al., 2010).

There is a need to establish standard semen evaluation protocols, develop a deeper knowledge in the basic aspects of the reproductive biology (Martinez et al., 2008), and evaluate the effect of ruminant reproductive pathogens in cervids. Studies of male white-

tailed deer (*Odocoileus virginianus*) semen quality and the effect on fertility in the state of Alabama does not yet exist. The specific objectives of this study were to: 1) investigate seroprevalence of infectious reproductive tract pathogens; 2) assess semen quality; 3) investigate correlations between semen traits, age, and male phenotype; 4) describe correlation between seroprevalence of pathogens and semen quality in an enclosed free-ranging population of white-tailed deer in Alabama.

Materials and Method

Study Facility

The study was conducted at Auburn University's Deer Research Laboratory, which is located in Tallapoosa County, Alabama, USA (Neuman et al., 2016). The deer laboratory was built in October 2007 and consisted of 174-hectare enclosed by a 2.6-meter steel fence, constructed to prevent white-tailed deer movement. During construction of the facility, approximately 40 individual white-tailed deer with offspring had been captured and these individuals became the population source. The rutting season of white-tailed was between December to February, with the peak rut occurring around 18 January (Neuman et al., 2016). The population size was regulated by natural and capture-related mortalities and approximately fifteen 6-month-old individuals were captured and released outside of the facility to control deer density and to maintain an adequate sex ratio (Newbolt et al., 2017). A stable water source was available through a creek with year-round water flow. An extruded pelleted feed of 16–18% extruded protein was fed ad libitum through deer feeders (Record Rack®, Nutrena Feeds, Abilene, Texas, USA). Corn

(2 kg/day) was distributed twice daily at timed feeders during October–March as part of capture procedures (Neuman et al., 2016).

Capture and Handling

Twenty-two adult male deer (~67% of the fertile population) and nineteen female were captured by chemical immobilization over two trapping seasons during October to March 2019-2021 (Figure 3.5). All captured animals were >1.5 years old. The Auburn University Institutional Animal Care and Use Committee approved all methods (protocol number 2019-3599). Immobilization of the deer was conducted using a combined intramuscular injection of Telazol® Tiletamine and Zolazepam (Zoetis US, New Jersey, USA; 100 mg/ml, 4.5 mg/kg) and Xylazine (Lloyd Laboratories, Shenandoah, Iowa, USA; 100 mg/ml, 2.2 mg/kg) and Tolazoline® (Lloyd Laboratories, 3000 Bulacan, Philippines; 100 mg/ml, 6.6 mg/kg) as reversal in accordance to (Miller et al., 2004). Cartridge-fired dart guns (Pneu-Dart, Williamsport, Pennsylvania, USA) with transmitter darts and night vision scopes, were used to immobilize the individuals at the feeding sites. The animals were hooded to minimize stress while handling.

Blood Collection and Body Measurements

Body measurements (chest girth, tail length, scrotal circumference, scull length, body length) were measured in centimeters. To evaluate the relationship between the male phenotype with the fertility data more easily, a principal component analysis (PCA) variable was developed using the first principal component (comp 1) from the PCA that included measurements of the chest girth, hind foot length, and body length (Newbolt et

al., 2017). The male body size is therefore referred to as a "PCA" variable generated by this principal component analysis in program R (Table 3.3).

Antlers of the male deer were measured using the Boone and Crockett scoring system (BCS) in inches (Wright, 2003). This scoring system includes measurements of the antler beam with circumference, the length of each tine, and inside spread of the antler. The Boone and Crockett scoring system (BCS) is set as a standard for measuring antler growth and it measures accurate antler size (Strickland et al., 2013).

Blood samples were obtained from a jugular vein of both male and female deer and was spun down for serum. The sera were stored in a -80°C freezer and brought later to the Alabama State Diagnostic Laboratory, Auburn, 36832, AL for testing.

The screening of antibodies against common ruminant reproductive tract pathogens was conducted using a bovine "abortion panel" that was a part of the laboratory serology services provided by the diagnostic laboratory. This panel screened for antibodies against *Anaplasma* spp *(A. marginale, A. centrale, and A. ovis)*, bovine herpes virus 1 (BHV-1) *Neospora caninum*, bluetongue virus (BTV), epizootic hemorrhagic disease virus (EHDV), bovine viral diarrhea virus (BVDV), and 6 *Leptospira* serovars (*L. grippotyphosa, L. canicola, L. pamona, L. hardjo, L. icterohaemorrhagiae*). The presence of antibodies against *Anaplasma* was evaluated using a competitive enzyme-linked immunosorbent assay (*Anaplasma* antibody test kit, cELISA v2, Veterinary Medical Research & Development, Pullman, Washington, 991673, USA). Antibodies against bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) were evaluated by agar gel immunodiffusion (AGID) (Bluetongue virus antibody test kit, Veterinary Medical Research & Development, Pullman, Washington, 991673, USA), and BHV-1 and BVD antibodies were detected by a virus neutralization (VN) (no kit, all

chemicals/reagents purchased commercially to State Diagnostic Laboratory, Auburn, Alabama, USA). Antibodies against *Leptospira* serovars were evaluated by a microscopic agglutination test (*Leptospira* microscopic agglutination test (MAT), National Veterinary Services Laboratory, Ames, Iowa, USA). *Neospora caninum* antibodies were evaluated through enzyme linked immunosorbent assay (Neospora caninum Antibody Test Kit, IDEXX Neospora X2, IDEXX Laboratories Inc, Westbrook, Maine, 04092, USA).

Preparation for Semen Analysis

The deer laboratory facility was located within 1–2-minute drive from the sample collection site and the deer laboratory was set up prior to semen collection. A slide warmer (Premiere XH-2002®, Science company, Lakewood, Colorado, 80227, USA) was preheated to 37°C and microscope slides, cover slips and 1 ml saline filled syringes, were placed to pre-heat on the slide warmer. The water bath (Shel Lab 7L SWB7®, Cornelius, Oregon, 97113, USA) was pre-heated to 37°C to prepare for storing the specimen tube with the obtained semen sample.

Semen Collection

Each male white-tailed deer was chemically immobilized (as described above), prior to semen collection. Semen samples were obtained using an electroejaculator (Pulsator IV®, Lane Manufacturing Inc, Denver, Colorado, 80231, USA), with a 3.2 cm in diameter tripolar rectal probe (22-2X Ram Probe®, Lane Manufacturing Inc, Denver, Colorado, 80231, USA), designed for sheep and goats. The small ruminant rectal probe was lubricated and inserted to its full length in the rectum

with the electrodes oriented ventrally. The pulsator with probe provided administration of low voltage (1-8 volts), low current, electrical pulses. Electrostimulation started at the lowest power and increased in stepwise fashion. Each stimulus was separated by a pause of about 2-5 sec. Peak voltage was heightened with each stimulation, applied for 2-4 sec, and then rapidly diminished to zero. About four stimuli were applied at each power set.

This process induces penis erection, semen emission, and eventually ejaculation (Brindley, 1981). The number of stimuli (7-15) were restricted according to the level of sedation of the buck and the responsiveness to the stimulus. A 10 ml plastic screw capped tube served to collect semen (Axygen™ Fisher Scientific, Waltham, Massachusetts, USA). The collector of the semen and the pulsator operator varied. The same veterinarian throughout the study either operated the pulsator or collected the semen, with one graduate or undergraduate student helping with the collection. In the colder winter months, hand warmers (HotHands® Kobayashi Healthcare Europe Ltd, Chiswick, London, United Kingdom) were used to pre-heat and post-heat the tube and sample to minimize cold shock to the spermatozoa. In warmer months, the sample was stored in a pocket close to the investigator's body to keep sample at body temperature. The sample was promptly transported to the neighboring deer laboratory for analysis.

Gross Semen Evaluation

The semen sample was placed in the warm water bath, and gross volume of semen was measured with a preheated 3 ml syringe. The color and opacity of each sample was recorded. The opacity was recorded as opaque or dilute, and the color of the ejaculate was recorded as either beige, white, or orange.

Semen Motility

Spermatozoal motility was evaluated immediately after arrival to the deer laboratory. After a quick visual inspection of the semen, a small semen drop was placed on a prewarmed microscope slide and observed under low-power (40–100× total magnification) microscopy for evaluation of gross motility. The collective gross movement of all spermatozoa, or their wave motion, which is also called mass activity, was evaluated. The intensity of mass activity was recorded as either poor, fair, good, or very good according to Hopper and King (2014). Dark, thick, and rapidly oscillating swirls indicates high sperm concentration that are progressively motile, which is indicative of excellent motility (Hopper & King, 2014). This type of sample was classified as "very good." A semen sample that showed slower moving swirls was classified as "good." A "fair" sample showed no swirls, but still had significant individual sperm movement. A "poor" sample had very little or no movement (Hopper & King, 2014). Individual and progressive spermatozoal motility was assessed using 400× total magnification microscopy of a sample droplet heavily diluted with warmed buffered saline. The progressive motility percentage and the general motility percentage of the individual spermatozoa were recorded. Individual motility was classified in the same way as gross motility, as very good >70%, good >50–69%, fair > 30–49%, and poor < 30%, as standardized in the bull breeding manual by the Society for Theriogenology (SFT).

Semen Morphology

As generally accepted protocol for semen quality assessment in deer is lacking, therefore a threshold of >60% for morphologically normal sperm was used for our study. This value was derived from previous work in domestic rams, in which >50% of normal sperm is used as the cut-off to classify satisfactory potential breeders (Edmondson&Shipley, 2021; Kimberling & Parsons, 2007) and the cut-off for bulls at >70% set by the STF.

