EVALUATION OF DIFFERENT METHODS OF INSEMINATION ON CONCEPTION RATE TO FIXED-TIME INSEMINATION OF EUROPEAN FALLOW DEER (*DAMA DAMA*)

A Thesis

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Master of Science

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by

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EVALUATION OF DIFFERENT METHODS OF INSEMINATION ON CONCEPTION RATE TO FIXED-TIME INSEMINATION OF EUROPEAN FALLOW DEER (*DAMA DAMA*)

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DEDICATION

As life has its twists and turns, the one person who has always supported my endeavors and been at the forefront of my personal growth is my father. When I was a teenager, he taught me all relationships are similar to the structure of a home. Each relationship, regardless of what kind it is, requires a solid foundation, and that foundation should be respect. Although houses come in many shapes, they all require at least four walls, and those walls should spell out L-O-V-E. Because as the relationships in your life begin to develop so will your love for the person who is contributing to a part of your story. Lastly, every house needs a roof to protect all your hard-earned valuables stored within, and the roof is built from trust. Without any of these three things the house will not survive the hardships of life. As there will be a number of unpredictable things in life that can destroy the relationships you've built but as long as you forge your relationships out of respect, love, and trust your house will withstand the challenges of time.

Creating this thesis was about more than researching topics I thought were relevant to the growth of the cervid breeding industry and highlighting the questions that need further exploration, which could lead to more conclusive results. Creating this thesis was more about personal growth and developing relationships with people in an industry I want to continue to contribute to for years to come. That is why I dedicate this research to my father, Rafael Ortega, for instilling the morals and values I live my life by that continue to aid in the development of life changing relationships forged over my lifetime.

ABSTRACT

Ortega, Sara J., *Evaluation of different methods of insemination on conception rate to fixed-time insemination of European Fallow deer (Dama dama)*. Master of Science (Agriculture), May, 2021, Sam Houston State University, Huntsville, Texas.

The objective of this research was to evaluate methods of insemination on conception rate to fixed-time insemination (FTAI) following estrous synchronization of fallow does. Mature European fallow does (n = 120) from an established herd (3-S Texas Outdoors, Bedias, Tx) were utilized to evaluate conception rate to fixed-time insemination using two different insemination methods. The does were synchronized with controlled internal drug release (CIDR) devices which were inserted for 14 d as a source of supplemental progesterone and received an intramuscular (i.m.) injection of equine chorionic gonadotropin (eCG; 0.25 mg) at the time of the CIDR device removal to stimulate follicle development and ovulation. The does were then inseminated through previously established cervical or laparoscopic insemination techniques. A subsample of does were implanted with embryos collected from donor females 6 d after laparoscopic intrauterine insemination. All does within the study group were evaluated by ultrasonography 65 days after artificial insemination (AI) and conception was confirmed by the presence of a fetus, fetal heartbeat, and fluid within the uterus. Pregnancy rate to laparoscopic insemination (30.2%) and cervical insemination (16.7%) were both lower compared to results from previous studies in New Zealand. The dates and times of conception for each method of AI and embryo transfer (ET) indicated there were no differences in conception rate between laparoscopic intrauterine insemination when compared to cervical AI ($P < 0.07$). Logistic regression also indicated there was no effect of method ($P < 0.63$) on pregnancy rate to FTAI. These data indicate no difference in conception rate when utilizing different AI methods within fallow deer when synchronization protocols are consistent. Pregnancy rate for embryo transfer was 37.8% which was lower than results from previous studies in New Zealand. The unsatisfactory conception rates to AI and ET indicate that more research is needed to determine the optimal protocol for estrous synchronization and AI in fallow does within the United States.

KEY WORDS: Fallow deer (*Dama dama*); Artificial insemination; Laparoscopic insemination; Reproduction; CIDR; Estrous synchronization.

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A special thanks goes to Kelli Stewart and her family for providing the animals used in this research and opening the doors for me to fall more in love with reproduction research. In addition, I could not have completed this research without the support from my chair, Dr. Kyle Stutts, and Dr. Mark Anderson. The hours they have spent mentoring me will continue to promote my intellectual desire to learn more about this line of study for years to come.

PREFACE

Less than a century ago, research to improve livestock reproductive efficiency was scarce. The predictions of drastic population growth within the United States compelled passionate individuals to meet the demands of the population growth trends through various reproductive strategies to prevent food shortages. This resulted in techniques like artificial insemination (AI), embryo transfer (ET), in vitro fertilization (IVF), somatic cell nuclear transfer (cloning), cryopreservation of various genetic material, and much more. Although these assisted reproductive techniques are vastly different, the one thing they all require to be successful is dedicated individuals to ensure the protocols are implemented accurately. My passion and commitment in the reproductive field stems from a combined interest in agricultural business and animal science with my current research interest on how to improve long-term productivity, sustainability, and profitability of these reproductive strategies.

The research examined in the following study are original ideas that stemmed from my committee chairman, Dr. Kyle Stutts. I am wholeheartedly grateful that he partnered with me on this project and introduced me to the exotic wildlife industry. Conducting research on this topic enhanced our collective reproductive knowledge regarding fallow deer (*Dama dama*), and enabled me to combine my vast interests in astronomy, business, and zoonotic disease with my love for reproduction.