To prepare the slide, a drop of Eosin-nigrosine stain (Hancock's stain®; Lane Manufacturing, Denver, CO) was laid on the end of a microscope slide, then a very small drop of semen was added and mixed with the stain. The slides were either air-dried or gently dried on the slide warmer for ~10 seconds. Individually, one veterinarian and two board-certified veterinary theriogenologists evaluated the semen morphology. The reported morphology are the mean results obtained by the three evaluators. Morphology was evaluated by examining a stained semen slide under 1000× total magnification with an oil immersion, counting 100 spermatozoa. The percentage of normal and abnormal spermatozoa was determined based on the absence or presence of either a tail, midpiece, or head abnormality, respectively.

Spermatozoal Concentration

To prevent movement for sperm count, the spermatozoa were killed by adding the semen to a container with formaldehyde diluent (Unopette® Becton-Dickinson, Rutherford, New Jersey, USA). The fresh live semen was first aspirated into a 0.02 ml Unopette capillary tube. This aliquot was then dispensed into a 1.98 ml Unopette with

formaldehyde to make a 1:100 semen dilution. A hemocytometer (Bright-Line™ Hemacytometer, Hausser Scientific, Horsham, Pennsylvabia, USA), was used to count the individual spermatozoa. The hemocytometer was charged with 10-15 ul of the diluted semen sample by placing the tip of a pipette in the V shaped groove of hemocytometer. A cover slip was placed on top of the evaluation area and the semen was allowed to settle. The hemocytometer was placed under a 400x total magnification microscope, and the counting area was visible as a grid with squares.

Statistical Analysis

A software program (R v4.1.0; R Core Development Team 2014) was used to generate a single principal component analysis (PCA) variable from body measurements. A two-sample t-test was performed, with a confidence level of 95%, assuming unequal variances. We compared the scrotum size, PCA variable, antler BCS and age between the deer with acceptable semen quality (groups with normal spermatozoa values >60%) and those with unacceptable semen quality (groups with normal spermatozoa values <60%).

A two-sample t-test was performed, assuming unequal variances, comparing the percentage of normal spermatozoa (quantitative variable) between either seronegative and seropositive deer for *Anaplasma* and *Neospora caninum*. The confidence interval for the t-tests was calculated at a confidence level of 95%, and the observed results were deemed statistically significant at a p-value of <0.05. We also performed a correlation analysis to look for associations between different semen quality variables (concentration, volume, morphology, and motility) and calculated the p-value from an online linear regression calculator.

Results

Serology and Body/Antler Measurements

We collected blood for a cattle reproductive tract pathogen serologic screening panel from all 41 individuals (Table 3.2). Of the 41 deer, 63% (26/41) were seropositive for *Anaplasma* spp, 27% (11/41) were seropositive for *Neospora caninum*, 100% (41/41) were seropositive for either BTV or EHDV, 5% (2/41) were seropositive for *L. grippotyphosa* and one deer (1/41) was positive for both *L.icterohemoragica,* and *L. grippotyphosa*. *Leptospira* MAT titers were 1:400 and 1:100 for *L. grippotyphosa* and 1:100 for *L. icterohaemorrhagiae.* A 100% seronegativity (41/41) was demonstrated for both BVDV and BHV-1.

When performing a two-sample t-test comparing the percentage of normal spermatozoa in our samples (quantitative variable) with the seropositive and seronegative groups of deer for *Anaplasma* and *Neospora caninum*, no statistically significant result was obtained ($p>0.05$) (Figure 3.10-3.11). The average normal spermatozoal percentage of deer positive for *Anaplasma* ($\mu= 49.9$ SD= 20.1) was not significantly different from the deer seronegative for *Anaplasma* ($\mu= 51.6$ SD= 17.6) ($p= 0.842$ $t= 2.093$ df= 19.0). Similarly, the average normal spermatozoal percentage of deer positive for *Neospora caninum* ($\mu= 59.8$ SD= 14.6) was not significantly different from the deer seronegative for *Neospora caninum* ($\mu=45.4$ SD= 19.6) ($p= 0.058$ $t= 2.093$ df= 19.0).

Semen Collection, Color and Opacity

Most of the ejaculates obtained 73% (16/22) had a beige color, 18% (4/22) had a white color, and one sample had an orange color. Of the ejaculates obtained, 77% (17/22) were classified as opaque and 23% (5/22) were classified as dilute.

Spermatozoal Concentration and Volume

The semen volume of the deer in our study varied between 0.1-2.5 ml, with a mean value of 0.7 ml and median of 0.5 ml. The semen concentration ranged from 35-2745 million/ml, with the mean value of 772 million/ml and median of 555 million/ml. The correlation analysis looking for associations between semen parameters (concentration, motility, morphology, and volume), demonstrated a strong positive correlation ($r= 0.70$ $p= 0.000$ $df =20$) between the sperm concentration (mil/ml) and spermatozoal head defect percentage.

Spermatozoal Motility

Median gross sperm motility was 70% and mean gross motility was 64.5%. The median progressive individual sperm motility was 65% and mean was 55.7%. Of the deer, 86% (19/22) had a > fair (30-49%) motility when assessing individual sperm motility and 50% (11/22) of the deer had > 70% individual sperm motility. When performing a correlation analysis to evaluate associations between semen variables (morphology, motility, concentration, and volume), a very strong positive correlation ($r= 0.93$ $p= 0.000$ $df= 20$) was identified between spermatozoal general motility percentage and sperm progressive individual motility percentage. We observed a moderate positive correlation

(r= 0.50 p= 0.017 df = 20) between spermatozoal progressive motility percentage and normal sperm percentage. The midpiece defect percentage had a moderate negative correlation (r= -0.55 p= 0.008 df= 20) with progressive motility percentage.

Spermatozoal Morphology

Of the deer sampled, 36% (8/22) would have passed a BSE, with the cutoff of normal spermatozoa at >60 %. Spermatozoa displaying secondary and primary morphological abnormalities, including acrosome defects (nuclear vacuoles and pouch defects), loose heads, abnormal midpieces (distal midpiece defect (DMR), thick midpiece, proximal droplet, dag-like defect) and tail defects (coiled-, bent- and double tails) were all identified in our study. The most common spermatozoa defect seen in our study was a midpiece defect, this was the most common spermatozoal defect in 95% (21/22) of the all the semen samples. While various midpiece defects were detected, and samples from individual deer had multiple specific midpiece defects, the most common specific defect was the distal midpiece reflex (DMR) (Figure 3.3).

The DMR was the most common midpiece defect in 85% (18/21) of the deer with a midpiece defect as the main morphological defect. Other common defects seen in several of the samples were a bent tail (tail defect) and a dag defect (mid-piece defect). The dag defect (Figure 3.4) was the most common mid-piece defect seen in 10% (2/21) of the deer with a midpiece defect as the main morphological defect. A swollen midpiece defect was the most common mid-piece defect seen in 5% (1/21) of the deer with a midpiece defect as the main morphological defect. A coiled tail (Figure 3.2) defect was the most common tail defect seen in 5% (1/22) of all the deer.

When comparing the deer-related parameters age, scrotum size, antler BCS, and PCA between deer with acceptable semen quality (normal spermatozoa >60%) and deer with non-acceptable semen quality (normal spermatozoa<60%) no statistically significant differences were detected (p= >0.05) (Figure 3.6-3.9). The average antler BCS (μ= 98.38 SD= 32.72) of deer with normal spermatozoa >60% was not significantly different from the average antler BCS (μ=110.50, SD=18.72) in deer with normal spermatozoa below 60% (p= 0.359 t= 2.23 df= 10). The average age (μ= 4.69 SD= 2.28) of deer with normal spermatozoa >60% was not significantly different from the average age (μ= 5.36, SD= 1.82) in deer with normal spermatozoa below 60% (p=0.491 t= 2.18 df= 12.0). The average PCA variable (μ= 4.53 SD= 13.57) of deer with normal spermatozoa >60% was not significantly different from the average PCA variable (μ=-2.59 SD= 8.82) in deer with normal spermatozoa below 60% (p=0.213 t= 2.23 df= 10.0). The average scrotum circumference (μ=18.50 SD=2.10) of deer with normal spermatozoa >60% was not significantly different from the average scrotum circumference (μ=17.93 SD=1.30) in deer with normal spermatozoa below 60% (p= 0.502 t= 2.23 df= 10.0).

When evaluating correlations between the semen variables (concentration, volume, morphology and motility) (Table 3.3), we observed a strong positive correlation (r= 0.70 p=0.000 df= 20) between the sperm concertation (mil/ml) and spermatozoal head defect percentage as well as a moderate positive correlation (r= 0.50 p= 0.017 df =20) between spermatozoal progressive motility percentage and normal sperm percentage. The midpiece defect percentage had a moderate negative correlation (r= -0.55 p= 0.008 df= 20) with progressive motility percentage and the head defect percentage (r= -0.51 p= 0.016 df=20), and a strong negative correlation (r= -0.86 p= 0.000 df= 20) with normal sperm percentage.

Discussion

In general, we observed good semen motility in our study population (average gross motility 64.5% and average progressive motility 55.7%), which is slightly less than reported by Prieto-Pablos et al. (2016), who described a mean gross motility of 70.2% and a progressive motility of 59% in roe deer (*Capreolus capreolus*). Our results are similar to those of (Gosch & Fischer, 1989; Malo. et al., 2005; Samsudewa et al., 2018), who reported that an average motility of >60% in fallow deer (*Dama dama*), 62.11% in Iberian red deer, and 59% in electroejaculated Rusa deer (*Rusa timorensis*), respectively. Our findings, however, are less than those reported in surgically collected white-tailed deer (78.6%) by Saenz (2007) and Rusa , deer by Fitri et al. (2017) who reported an average gross motility of 82.9% and progressive motility of 76%. According to Kimberling and Parsons (2007), a satisfactory general sperm motility in ram semen is >40% and according to Edmondson&Shipley (2021), the value should be >30% for satisfactory motility with an exceptional ram semen motility value of >70%. In bulls, an individual motility is classified as very good if the sample has a motility of > 70%. We conclude therefore that the sperm motility would be categorized as very good in 59% (13/22) of the deer in our study.

The semen volume of the deer in our study varied between 0.1-2.5 ml, with a mean value of 0.7 ml and median of 0.5 ml. The semen volume range and mean obtained in our study compares with the volumes of electroejaculated fallow deer semen with few drops up to a maximum of 2-5 ml reported by Gosch and Fischer (1989) and 0.10-2.10 ml reported in Rusa deer by Fitri et al. (2017). Our results are slightly less than the mean of 1.1 ml in fallow deer (Gosch & Fischer, 1989) and less than the average ~1.5 ml in Timur

deer (Samsudewa et al., 2018). However, the results correlated with the findings by Fitri et al. (2017) where the mean volume of electroejaculated Rusa deer was 0.86 ml and 0.7 ml in electroejaculated roe deer (Prieto-Pablos et al., 2016). As demonstrated in different sized dogs, the volume collected through electroejaculation can depend on the size and the breed of dog (Tesi et al., 2018). It is therefore difficult to assess and compare the semen volume of the deer in our study to values from different cervid species, due to a presumptive variability in size of the deer species. There was also a variability of age among our individuals, which possibly contributed to a variability of volume in the ejaculates obtained.

The range of semen concentration was 35-2745 million/ml in our study, with the mean value of 772 million/ml and median 555 million/ml. The semen concentration range and mean obtained in our study is lower in comparison to the reports of electroejaculated Rusa deer Fitri et al. (2017) with a range of 94-4825 million/ml and a mean of 1194.2 million/ml. It was also less than a mean value of 981.1 million/ml reported in roe deer by Prieto-Pablos et al. (2016). The mean sperm concentration in our study was greater than reported in the study by Martinez et al. (2008) where the mean concentration of sperm in Iberian red deer was 444 million/ml. The good average motility (64.5%) along with the adequate concentration and volume, suggests that we had a good electroejaculation technique in our study. The ejaculate volume in deer semen is usually greater, and sperm concentration is somewhat less in electro ejaculated semen in comparison to naturally ejaculated semen (artificial vagina) (Lambiase et al., 1972). A study with rams by Matthews et al. (2003), concluded that the electroejaculation method produces semen of lesser quality (lower concentration and the percentage of live sperm) than an artificial vagina. Similar studies do not exist for deer (Asher et al., 2000). Semen has been

collected from a wide variety of deer species through electroejaculation, often with no lesser quality or volume compared with natural collection (Asher et al., 2000). The comparatively sperm free pre-sperm and post-sperm fractions of an ejaculate obtained by electroejaculation is influenced by the technique of the operator (Lambiase et al., 1972). The sperm count and volume can also be dependent on whether a particular animal has bred earlier, and has done so only before being evaluated (Lambiase et al., 1972).

Because the deer evaluated in this study are a free-ranging population and the mating is not monitored during the trapping season, the possibility of an individual variation in semen volume and concentration due to recent mating or abstinence, is possible. An operator influence on the sample quality in our study cannot be ruled out as the collector of the semen and the pulsator operator varied. We observed a strong positive correlation ($r= 0.70$ $p= 0.000$ $df= 20$) between the sperm concertation (mil/ml) in a semen sample and spermatozoal head defect percentage. Semen that has been in the ampullae/epididymii longer (less frequent ejaculation), tend to have a lesser acrosome quality in bulls (Wells et al., 1971).This could be one explanation why we found a strong positive correlation between head defects and sperm concentration in our study.

The deer in the study had a significant variation in percentage of morphologically normal sperm. The range of normal spermatozoa percentage was 17-81% in our study with the mean number of normal spermatozoa of 50.6% and a median of 53%. The range in our study is close to the range of morphologically normal sperm reported for surgically collected red deer (12–97%) (Malo et al., 2005), but differed from a study with electroejaculated Timur deer (~55-91%) (Samsudewa et al., 2018). The mean morphologically normal sperm count of 50.6% in our study, was less than described in other cervid studies (Gosch & Fischer, 1989; Malo. et al., 2005; Samsudewa et al., 2018),

where the mean number of normal spermatozoa was >60% in electroejaculated fallow deer, 77.02% in surgically obtained sperm from Iberian red deer and ~85% in electroejaculated Timur deer, respectively. Our results do, however, correlate fairly well with a study on white-tailed deer in Louisiana by Saenz (2007), who reported a mean value of 58.2% morphologically normal sperm in post mortem semen collections.

To our current knowledge a standard protocol for evaluation of deer semen does not exist and data concerning several aspects of basic semen collection in cervids are lacking (Martinez et al., 2008). If using the standard protocol for bovine bulls by SFT to evaluate sperm morphology (acceptable threshold of normal spermatozoa >70%), then 13% (3/22) of the deer in our study would have passed a BSE. However, when using the ram standards (acceptable threshold of normal spermatozoa >30%) by Tibary et al. (2018), our data indicate that 81% (18/22) of the deer would have passed a BSE. Kimberling and Parsons (2007) recommends a >70% threshold in yearling rams, with an unsatisfactory cutoff of >50% normal spermatozoa. This is in accordance with Edmondson&Shipley (2021) who recommend a threshold of normal ram spermatozoa at >50%. If we would use the unsatisfactory classification of >50% by (Edmondson&Shipley, 2021; Kimberling & Parsons, 2007) then 62 % (13/21) of the deer in our study would have passed a BSE. It is evident that the standard protocols differ between species but also among species. This makes the accurately assessing fertilization data in deer a challenge. It also raises the question of whether there will be enough research in the future to validate and set a standard protocol for assessing deer semen quality.

Spermatozoa displaying morphological abnormalities, including, acrosome defects, loose heads, abnormal midpieces, nuclear pouches, and tail defects, were all

identified in our study. Very few secondary abnormalities <1% are usually seen in deer (Bringans et al., 2007). Only one subfertile red deer male and two subfertile wapiti males with >50% secondary abnormalities, in a mid-breeding season have been reported by Bringans et al. (2007). We found that 27% (6/22) of the deer in our study had >50% secondary abnormalities, which is considerably greater than reported by Bringans et al. (2007). The most common secondary abnormality seen in deer according to Bringans et al. (2007), are bent tails, and this was reported in Rusa deer by Fitri et al. (2017). Bent tails are usually the result of cold shock and can also result from urine contamination (Bringans et al., 2007). A bent tail is considered a spermatozoal tail defect and this defect was the second most common specific defect seen in our samples however, it was never the main defect seen in any of deer in our study.

The most common spermatozoal defect in our study was a mid-piece defect (mean 36.9% and median 34%). Knight et al. (2017) had similar findings, that a mid-piece defect is the most common defect in wapiti. The DMR defect was the most common midpiece defect seen in 85% (18/21) of the deer with a midpiece defect as the main morphological defect. The DMR defect in bulls is the most common abnormality seen (Barth, 1989), and originates from the epididymis. It is therefore considered a minor secondary defect and is classified as a compensable defect due to the lack of progressive motility (Barth, 1989; Hopper & King, 2014). The DMR can be observed within a few days of a thermal insult in bulls (Hopper & King, 2014), and is also the most notable defect associated with stress in bulls (Koziol, 2021). Depressed testosterone levels can cause a compromised epididymal function, which is often the result of stress (Barth, 1989; Boakari et al., 2022; Koziol, 2021), or induced hypothyroidism or exogenous estrogen (Barth, 1989). A DMR defect can be observed in up to 25% of the sperm count

in a fertile bull ejaculate, and is considered a compensable defect (Barth, 1989). The median and mean value of mid-piece defects in our study (with DMR as the dominant mid-piece defect) is greater than what would be acceptable for fertile bulls (<25%) and may have a negative effect on the post-copulatory fertility in the deer.

A variety of other defects can be found following a stressful event including proximal droplets, detached heads and mitochondrial disturbances, knobbed acrosomes, nuclear vacuoles, coiled principal pieces, and pyriform heads (Koziol, 2021). We observed several morphological sperm defects as well and all our semen samples had simultaneously both head-, tail- and midpiece abnormalities. Acrosome defects (nuclear vacuoles, diadem defect and pouch defects), abnormal midpieces (DMR, thickened midpiece, proximal droplet, dag defect), loose heads, and tail defects (coiled-, bent- and double tail), were other specific defects identified in our study. Understanding the significance of specific types and number of sperm abnormalities in semen allows prediction of its fertilizing ability in bulls (Barth, 1989). It is also important to understand how and why various defects of spermatozoa occur. A diagnostician with this knowledge would be able to establish a prognosis for future fertility, and assist in the treatment/recovery of a bull with abnormal sperm production (Barth, 1989). It is not unlikely, when referring back to the statements of (Barth, 1989; Hopper & King, 2014; Koziol, 2021), that the unusually hot autumns and winters during our study period might have negatively affected the sperm quality in our deer, more specifically the amount of morphologically abnormal sperm such as DMR defect.

Alabama is situated at subtropical latitudes between the southern end of the plains of central North America and the Gulf of Mexico (Runkle J., 2022). Diverse air masses, including the moist, and warm air from the Gulf of Mexico and dry continental air masses

are affecting the climate of Alabama. Hot summers, relatively mild winters and year-round rainfall characterize the state's climate (Runkle J., 2022). Temperatures in Alabama have not increased since the beginning of the 20th century; however, recent years have been very warm, and the warmest consecutive 5-year interval was the most recent, 2016–2020 (Runkle J., 2022) (Figure 3.1). Heat stress might therefore be one plausible theory to explain the relative high number secondary DMR defects that we describe in our study.