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CHAPTER I

INTRODUCTION

Background

Fallow deer are seasonal breeders with mating coinciding with decreasing photoperiod in autumn and fawning occurring in the summer (Chapman and Chapman, 1975; Asher, 1985; 1986). As urbanized communities continue to expand, fallow deer habitats continue to decline leading to small and fragmented populations, lower genetic variability, and a decline in fertility overtime. Utilization of assisted reproductive techniques are important tools to cope with the dramatic loss of biodiversity (Uccheddu et al., 2020). Over the past three decades, interest in the application of controlled breeding and artificial insemination (AI) technology has grown rapidly (Jabbour et al., 1993), which has improved the avoidance of inbreeding depression within various cervid species (Asher, 2011).

Currently, fallow deer are successfully impregnated through natural mating, AI, and embryo transfer (ET). Utilization of estrous synchronization for AI methods requires the synchronization of both luteal and follicular functions within a female's estrous cycle (Patterson et al., 2013) and remains an effective means of disseminating genes from bucks of high genetic merit more widely and rapidly than through natural mating (Asher, 1986; Mulley et al., 1988; Asher et al., 1990a; 1992). Application of estrous synchronization techniques also enables animal husbandry producers to select which traits align with their individual goals to improve the overall breed quality, fecundity rates, and economic return. Whereas producers that choose to not utilize estrous synchronization techniques make it financially impractical to use AI or other assisted reproductive techniques because of the labor required for detecting estrus (Patterson et al., 2013).

Additionally, implementing effective estrous synchronization programs offer the following advantages (Patterson et al., 2013): (1) does come into estrus at a predicted time that facilitates AI, ET, or other assisted reproductive techniques; (2) the time required to detect estrus is reduced, thus decreasing labor expenses associated with detecting estrus; (3) does will conceive earlier during the breeding period reducing the labor and vet expenditures associated with multiple conception attempts, shortening the fawning seasons, and giving birth to more uniform fawns; (4) does that conceive will fawn earlier in the fawning season and wean heavier fawns throughout their lifetime than those from a non-synchronized doe (Dziuk and Bellows, 1983; Patterson et al., 2013); (5) fawns will be older and heavier at weaning which could assist in the prediction of the animal's expected structural correctness and size at maturity; (6) AI and other reproductive techniques become more practical to achieve herd objectives and can increase reproductive rates.

The preferred method of AI in conjunction with estrous synchronization protocols for fallow deer is laparoscopic intrauterine insemination, as it allows for the precise placement of small quantities of semen close to the site of eventual fertilization (Asher et al., 1990b; 1992; Willard et al., 1998). Though these techniques have been used on various cervid subspecies, timing of insemination and hormone administration to control ovulation in one subspecies commonly results in significantly different conception rates in another subspecies (Asher et al., 1999; Whitley and Jackson, 2003).

The utilization of AI methods also facilitates the maintenance of genetic variation within herds because safe and cost-effective techniques like cryopreservation can be used to import viable spermatozoa from different countries without risking introduction of diseases associated with the importation of live animals (Mylrea et al., 1991). The overall costs associated with breeding fallow deer is notably higher in comparison to native breeds, because they are classified as exotic and limited research has been directed towards them. Therefore, it is important that AI methods are analyzed so producers can be more educated when selecting the methods that are more cost-efficient for their operation.

Research Objectives

The objective of this research was to evaluate methods of insemination on conception rate to fixed-time insemination. The long-term implications of this study include:

- Establishment of an effective AI protocol for captive fallow deer.
- Increasing the fallow deer population within the United States.
- Improving the genetic merit of the fallow deer population.

CHAPTER II

LITERATURE REVIEW

Cervid Species Differentiation

There are over 40 species and 200 subspecies of deer with notably different behavior, physiology, ecology, morphology, and geographic distribution (Asher, 2011). All deer are part of the Cervidae family and are ruminant animals (Cadman, 1971). In many respects, cervids are reproductively similar to domestic ruminant species. Therefore, when developing procedures for reproductive artificial synchronization in cervids, previously established sheep, goat, and cattle artificial synchronization procedures are often referred to as a foundation for research. Leading to the development of several successful technologies, including artificial insemination (AI) and embryo transfer (ET) (Asher, 2011).

However, when evaluating reproductive performance within cervids there is not one species that can be regarded a "typical" deer model. Characteristics that very among cervids include, how many offspring they can conceive yearly, what season they become reproductively receptive, what temperatures initiate the fawning season, and the development process of the embryo and placenta (Asher, 2011). It is understood that these reproductive differences within the various cervid species are essential to obtain optimal survival and growth for their offspring within their native geographic location. However, it is also what makes conducting the same experiments across different species key to understanding cervids in general.

Physical Characteristics

Fallow deer are classified with all other species of deer, antelopes, goats, sheep, and similar large herbivorous mammals with structural dynamics for fast movement (Taylor Page, 1962). No other deer have similar antlers to an adult fallow buck (Cadman, 1971). Fallow bucks are distinguished from other deer because their antlers are broadly palmated with spellers and have brow and trey tines present, which are connected to the skull by the coronet (Fig. 1). Male fallow deer have a tuft of hair hanging from their sheath, which is useful in distinguishing young males and females from a distance (Langbein and Chapman, 2003; Miller and Sykes, 2016; Mungall and Sheffield, 1994), yet fallow males do not have a mane like other cervid species (Cadman, 1966). A fallow buck reaches physical maturity at 6 yr and will typically live from 11 to 15 yr, although records indicate a captive fallow buck in Texas once lived to be 25 yr (Mungall and Sheffield, 1994).

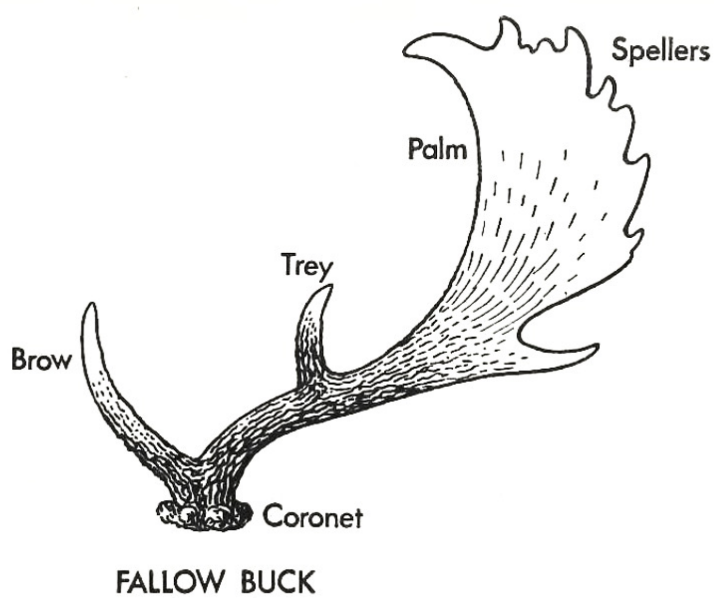


Figure 1

Diagram of a male fallow deer antler.

Note: Illustration obtained from Dawn, Dusk and Deer (1966), and created by C. F. Tunnicliffe.

Bucks in good condition can weigh approximately 168 to 242 pounds but will lose roughly 20 to 50 pounds during the breeding season. Meanwhile, does weigh approximately 65 to 121 pounds and maintain their weight during the breeding season (Taylor Page, 1962; Mungall and Sheffield, 1994; Langbein and Chapman, 2003; Hughes et al., 2017). Additionally, male fallow deer are approximately 3 feet tall at the shoulder, but the does are approximately 3 to 4 inches shorter (Taylor Page, 1962; Cadman, 1966; Mungall and Sheffield, 1994; Langbein and Chapman, 2003). With considerable length variations, the distance from the nose to the last vertebra measured along the mid-dorsal line for a buck is approximately 5 feet 3 inches and approximately 7 inches shorter for a doe (Langbein and Chapman, 2003). Both male and female fallow deer develop a predominate Adam's apple (Mungall and Sheffield, 1994) and have no upper front teeth. Instead, they have a dental pad that closes against the lower teeth (Cadman, 1971; Chapman and Chapman, 1975). The tail of both sexes is approximately 9 inches, which is unusually long when compared to native species (Cadman, 1966; 1971; Chapman and Chapman, 1975; Langbein and Chapman, 2003). Often, a mature fallow's tail will be carried upwards during the first moments of alarm while fawns curl their tails upwards when suckling (Cadman, 1971; Chapman and Chapman, 1975). Unlike roe and sika, fallow do not have the ability to flare the fur on their rump when alarmed (Chapman and Chapman, 1975).

Fallow deer often have long and ragged coats (Miller and Sykes, 2016) and can sometimes display curls on the forehead and tiny tufts of hairs within the ears (Langbein and Chapman, 2003). Fallow are frequently seen in black, white, and menil, commonly referred to as spotted, colors (Cadman, 1966; 1971; Chapman and Chapman, 1975; Langbein and Chapman, 2003; Miller and Sykes, 2016; Hughes et al., 2017).

Menil is the most common color of fallow deer and appears as a rusty shade of brown with large white spots along the ribs, a white line along the rump on the haunches, and solid white along the rump and belly. The white along the rump is emphasized by a bordering line of black and extends to outline the tail (Taylor Page, 1962; Miller and Sykes, 2016) (Fig 2). In Old English, "fealou" means "pale brown or reddish yellow." It is believed this is how fallow deer received their name (Miller and Sykes, 2016). In addition, during the winter months, the spots on a menil-colored fallow fade and appear gray (Taylor Page, 1962; Langbein and Chapman, 2003; Miller and Sykes, 2016).

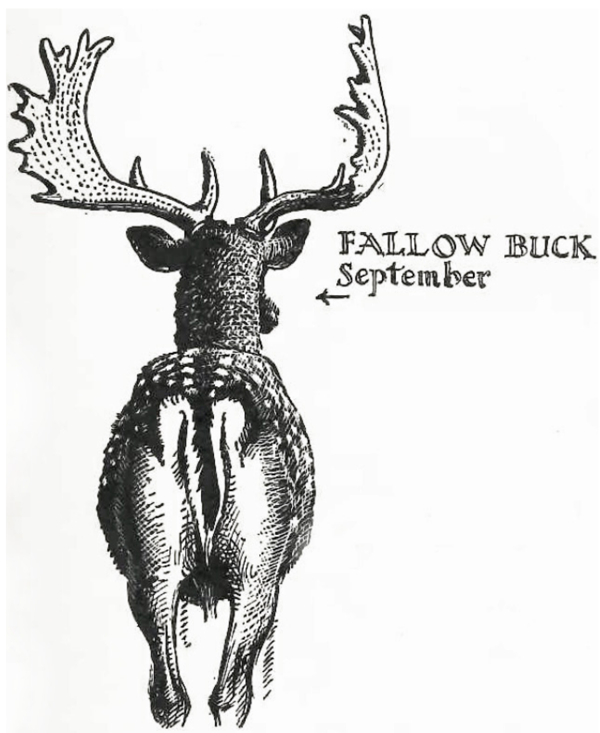


Figure 2

Menil (common) colored fallow buck.