Other male reproductive tract characteristics that negatively affect sperm quality must also be considered as testicular degeneration is not only associated with thermal stress (Koziol, 2021). The etiology of testicular degeneration, whether due to thermoregulatory issues or other stressful events, is difficult to differentiate without a sufficient history and physical exam. Physical examination findings and semen evaluation is the basis of a testicular degeneration diagnosis. Testes, in the case of testicular degeneration, are often palpably softer than normal (Barth, 1989; Koziol, 2021). Systemic illness, excessive fat in the neck of the scrotum, scrotal dermatitis, insulation of the scrotal contents due to trauma, inguinal hernia, or hydrocele have also shown to be causes of testicular degeneration (Barth, 1989). Local inflammatory processes, such as periorchitis or orchitis, and lameness (Boakari et al., 2022; Koziol, 2021) may also impair normal testicular thermoregulation in males (Koziol, 2021). No lameness was observed in our study population, nor were any scrotal abnormalities such as excessive fat in the neck of the scrotum, scrotal dermatitis, softness, insulation of the scrotal contents due to trauma, inguinal hernia, or hydrocele observed or palpated upon testicular manipulation. The periorchitis or orchitis, and prolonged recumbency cannot however be ruled out completely in our study.

It is important to remember that some types of constant and large number of sperm defects occur without any evidence of environmental impact in bulls (Barth, 1989). Such defects can be due to a genetic etiology, and the future prognosis for improvement in semen quality is believed to be prognostically very poor (Barth, 1989). A hereditary association has been documented for several types of defects in bulls like the dag defect (Koefoed-Johnsen et al., 1980). It is noteworthy that two deer 9% (2/22) in our study had a dag defect, considered an abnormality of the spermatozoal midpiece, as the main defect (Figure 3.4). The dag defect is considered a primary abnormality of epididymal origin (Wenkoff, 1978). It would be interesting to evaluate if there would be a hereditary component to the dag defects seen in our deer population, as it has been demonstrated to be hereditary in bulls (Koefoed-Johnsen et al., 1980). A swollen midpiece and terminally coiled tail, both considered secondary and compensable defects, were two other main defects in two deer in our study. Of note, the oldest buck, >9-year-old, had the coiled tail defect (Figure 3.2), as the main defect which represented about 34% of the sperm morphology count in this individual.

Our study results, together with previous studies in deer, suggest there exists a great variety in the main spermatozoal defects in deer semen. Bent tails secondary to cold shock, must be considered in deer semen samples, as well as other factors such urine contamination and osmolarity changes in stain preparation of the sample (Fitri et al., 2017). The semen laboratory is often located far from where the animals are sampled in wildlife research, with an inevitable longer transportation time than is found in most domestic animal settings (Prieto-Pablos et al., 2016). This was also true for our study, in which the site of collection was in the field, and samples had to be transported to the

laboratory location. Sample handling was challenging especially during the winter months.

We observed a moderate positive statistical correlation between spermatozoal progressive motility percentage with the normal sperm percentage (r= 0.50 p= 0.017 df =20). The midpiece defect percentage had a moderate negative correlation with the progressive motility percentage (r= -0.55 p= 0.008 df= 20). This makes sense as the spermatozoal defects were mostly secondary in nature and therefore contributed to a lower general motility score in our samples. The midpiece defect percentage had a moderate negative correlation with the head defect percentage (r= -0.51 p= 0.016 df= 20), and a strong negative correlation with normal sperm percentage (r= -0.86 p= 0.000 df= 20). It is logical that with a rise in midpiece defects in a sample, the amount of normal sperm will fall simultaneously. However, why the number of head defects would decrease with the rise in mid-piece defects, is yet to be evaluated.

Our study showed a 100% prevalence of BTV in the deer. A cross reaction between BTV and EHDV using the AGID serologic test has been demonstrated (Work et al., 1992). It is therefore not possible to know what specific orbivirus is the cause of the 100% seroprevalence found in our study. A more specific test like a RT-PCR would be appropriate to serotype EHDV or BTV (Maan et al., 2010). If the male deer population would be seropositive to BTV, it is theoretically possible, when reading the BTV study in rams by (Puggioni et al., 2018), that BTV could induce testicular degeneration and disruption of spermatogenesis in deer, and therefore temporarily affected male fertility. When reading the study by (Müller et al., 2010), an established BTV infection decreased sperm motility in thawed semen, as well as contribute to an abnormal sperm morphology in both in fresh and thawed semen. Infertility has been shown following EHDV recovery

in white-tailed deer males (Haigh, 2007b), it is therefore not entirely unlikely that if the deer in our study population would be 100% seropositive to EHDV, that this could impact fertility. It would be interesting to evaluate a correlation between the 100% seroprevalence for BTV/EHDV in our study population with semen quality.

In our study, 63% of the deer were seropositive (26/41) for *Anaplasma. A. marginale* and *A. phagocytophilum* have been reported to cross-react in the bovine using ELISA (Dreher et al., 2005). Bovine. *A. marginale, A. centrale and A. ovis* are the *Anaplasma* spp that our serologic cELISA was testing for in the study. It is not unlikely that cross reactivity occurred in our samples. It is therefore unclear what exact *Anaplasma* spp our study population have antibodies against. A PCR of whole blood in cattle has shown to be an option to type *Anaplasma* spp (Torioni de Echaide et al., 1998). A PCR would be an acceptable diagnostic test to determine the precise *Anaplasma* spp in our study population.

When considering the Alabama weather data from (Runkle J., 2022), it is not unlikely that the global warming might influence future prevalence of vector-borne diseases such as BTV/EHDV and *Anaplasma*. Global warming is of concern (McIntyre et al., 2017). Vector-borne diseases will expand due to the ongoing global warming, as they are the most sensitive to climate drivers (McIntyre et al., 2017). Numerous pathogens have shown to be climate sensitive with increased rainfall and temperature as primary drivers (McIntyre et al., 2017). The results from a detailed spatial transmission model (Jones et al., 2019), suggests that to limit the spread of vector-borne diseases, an efficient detection and control measures, will be important in the future due to global warming. Rapid and precise disease detection and effective control strategies of vector-borne diseases will be increasingly important in the future (Jones et al., 2019). Global warming

and heat stress has also been suggested to affect the weight gain in young Iberian red deer males (Pérez-Barbería et al., 2020), and affect the semen quality in animals (Boni, 2019). Therefore, we believe that weather monitoring will also be a crucial component in wildlife fertility research in the future.

Our study demonstrated a prevalence of 27 % (11/41) of *Neospora caninum*, which is lower than reported in US white-tailed deer previously (Dubey et al., 1999; Lindsay et al., 2002), and higher than the reports by (Anderson et al., 2007), but close to the seroprevalence findings by (Gondim. et al., 2004). It makes one consider if rural dogs or other canine species such as coyotes are the definitive hosts of *Neospora caninum* in our study area, and therefore contributing to the apparent prevalence. Further research is necessary to understand what canine species would act as the maintaining host and hence linked to the prevalence seen in our white-tailed deer population.

Conclusion

Electroejaculation appears to be a reliable technique for routinely obtaining semen from free-ranging captive white-tailed deer in Alabama. DMR, bent tail and dag defect, were the most prevalent spermatozoal defects found in our study and they should be considered as true abnormalities until proven otherwise. How the variation in deer fertility correlates with male breeding success would need to be studied further in natural populations (Gomendio et al., 2007). This is especially important as the basic aspects of semen collection in cervids is lacking (Martinez et al., 2008), which makes interpretation of fertility data in deer challenging. It is therefore necessary to establish standard testing protocols to evaluate semen quality in different cervids species, and to expand the basic knowledge of the reproductive biology (Rola et al., 2021). We would like to point out

with our study findings that when assessing breeding success in deer, the semen quality is equally important.

Our study demonstrated a variety of sperm morphological abnormalities, as well as a high number of secondary sperm abnormalities from electroejaculated semen obtained from white-tailed deer of Alabama. Poor semen morphology was not linked to deer age, scrotum size, PCA variable, antler BCS, or being seropositive or negative to *Anaplasma* or *Neospora caninum*.

We conclude that there is an established seropositivity of both *Neospora caninum*, *Leptospira*, BTV or EHDV and *Anaplasma* in free-ranging white-tailed deer in Alabama. We recommend the monitoring for any negative effects of these pathogens on fertility and continue disease monitoring in the white-tailed deer. This is especially crucial as there is an ongoing global warming, that is now prominent in Alabama. Global warming could have a potential negative effect on sperm quality, growth of off-spring, and the prevalence of vector borne diseases (Barth, 1989; Boni, 2019; Jones et al., 2019; Pérez-Barbería et al., 2020; Runkle J., 2022).

The study limitation was the small sample size. Caution is advised when drawing any conclusions of the exact etiology of the spermatozoal defects found. Sampler and methodology bias, such as cold shock to the semen, urine contamination and semen stain imperfections, must be considered. These pitfalls need to be controlled and addressed in future semen evaluation in the deer. The study findings reported here may act as a foundation for future fertility research in white-tailed deer in Alabama.

Figure 3.1 Alabama average temperature Oct-Feb 2002-2022

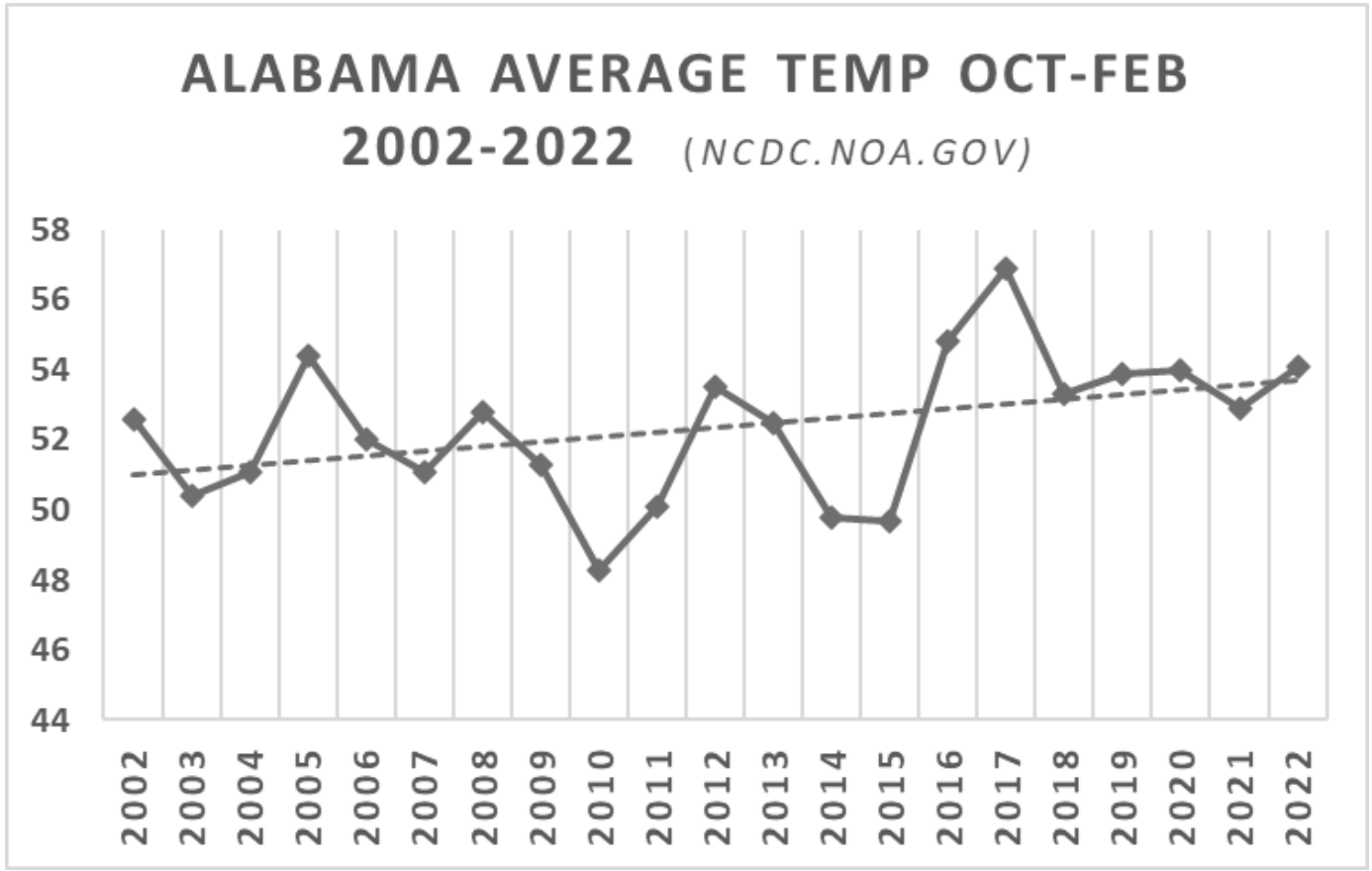


Figure. 3.2 Coiled tail defect

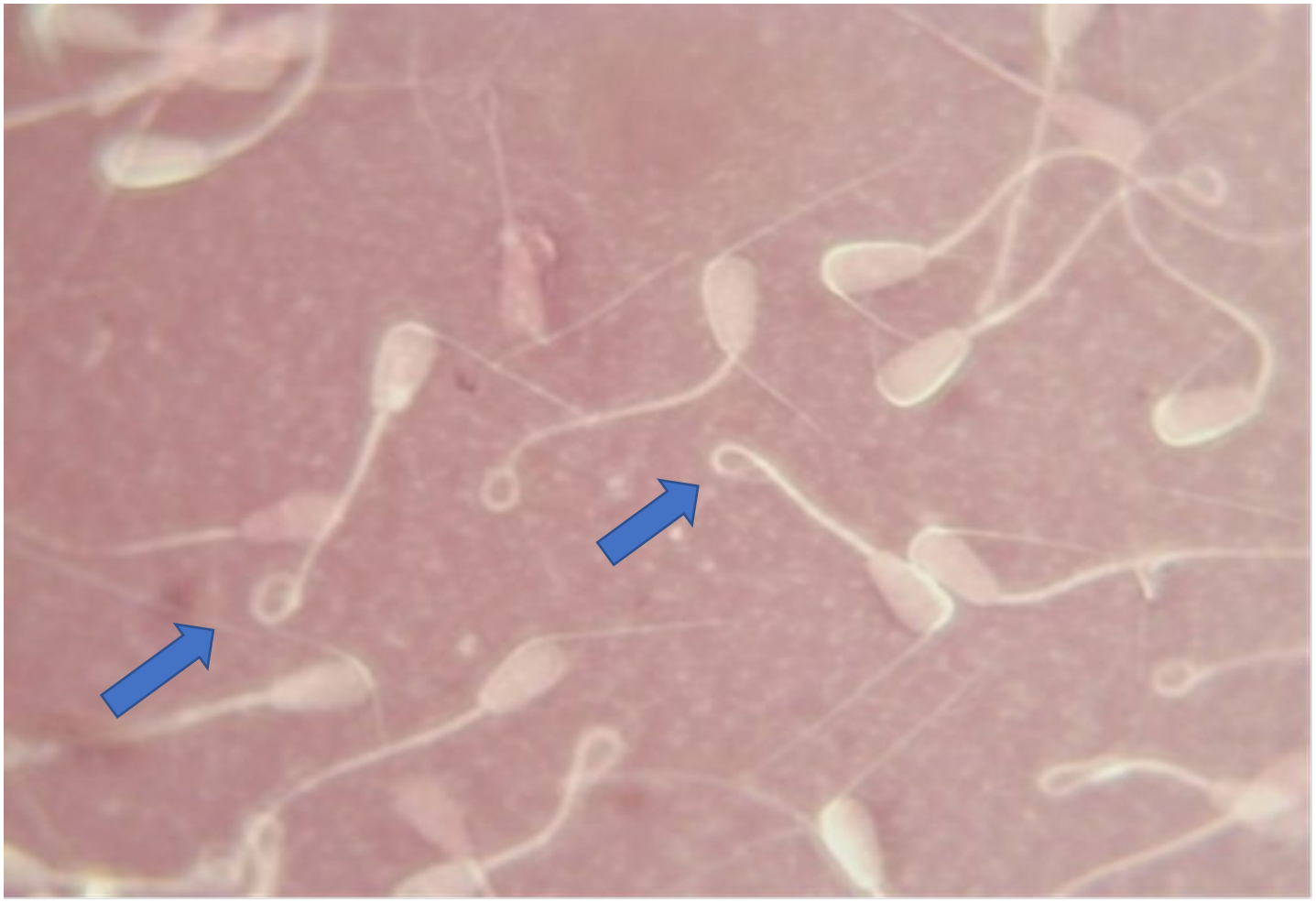


Figure 3.3 Distal midpiece reflex (DMR) defect

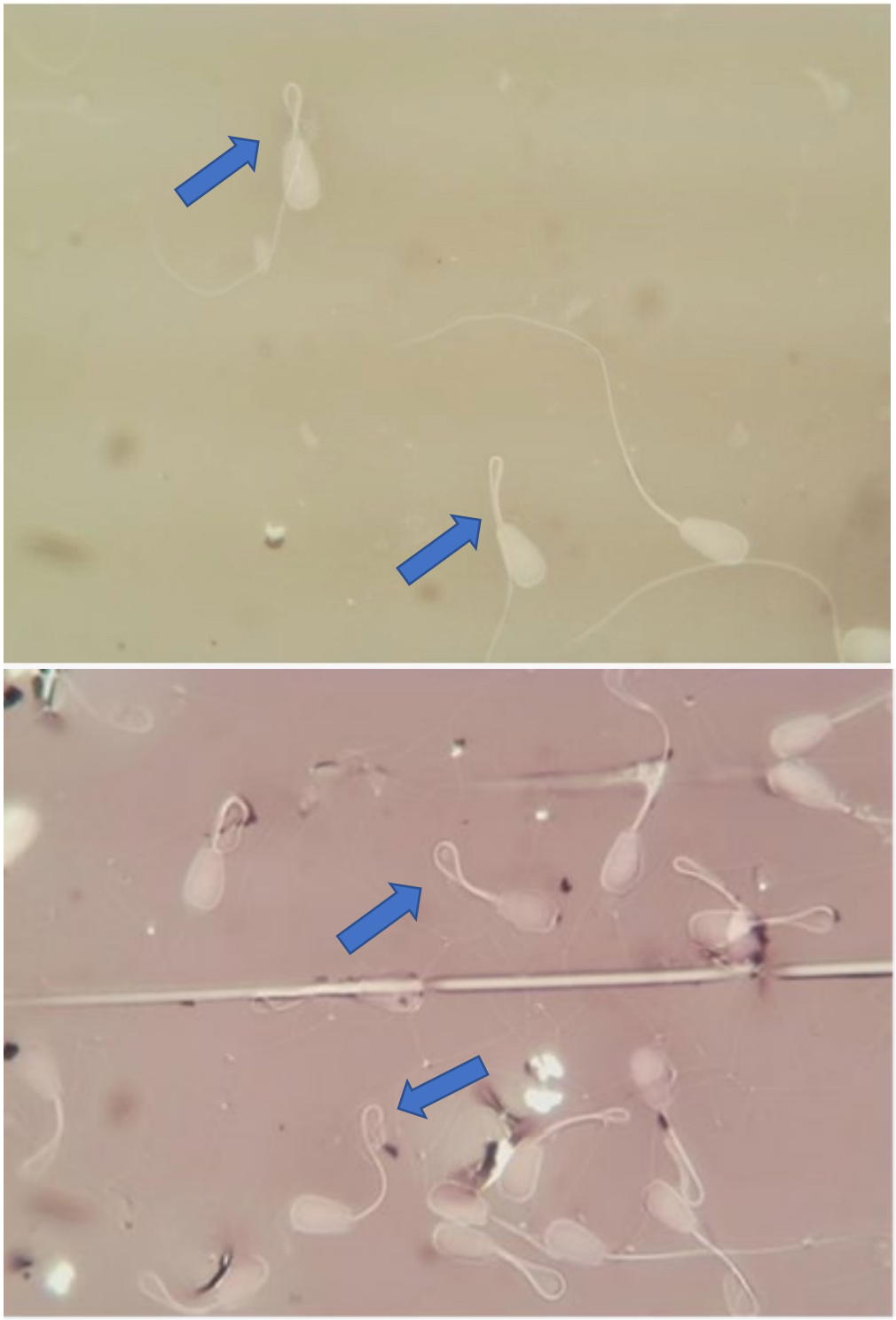


Figure 3.4 Dag defect

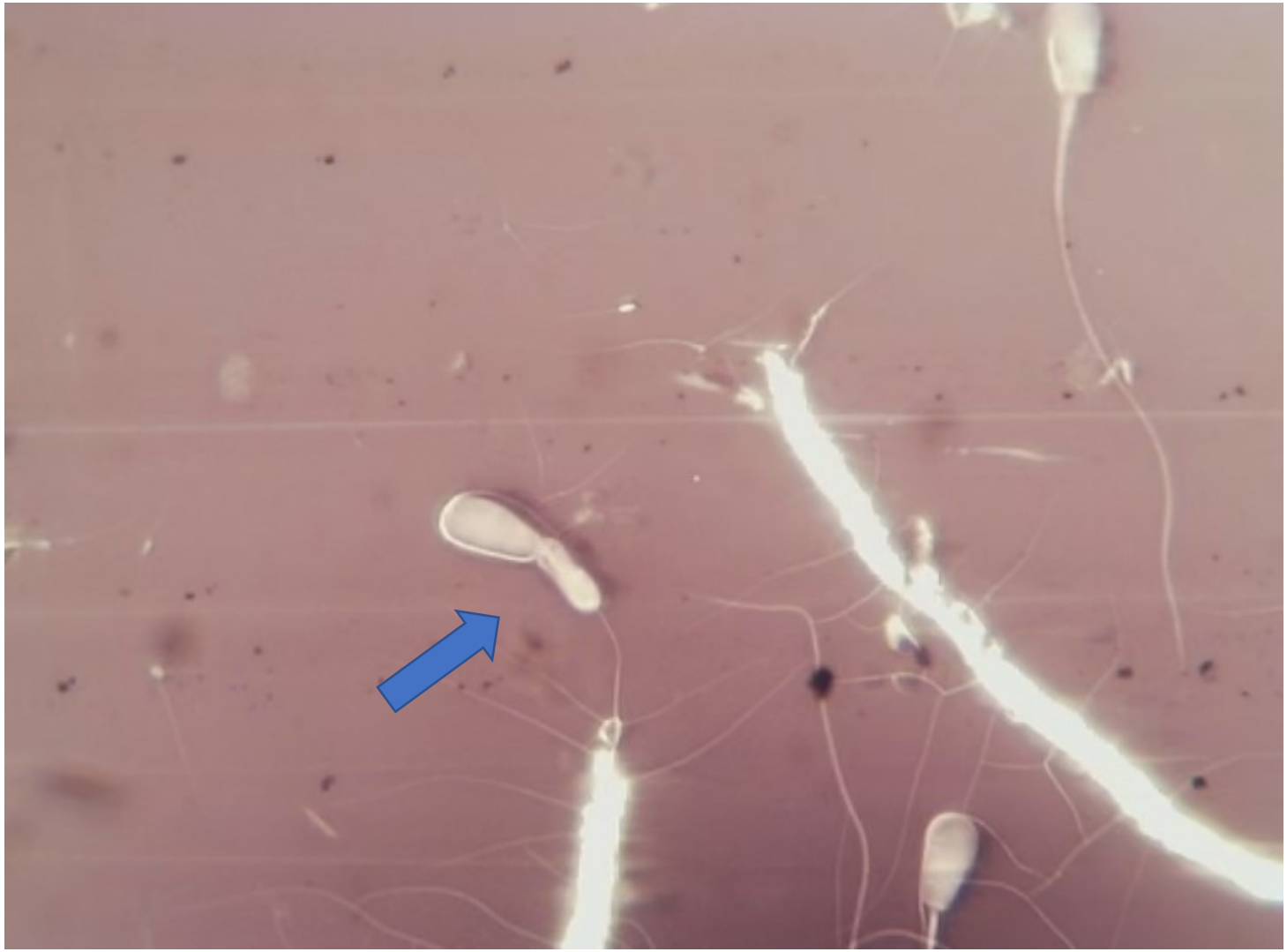
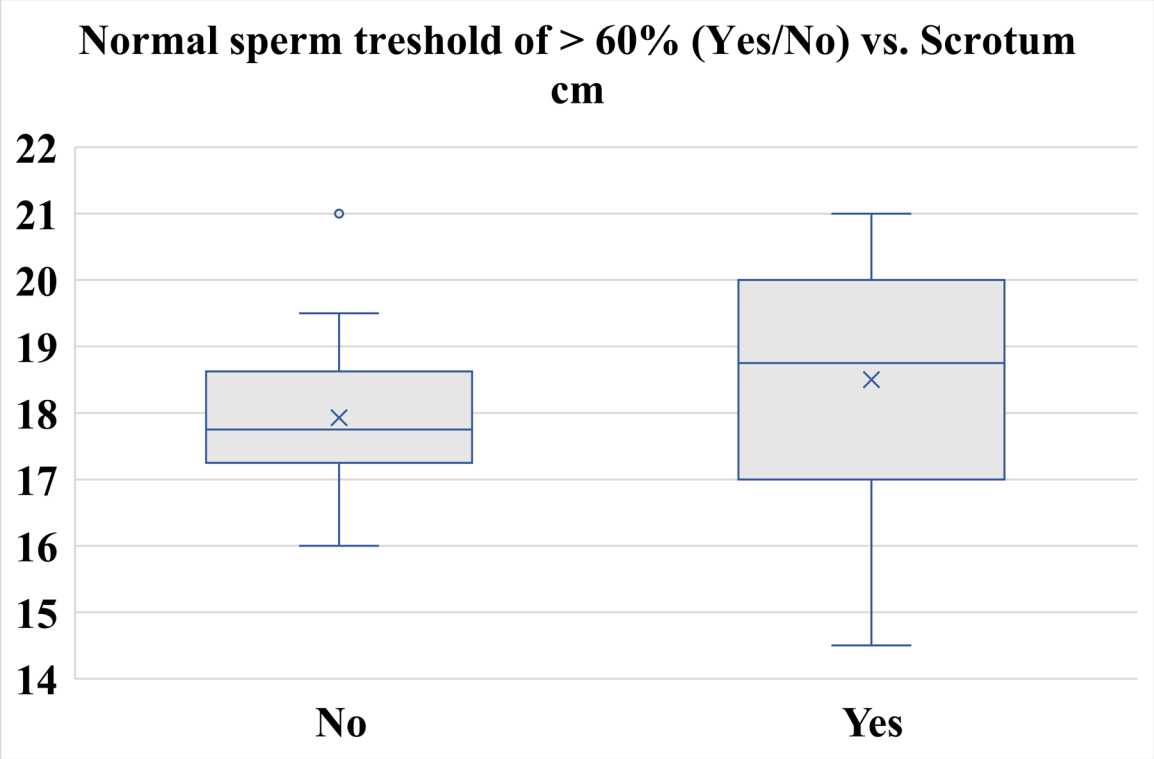


Figure 3.5 White-tailed deer buck sedated at the collection site