Note: Illustration obtained from Dawn, Dusk and Deer (1966), and created by C. F. Tunnicliffe.

A black fallow is not truly black. During the summer months, a black fallow almost appears dark chocolate with grayish-brown spots that are hardly visible. Then, during the winter months, a black fallow loses its shiny coat and appears more of a dusky brown (Langbein and Chapman, 2003; Miller and Sykes, 2016).

Lastly, white fallow have dark eyes and are born a cream color which whitens as they mature, sometimes maintaining their creamy tan color for half a decade or longer (Chapman and Chapman, 1975; Mungall and Sheffield, 1994). The hooves and nose of a white fallow are less pigmented than normal appearing orangish in color, whereas all other color variations are black (Langbein and Chapman, 2003).

Albinos have been recorded but they are rare. Only the fallow which are true albinos have pink eyes and are a pure white at birth (Chapman and Chapman, 1975; Mungall and Sheffield, 1994). While most deer do not typically develop color variations, fallow are the most variable of any species (Hughes et al., 2017). Although all color variations interbreed with one another, black is expressed as the homozygous dominate, menil is heterozygous, and white is homozygous recessive (Langbein and Chapman, 2003).

Distribution and History

Fallow deer are one of the most widely distributed species of deer in the world (Chapman and Chapman, 1975; Mungall and Sheffield, 1994; Langbein and Chapman, 2003; Hughes et al., 2017), as they are currently found on all inhabited continents (Mungall and Sheffield, 1994). This includes nearly all the counties in England, most counties in Wales, several counties in Scotland, and most counties in Ireland (Chapman and Chapman, 1975; Langbein and Chapman, 2003). Allegedly George Washington brought the first fallow deer to the United States from Europe to lavish his Mount Vernon estate (Mungall and Sheffield, 1994).

Fallow deer are believed to have been originally introduced to new countries by the Phoenicians and, subsequently, on a wider scale by the Greeks and Romans (Cadman 1966; Chapman and Chapman, 1975; Mungall and Sheffield, 1994). In Greek and Roman mythology, fallow deer are portrayed as the protectors of the forest, resulting in Greek and Roman writers and artists depicting fallow bucks on ancient coins, pottery, statutes, and often illustrating them next to the goddesses of hunting, Artemis and Diana, as their companion. Fallow deer's distinct antlers have also been identified on rock carvings, mosaics, treasures, statues, and monuments from the Bronze Age, Asia Minor, and ancient turkic periods. Fallow deer were also present in Britain in the Pleistocene interglacial periods, though it is likely they were reintroduced after some time (Chapman and Chapman, 1975).

Supposedly, fallow deer originate from the Mediterranean region of southern Europe, Turkey, and Asia Minor; however, the precise location is indeterminable (Chapman and Chapman, 1975; Hughes et al., 2017; Mungall and Sheffield, 1994). Deer colonization occurs through intentional introduction by man or by natural spread due to continual changes in rural environments (Chapman and Chapman, 1975). One of the first recorded legends regarding fallow deer documented that they were initially transported around the Mediterranean region because of their ability to eradicate snakes and the healing properties of the bone marrow in their antlers, though neither have been proven to be more than myths. (Miller and Sykes, 2016).

It is believed that the value placed upon fallow deer centuries ago is how they have not only survived for thousands of years, but also led to the expansion of their geographic base across continents. As they are predominately grazing animals often feeding on shrubs and trees, fallow deer have been seen in an assortment of varying habitats with the most frequent places being woods, meadows, copses, pastures, overabundant vegetation, mountainous areas, and arable fields (Chapman and Chapman, 1975). However, they have also been observed in waste lands that are not afforested and still managed to thrive (Cadman, 1971).

Factors such as the sex of the animal, the degree of disturbance, and weather all effect the locality in which a fallow will choose to reside (Chapman and Chapman, 1975). Fallow deer continually prove to be adaptable to a wide range of pastoral and climatic environments even if the genetic base of the founding stock is limited (Cadman, 1966; Mulley et al., 1987; Asher et al., 1990b; Morrow et al., 1994; Mungall and Sheffield, 1994).

In the 11th century, Canute, the Danish king of England, drew up the code of forest laws which preserved fallow deer and made the punishment for killing one the amputation of a right hand, removal of an eye, or 2 pounds of flesh. Additionally, domesticated dogs caught within fallow-populated forests were crippled by having their two center toes removed from their front feet (Lascelles, 1886). The first specific mention of hunting fallow on horseback with Royal Buckhounds occurred in the 14th century, but was a sport limited to royalty (Chapman and Chapman, 1975).

It was not until the early nineteenth century that fallow deer became hunting game for venison for the general public, but it was only allowable in March and April (Lascelles, 1886). For the centuries following, fallow deer were a continual source of food, sport, and pleasure for man (Chapman and Chapman, 1975; Langbein and Chapman, 2003). The same is still true today, though fewer people regularly eat venison. The form of the sport has varied over the centuries, from stalking with a bow and arrow while coursing with dogs to hunting on horseback with horns and hounds to stalking with a rifle today (Chapman and Chapman, 1975).