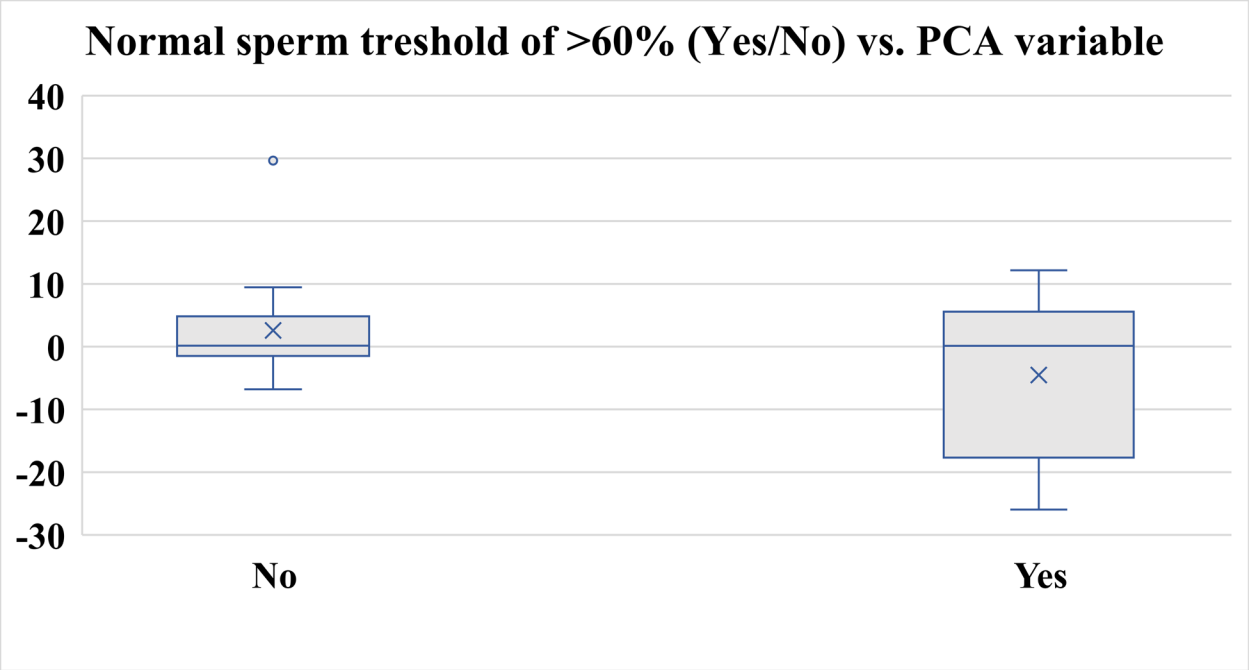
Figure 3.6 T-test, Acceptable semen quality (normal sperm > 60%) vs. Scrotum circumference (cm)



t-Test: Two-sample assuming unequal variances

Scrotum cm	No (<60%)	Yes (>60%)
Mean	17,93	18,50
Variance	1,69	4,43
Observations	14,00	8,00
Hypothesized Mean Difference	0,00	
df	10,00	
t Stat	-0,70	
P(T<=t) one-tail	0,25	
t Critical one-tail	1,81	
P(T<=t) two-tail	**0,50**	
t Critical two-tail	2,23	