Present Status of the Question

Within the past three decades, domestication of fallow deer for venison and trophy antler production has become increasingly popular in the United States and Canada (Frosch et al., 2008). Currently, the world record antlers measure 37 $\frac{2}{5}$ inches long and are from Turkey. The biggest fallow antlers within the United States are from Texas, measuring 29 to 35 inches long with palms 5 $\frac{1}{4}$ to 10 $\frac{3}{8}$ inches wide. A good set of fallow antlers within Texas is approximately 23 inches or longer, with a palm width of at least 3 $\frac{1}{2}$ inches wide (Mungall and Sheffield, 1994). Although bucks are the only sex to grow antlers the character trait is transmitted by both sexes (Chapman and Chapman, 1975).

There are approximately 8,000 cervid farms within the United States that generate an estimated breeding economic activity of $523 million and an estimated hunting economic activity of $73 million in direct impacts. The combination of secondary impacts for items such as such as fuel, feed, vehicles, labor, ammo, rifles, and sporting goods totals approximately $3 billion (Frosch et al., 2008). Despite the interest shown in deer, there is sparse reproductive research available regarding fallow deer. Since the middle of the nineteenth century, limited research efforts have been conducted on various aspects of fossil or living fallow deer; however, numerous anecdotal articles have been published (Chapman and Chapman, 1975).

Reproductive characteristics can vary significantly within different species. While some mammals can reproduce during the whole year, others are only reproductively receptive during a defined season. Environmental conditions (Monniaux et al., 1997), presence of pheromones from either males or other females (Asher, 1986), and photoperiodism can affect the initiation and duration of mammalian cycles. Ruminants, carnivores, and rodents are all seasonal breeders, and the length of their cycle is correlated with their mature size. Small rodents will have short cycles lasting approximately 4 to 5 d, whereas larger farm animals like sheep have cycles approximately 17 d long. The number of ovulations a mammal has during one cycle differs between species and breeds within the same species (Monniaux et al., 1997).

Fallow deer are seasonally polyestrous (Asher, 1985; 1986), which is a term used for mammals that are reproductively receptive during one specific season each year. Observations at various latitudes have provided evidence of the importance of light in initiating the breeding season of fallow deer (Chapman and Chapman, 1975; Asher, 2011). In the northern hemisphere, the onset of rutting activity in bucks and the first estrus within does occurs in autumn (Asher et al., 1990b; Mungall and Sheffield, 1994; Asher, 2011) and fawning occurs in summer (Chapman and Chapman, 1969a; 1975; Mungall and Sheffield, 1994; Asher, 2011). Even though the forage produced is considerably different between the northern and southern hemisphere, according to Asher et al., (2011) transferring seasonal breeders across the equator results in an exact 6 mo estrous cycle change.

The “rut” is a term used within various deer species to indicate the peak period in the breeding season when males exhibit intense overt sexual behavior, during which period most conceptions occur. Previously conducted studies identified the occurrence of the first oestrus within does to stimulate groaning activity within fallow bucks and limit the activity to only the duration of the mating period. Unlike red deer that "roar" at least two weeks before the onset of mating activity (Asher, 1986). It is believed the first fallow buck begins his mating call in the northern hemisphere around September 27th and the peak of the rut is thought to occur from October 18th to October 25th, yet multiple observations have recorded fallow buck's groaning well into the first week of November (Cadman, 1971; Mungall and Sheffield, 1994).

However, the breeding season of fallow does is considerably longer than indicated by the length of the rut (Asher, 1986). Observations have recorded fallow breeding from timeframes as long as September to February within Texas (Mungall and Sheffield, 1994). Most published research regarding conception efforts within fallow deer occur throughout October (Asher et al., 1999); however, research results continuing into mid-November have higher than the average conception rates when compared to October (Asher et al., 1992).

The average estrous cycle of a mature fallow doe is between 21 to 23 d (Asher, 1986, 2011; Asher et al., 1990b; 1999; Polegato et al., 2018) with approximately 5 to 8 cycles expressed, including the silent ovulations, within a breeding season (Asher, 2011). According to research conducted by Asher (1986), first time cycling fallow does have fewer cycles than older does. The approximate mean number of true cycles for does 16 mo, 28 mo, and 40+ mo is 3.56, 4.24, and 5.43 cycles a year, respectively. In addition to fewer cycles, as the season progresses the number of days within each fallow doe cycle significantly decrease until the extending daylight of spring ceases the cycling all together.

The gestation period for fallow does is between 228 to 257 d (Chapman and Chapman, 1975; Asher, 1986; Mungall and Sheffield, 1994; Bériot et al., 2014; Polegato et al., 2018) with the overall mean (+sd) length around 234.2+-2.7 d (Asher, 1986), and an approximate prenatal mortality rate can occur as high as 10% (Chapman and Chapman, 1975). Fallow births can begin around mid-May, but most fallow fawns are born in June, and reports have recorded the occasional fawn being born as late as October or November, which suggests fallow does are still capable of conceiving until March (Chapman and Chapman, 1975; Mungall and Sheffield, 1994). The average estrous cycle durations among other species is 17 d in sambar and axis deer, 18 to 20 d in Eld's and red deer, and 24 to 27 d in moose and black-tailed deer. However, the cycle mean duration tends to increase as the breeding season progresses in most deer species (Asher, 2011).

Fallow deer differ from native deer species in their mating behavior as well. As most male cervids will round up and hold a group of does until they are receptive, this does not occur within fallow deer herds. It is the does which come to the buck and then lead them into a mating chase often 100 to 150 yards from their rutting grounds (Cadman, 1971; Mungall and Sheffield, 1994). Most observations of a doe being serviced have occurred between 9 AM and 3 PM, although it is rare to witness, because the majority of mating attempts are believed to take place at night (Cadman, 1971).