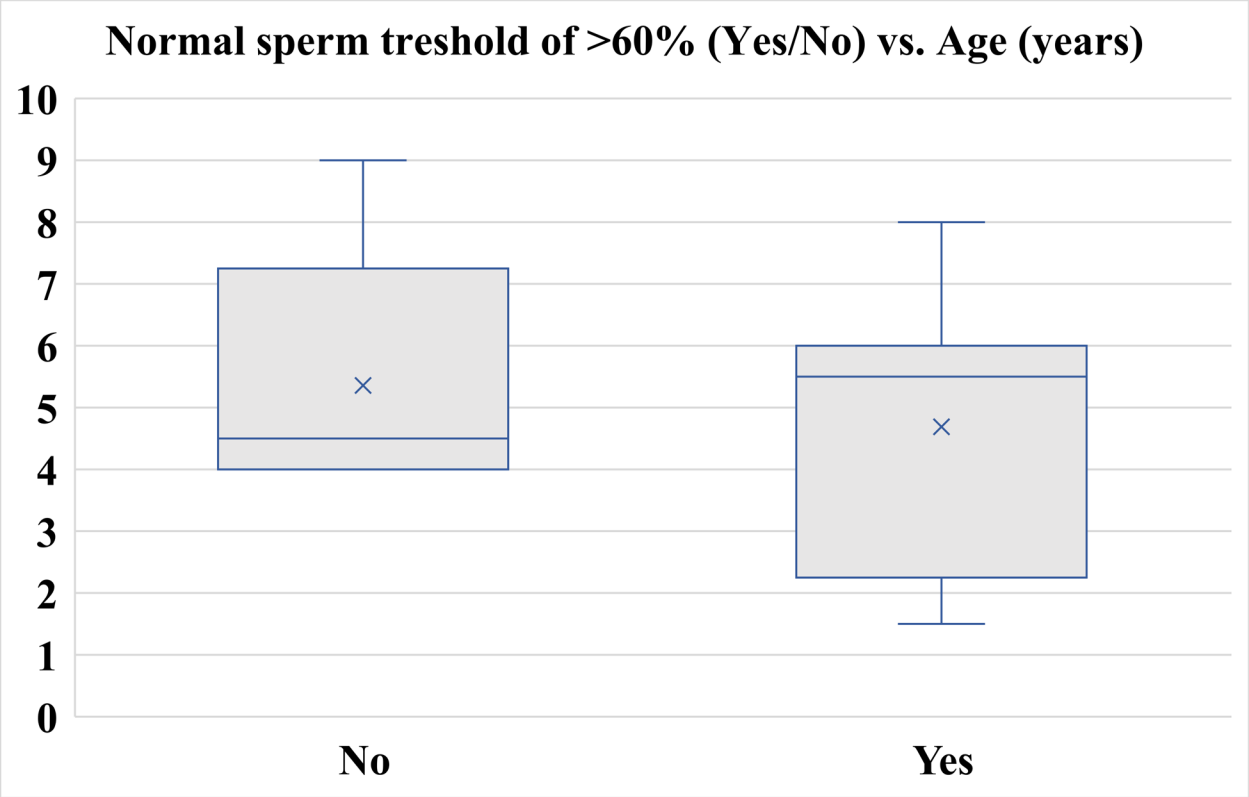
Figure 3.7 T-test, Acceptable semen quality (normal sperm >60%) vs. PCA variable



t-Test: Two-sample assuming unequal variances

PCA variable	No (<60%)	Yes (>60%)
Mean	2,59	-4,53
Variance	77,86	184,21
Observations	14,00	8,00
Hypothesized Mean Difference	0,00	
df	10,00	
t Stat	1,33	
P(T<=t) one-tail	0,11	
t Critical one-tail	1,81	
P(T<=t) two-tail	**0,21**	
t Critical two-tail	2,23	

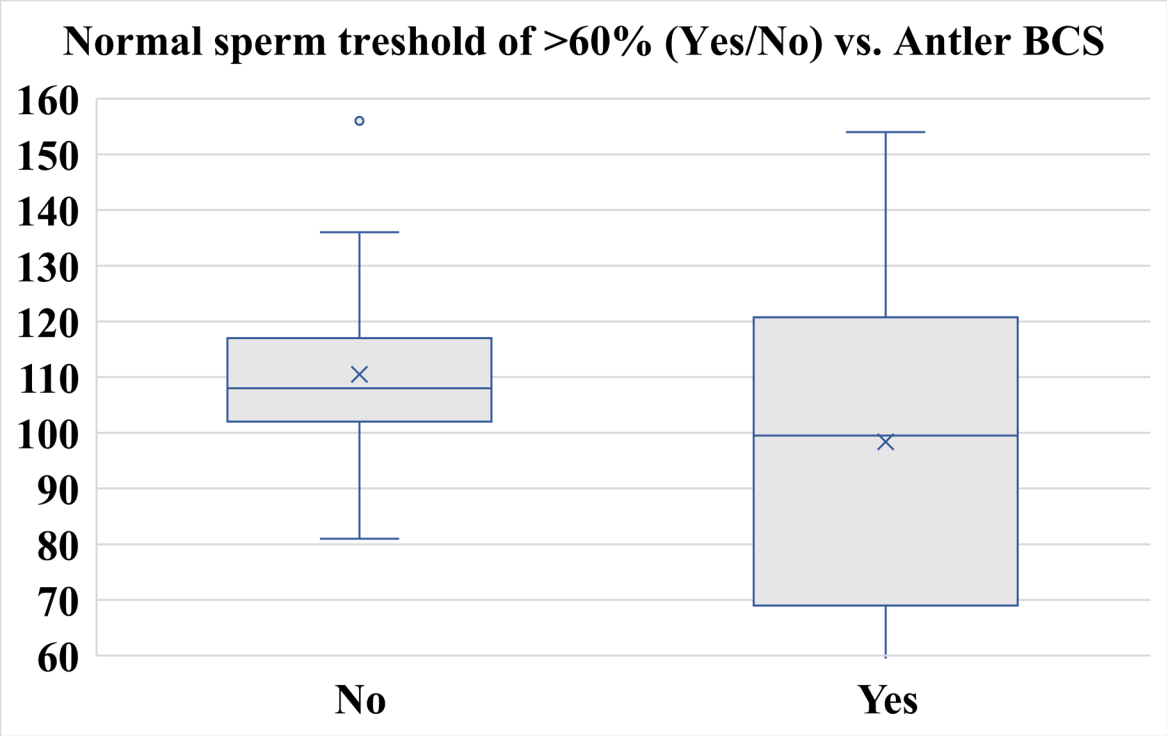
Figure 3.8 T-test, Acceptable semen quality (normal sperm >60%) vs. age



t-Test: Two-sample assuming unequal variances

Age Years	No (<60%)	Yes (>60%)
Mean	5,36	4,69
Variance	3,32	5,21
Observations	14,00	8,00
Hypothesized Mean Difference	0,00	
df	12,00	
t Stat	0,71	
P(T<=t) one-tail	0,25	
t Critical one-tail	1,78	
P(T<=t) two-tail	**0,49**	
t Critical two-tail	2,18	

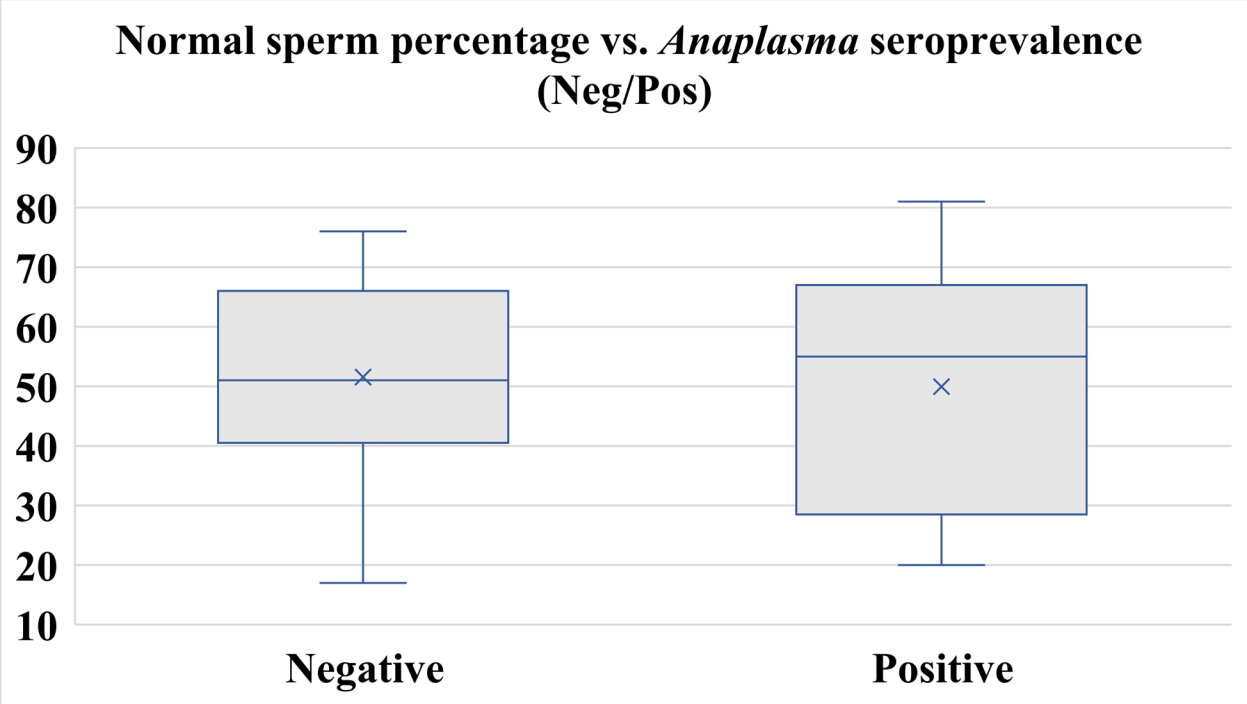
Figure 3.9 T-test, Acceptable semen quality (normal sperm >60%) vs. antler BCS