Although both male and female fallow deer reach puberty at around 16 mo (Chapman and Chapman, 1969a; 1975; Mungall and Sheffield, 1994), it is unknown at what age males start to breed because the presence of dominant bucks prevents yearlings from breeding and limit their efforts for subsequent years (Mungall and Sheffield, 1994). However, female fallow does can conceive at this age (Cadman, 1971; Chapman and

Chapman, 1975; Asher, 1986), yet it is common for does to conceive their first pregnancy closer to 2 yr (Cadman, 1971; Mungall and Sheffield, 1994). A female fallow can breed continually for at least 12 yr (Mungall and Sheffield, 1994). The ovaries of a fallow fawn are approximately the size of a gain of rice. As they approach puberty, the ovaries grow to roughly the size of a large pea (Chapman and Chapman, 1975). Follicular growth occurs in a cyclic pattern within mammals and as one follicle gradually becomes more dominant all others will regress (Monniaux et al., 1997). Therefore, fallow does usually shed only a single ovum following the rupture of a follicle, and previous studies indicate the occurrence of twins has less than a 1% possibility (Chapman and Chapman, 1975; Mungall and Sheffield, 1994).

Anoestrus is a period of time during which a female is incapable of reproducing and is characterized by low peripheral plasma concentrations of progesterone indicative of complete ovulatory arrest, which may persist for 4 to 6 mo typically from spring to early autumn (Asher, 2011). The female fallow deer's transition into the breeding season from anoestrus is characterized by one or more silent ovulations and 8 to 10 d *corpora lutea*, followed by the first true cycle (Asher, 1986; Youngquist and Threlfall, 2006). True estrus is defined as the period when a receptive female voluntarily stands to be mounted. This behavioral strategy ensures mating occurs at the optimal time for sperm to reach the site of fertilization (Morrow et al., 2009). The occurrence of silent ovulations can also characterize the end of the breeding season in some fallow does (Asher, 1986).

Seasonally polyestrous ruminants, like sheep and goats, also have anoestrus phases to which are commonly referred to when a reproductive study under consideration has not yet been conducted with deer. The ovine and goat industries regularly use progesterone and its synthetics to synchronize estrous cycles by oral administration or implantation of intravaginal sponges (Whitley and Jackson, 2003; Koyuncu and Ozis Alticekic, 2010). Though cattle are also ruminant mammals, they are not typically referred within deer research because studies within the last few decades indicate the use of common superovulatory drug used in beef cattle, like prostaglandin, decline the overall fertility and conception rates within goats as the dosage increases. The beef cattle industry commonly uses controlled internal drug release (CIDR) devices in combination with superovulatory drugs, yet the goat industry only uses CIDR devices outside the United States (Whitley and Jackson, 2003).

Synchronization of the estrous cycle in the deer industry is commonly induced by utilizing progesterone releasing CIDR devices (Youngquist and Threlfall, 2006), which decreases the time and stress involved in handling animals for multiple insemination attempts (Mulley et al., 1988). The CIDR devices are typically inserted intravaginally for 10 to 14 d and then removed manually (Asher, 1986; Jabbour et al., 1993; Youngquist and Threlfall, 2006; Thomas et al., 2016). Achieving a highly responsive estrous synchronization response after CIDR removal is dependent on uniformity in the development of the dominant follicle during the period of CIDR treatment (Thomas et al., 2016). Upon removal of the CIDR device, the blood progesterone concentration levels decrease resulting in a surge in luteinizing hormone (LH), which induces ovulation (Youngquist and Threlfall, 2006), and insemination typically occurs between 60 to 72 h after the CIDR device removal in fallow deer (Mylrea et al., 1991; Asher et al., 1992).

Generally, when a program wants to ensure the buck they have chosen is responsible for impregnating the desired does through potentially multiple AI attempts, an intact buck is kept in the adjoining enclosure or a vasectomized buck is included into the group of recently AI does 10 to 14 d after insemination (Asher et al., 1990b; 1992; Youngquist and Threlfall, 2006). If the initial AI does not result in a pregnancy, the buck will prompt the females to return to estrous until she conceives or until her estrous ceases from the extending daylight of spring. However, most operations will use a “cover buck”, also known as a “clean-up buck”, to breed females that did not conceive to AI. Research indicates the mortality rate for fawns is highest in the winter and early spring months, most likely due to environmental conditions (Chapman and Chapman, 1975). Therefore, a common practice is to remove any intact males from the breeding group after three cycles if there is still not a confirmed pregnancy to avoid fawns being born late in the fawning season (Youngquist and Threlfall, 2006).

There have been many research articles published over the last few decades that describe in detail methods of AI and ET to enhance the breeding conception rates of various cervid species. To date, the standard protocol for laparoscopic intrauterine insemination of fallow deer yields 60 to 70% conception rates (Mulley et al., 1988; Asher et al., 1990b; 1992; Jabbour et al., 1993; Youngquist and Threlfall, 2006); however, as previously mentioned, results in earlier research for mid-November presented an 80% average conception rate (Asher et al., 1992). Records consistently report the pregnancy rate for natural conception in fallow deer at approximately 83 to 85% from the first oestrus mating (Asher, 1986; Asher et al.,1999), and the conception rate increases to approximately 96% by the second or third oestrus mating (Asher, 1986), which is repeatedly a higher conception rate than the assisted reproductive techniques utilized currently.

Within the fallow deer industry, high conception rates have been reported of 81% and 78% for intrauterine insemination with 7.5×10^6 fresh and 25×10^6 frozen-thawed spermatozoa, respectively. However, one reproductive fallow deer study reported that the concentration of spermatozoa deposited in the cervix had no effect on conception rate (Jabbour et al., 1993). These techniques have neither proven to be consistent in producing offspring nor have not been integrated effectively into current fallow deer breeding programs. The results are sporadic, and ET, in particular, has consistently low conception rates in comparison to other cervid species (Asher et al., 1999) due to erratic superovulatory responses to exogenous gonadotropin regimens and poor embryo recovery rates (Morrow et al., 1994).

Few protocols are presently generating the same degree of synchronization required to achieve optimal pregnancy rates to fixed-time AI within fallow deer. Various strategies have been published to evaluate follicular synchronization, and studies evaluating the utilization of pregnant mare's serum gonadotropin (PMSG) (also known as equine chorionic gonadotropin, eCG) or gonadotropin-releasing hormone (GnRH) in combination with CIDR devices continually have inconsistent results. There have also been several studies conducted on manipulating the estrous cycles using CIDR and PMSG to induce various seasonal breeding species to conceive during anoestrus.

There are multiple factors to consider when choosing which method to use to detect pregnancies early within the breeding season. The diagnostic technique must be accurate, safe, rapid, relatively simple to use, and easy to transport (Lane and Lewis, 1981). Accurately detecting pregnancy early will allow producers to breed the female again during the next estrous cycle or enable producers to cull females from the herd that did not conceive to their ideal management practices.

Approximately 12 d after conception, the blastocysts elongate and begin the implantation process within the uterus. The earliest form of detection can be through laparoscopy around 16 d. However, it is most likely uneconomical for the average producer to detect pregnancy through laparoscopy because of the necessary time to evaluate each female and the cost associated with hiring trained professionals and transport the equipment required for the procedure (Cole and Foxcroft, 1982). Analyzing blood and plasma progesterone is the second earliest form of detecting pregnancies within swine and ewes. As with laparoscopy the diagnosis per female requires a considerable amount of time, trained technicians, and a laboratory in close proximity to conduct the analysis (Lane and Lewis, 1981; Cole and Foxcroft, 1982). Rectal palpations are rapid and accurate in ewes after 65 d of conception but are associated with damage to the rectal wall, which can cause peritonitis and, in extreme cases, result in death (Lane and Lewis, 1981).

Conception is regularly confirmed by ultrasonography within various species the animal breeding industry (Lane and Lewis, 1981; Cole and Foxcroft, 1982; Mulley et al., 1987) through doppler (detection of a fetus pulse) or a-mode (detection of embryonic fluid within the uterus) analysis (Cole and Foxcroft, 1982). In the 1950s ultrasonography was developed to composite images of organs and connective tissue within humans. This imaging process uses a piezoelectric crystal as a medium to send and receive acoustic pulse echoes derived from sonar waves. The waves bounce from connective tissue, allowing images to be captured of the organs that are outlined. Ultrasonography is an invaluable tool when assessing the presence, development, and age of a fetus because the acoustic radiation admitted when compositing images appears to have no significant biological effects on the fetus. Additionally, up to 60 images per second can be obtained making ultrasonography ideal for accurately assessing a fetus. Because of the low cost and absence of radiation effects, ultrasonography has become the most widespread method used when evaluating a fetus (Jaffee, 1982).

The most common errors associated with estimating conception rates by confirmed ultrasonography pregnancies are due to operator inexperience and abortions after examination, but these combined errors occur in less than 3% of the females evaluated. Also, weather and temperature do not affect the accuracy of ultrasonography to detect a viable fetus (Lane and Lewis, 1981). As the fetus continues to develop, the accuracy of pregnancy detection increases within all diagnostic methods and approaches 100% accuracy after the first trimester, excluding the occurrence of diseases that may result in abortions (Cole and Foxcroft, 1982).

As a whole, fallow deer are healthy animals, and it is uncommon to hear of large numbers of fallow dying from any sickness. There are several diseases that have been recorded in captive fallow deer including rabies (Chapman and Chapman, 1975; Zhu et al., 2015), spinal ataxia (Chapman and Chapman, 1975; Geisel et al., 1997), T-cell lymphocytic leukemia (Madson and Opressnig, 2009; Kleinschmidt et al., 2012; Suedmeyer et al., 2015), cerebrocortical necrosis (Markson and Giles, 1973; Chapman and Chapman, 1975), and Johne's disease (Jackson et al., 2020) but almost always involve a single animal.

Bone abnormalities ranging from minor deviations to chronic arthritic conditions are more common within fallow deer (Chapman and Chapman, 1975; Baker et al., 1979).

Defects of bone growth are not the same in every case on the cellular level and are commonly identified in cattle, sheep, and goats (Baker et al., 1979; Chapman et al., 1984). Typically, bone growths frequently occur on the edge or surface of joints, but they also can be found on the vertebrae, ribs, hips, knees, shoulders, elbows (Chapman and Chapman, 1975), wrists, metacarpals, metatarsals (Chapman and Chapman, 1975; Chapman et al., 1984), and mandible (Chapman and Chapman, 1969b). Joints that are severely engrossed with bone abnormalities can render an animal incapable of movement (Chapman and Chapman, 1975).