t-Test: Two-sample assuming unequal variances

Antler BCS	No (<60%)	Yes (>60%)
Mean	110,50	98,38
Variance	350,42	1070,55
Observations	14,00	8,00
Hypothesized Mean Difference	0,00	
df	10,00	
t Stat	0,96	
P(T<=t) one-tail	0,18	
t Critical one-tail	1,81	
P(T<=t) two-tail	**0,36**	
t Critical two-tail	2,23	

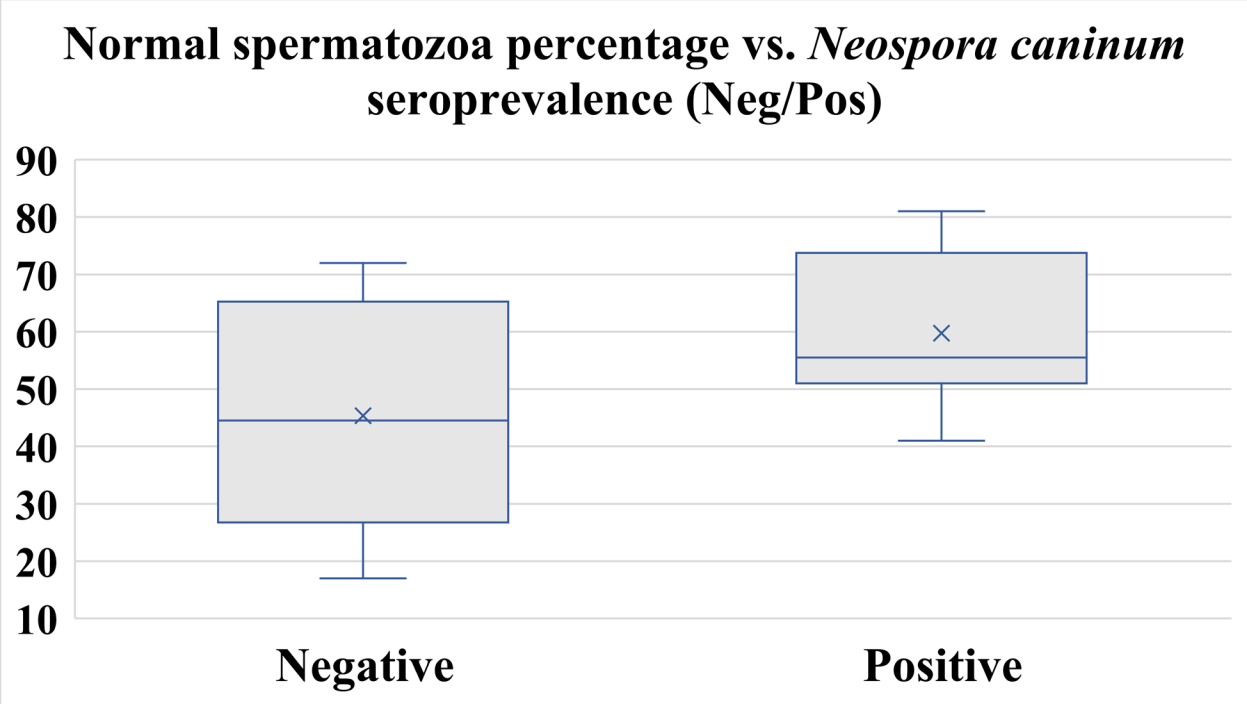
Figure 3.10 T-test, Normal spermatozoal percentage vs. *Anaplasma* seropositivity/seronegativity



t-Test: Two-sample assuming unequal variances

Normal sperm %	Negative for Anaplasma	Positive for Anaplasma
Mean	51,56	49,92
Variance	309,53	404,74
Observations	9,00	13,00
Hypothesized Mean Difference	0,00	
df	19,00	
t Stat	0,20	
P(T<=t) one-tail	0,42	
t Critical one-tail	1,73	
P(T<=t) two-tail	**0,84**	
t Critical two-tail	2,09	

Figure 3.11 T-test, normal spermatozoal percentage vs. *Neospora caninum* seropositivity/seronegativity



t-Test: Two-sample assuming unequal variances

Normal sperm %	Negative for Neospora	Positive for Neospora
Mean	45,36	59,75
Variance	383,32	187,07
Observations	14,00	8,00
Hypothesized Mean Difference	0,00	
df	19,00	
t Stat	-2,02	
P(T<=t) one-tail	0,03	
t Critical one-tail	1,73	
P(T<=t) two-tail	**0,06**	
t Critical two-tail	2,09	

Table 3.1 Mean, median and range for numeric data

	Mean	Median	Min	Max
Age (years)	5.1	5	1.5	9
Volume (ml)	0.7	0.5	0.1	2.5
Concentration (mil/ml)	772.3	555	35	2745
Gross Motility %	64.5	70	0	95
Progressive %	55.7	65	0	90
Normal Spermatozoa %	50.6	53	17	81
Head defect %	3.76	3.5	0	11
Midpiece defect %	36.9	34	13	80
Tail defect%	8.8	5.5	1	34
Chest (cm)	91.9	91.8	79	100
Body (cm)	147.3	148	122.5	177
Foot (cm)	43.2	43.5	39	45
Skull (cm)	32.4	33	24	37.5
Antler BCS (inches)	106.1	106.8	52.9	156.4
Scrotum (cm)	18.1	18	14.5	21

Table 3.2 Results from serologic screening for reproductive tract pathogens

Sex	Deer #	Anaplasma	BTV/ EHDV	Neospora caninum	BVD	BHV-1	L. grippo	L. canicola	L. pamona	L. hardjo	L. ictero
M	1	Negative	**Positive**	**Positive**	Negative	Negative	**1:400**	Negative	Negative	Negative	**1:100**
M	2	**Positive**	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
M	3	**Positive**	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
M	4	**Positive**	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
F	5	Negative	**Positive**	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative
M	6	Negative	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
M	7	Negative	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
M	8	**Positive**	**Positive**	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative
M	9	**Positive**	**Positive**	Negative	Negative	Negative	**1:100**	Negative	Negative	Negative	Negative
M	10	**Positive**	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
M	11	**Positive**	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
M	12	**Positive**	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
M	13	**Positive**	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
M	14	Negative	**Positive**	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative
M	15	**Positive**	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
M	16	Negative	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
M	17	**Positive**	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
M	18	Negative	**Positive**	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative
M	19	**Positive**	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
M	20	**Positive**	**Positive**	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative
M	21	**Positive**	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
M	22	**Positive**	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
M	23	**Positive**	**Positive**	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative
M	25	Negative	**Positive**	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative
M	26	Negative	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

M	27	**Positive**	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
M	28	**Positive**	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
M	29	**Positive**	**Positive**	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative
M	30	Negative	**Positive**	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative
F	31	**Positive**	**Positive**	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative
F	32	Negative	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
F	33	**Positive**	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
F	34	**Positive**	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
F	35	**Positive**	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
F	36	**Positive**	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
F	37	Negative	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
F	38	**Positive**	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
F	39	Negative	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
F	40	Negative	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
F	41	Negative	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
F	42	**Positive**	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

Table 3.3 PCA variable from chest, body and hind-foot measurements (cm)

Chest girth	Body length	Hind foot length	Comp.1 (PCA score)
97	150	44	4,49
88	142	40	-6,79
91	147	45	-0,77
94	145	45	-1,27
92	150	45	2,36
92	149	42	1,33
98	158	45	12,17
88	147	44	-2,12
91	149	41	0,86
94	144	45	-2,17
90	148	39	-0,56
89	148	45	-0,74
98	150	43	4,88
99	177	44	29,65
83	123	42	-25,97
98	151	43	5,78
95	148	43	1,78
82	136	44	-14,62
100	150	44	5,80
96	156	44	9,46
92	142	44	-4,88
79	133	42	-18,71

Table 3.3 Correlation analysis of semen parameters

	Volume (ml)	**Concentration (mil/ml)**	**Sperm motility (%)**	**Sperm progressive motility (%)**	**Normal Sperm (%)**	**Head defect (%)**	**Midpiece defect (%)**	**Tail defect (%)**
Volume (ml)	1,00							
Concentration (mil/ml)	-0,09	1,00						
Sperm motility (%)	-0,22	0,18	1,00					
Sperm progressive motility (%)	-0,2	0,12	**0,93**	1,00				
Normal Sperm (%)	-0,280	0,27	0,37	**0,50**	1,00			
Head defect (%)	-0,07	**0,70**	0,20	0,17	0,27	1,00		
Midpiece defect (%)	0,18	-0,45	-0,45	**-0,55**	**-0,86**	**-0,51**	1,00	
Tail defect (%)	0,25	0,15	0,07	0,02	-0,43	0,19	-0,08	1,00

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