The primary cause of bone abnormalities appears to be a defect in calcification of the cartilage due to inadequate nutrition, resulting in abnormal bone lengths or closure inability of the epiphyseal plates. Depending on the type of bone, the epiphyseal closure may be delayed by approximately 1 yr, which can be an indication of when inadequate nutrition occurred. Further causes within fallow include trauma or compression to the growth plates (Chapman et al., 1984). Abnormalities most of the time reduces the agility of an animal and shortens their overall lifespan (Chapman and Chapman, 1975; Baker et al., 1979; Chapman et al., 1984). Though slight abnormalities can occur in younger fallow, it is most extensive in aged bucks and does (Chapman and Chapman, 1975), and the same frequency appears in males as in females (Chapman and Chapman, 1969b).

It is uncommon for captive fallow to develop tumors, either malignant or benign (Chapman and Chapman, 1975; Kidd and Reuter, 1989; Sakai et al., 2001; Hughes et al., 2017). However, recorded abscesses in captive fallow have arisen in several locations, including the brain (Kidd and Reuter, 1989; Hughes et al., 2017), eyelid (Sakai et al., 2001; Hughes et al., 2017), perineal, lymph nodes, neck tissue, spinal cord (Hughes et al., 2017), nasal cavity, and mandible (Chapman and Chapman, 1975). Historically, tumors can have varying appearances. The most common tumors reported in fallow deer are types of fibromas, which are more commonly seen as a result of the deer papillomavirus. Documentation of this infection has been made within white-tailed deer, mule deer, European elk, reindeer, red deer, roe deer, sika, black-tailed deer, and caribou (Hughes et al., 2017).

Fallow deer can also be the host for ectoparasites like lice, ticks, and mites, though none will affect a female's ability to reproduce. Likewise, both does and bucks can be hosts to a wide range of endoparasites with the main four groups being roundworms, tapeworms, lungworms (Chapman and Chapman, 1975), or liver fluke (Chapman and Chapman, 1975; Jenkins et al., 2020). Recent research indicates fallow deer tend to have a lower lymphocyte count than other wildlife species, but this does not affect their innate immune response system to kill bacteria (Dugovich et al., 2019).

Although such occurrences are important, surveys conducted within various zoos indicate that fallow are overall healthy animals and not likely to transmit diseases to one another or domestic stock. There has even been a case where a female fallow had a leg amputated in a car accident and still managed to give birth that season (Chapman and Chapman, 1975).

In addition to diseases and some parasites, most mammals will not reproduce without having their nutritional requirements meet. In research conducted on the effects of starvation in rats, the number of developing follicles decreased indicating metabolic hormones, such as insulin and insulin-like growth factors, have an impact on reproduction (Monniaux et al., 1997).

To improve AI effectiveness, with or without the use of CIDR devices, detailed information should be gathered on the general biology and habits of the females to allow manipulation of their ovulation cycles and proper deposition of spermatozoa (Cadman, 1971; Asher, 1986; Asher et al., 1990a). Synchronization of estrus is largely dependent on synchronizing ovarian follicular development (Thomas et al., 2016). In addition to focus on the female reproductive cycle, fresh spermatozoa should be collected from bucks within the optimal time frame to heighten pregnancy rates to AI.

CHAPTER III

MATERIALS AND METHODS

Ethical Statement

The use of animals in reproductive related scientific research continues to utilize advancements in technologies to enable the growth of various species within our country and in the global community. Through reproductive research involving various native and exotic animals, scientists continue to develop sound methods of artificial insemination (AI), embryo transfer (ET), in vitro fertilization (IVF), estrous synchronization, hormone treatments, and cryopreservation of sperm, oocytes, and embryos, all while prioritizing high standards of animal safety and well-being throughout the trials.

The scientific community is committed to ensuring all research involving animals conforms to ethical, legal, and safety regulations created by federal guidelines. To fulfill this commitment, ongoing training and education is recommended within the animal community for personnel handling, managing, or operating on animals during research experiments.

Throughout the duration of the research conducted in this investigation, all procedures were performed by a licensed veterinarian and technicians with no physical assistance from the authors. Because the research conducted was focused on analyzing data gathered on comparing the conception rates of cervical AI to laparoscopic AI and evaluating the success of ET, it was not necessary for the authors to assist in a physical capacity. In addition, no animals were harmed or perished due to the research conducted within this trial.

Data Collection

Mature European fallow does (n = 120) from an established herd on a working deer ranch (3-S Texas Outdoors, Bedias, TX) were utilized to evaluate conception rate to fixed-time insemination. Each doe was marked with an ear tag containing a unique identification number, which assisted in maintaining records during this study. Alfalfa hay was provided *ad libitum* in hay racks to supplement Bermudagrass pasture intake throughout the study period. Also, approximately 2.5 pounds of Sportsman's Choice Record Rack Breeder Pellets (Cargill, Inc., Minneapolis, MN) was fed per head per day, with an occasional feeding of whole peanuts to accustom the deer to the presence of humans.

Estrous synchronization to facilitate AI and ET was accomplished using CIDR devices and eCG. Controlled internal drug release (CIDR) devices were inserted for 14 d as a source of supplemental progesterone and does received an intramuscular (i.m.) injection of equine chorionic gonadotropin (eCG; 0.25 mg/per animal) at CIDR device removal to stimulate follicle development and ovulation. Insemination took place 69 to 70 h after withdrawal of the CIDR device. Fresh semen was collected from one buck on-site at approximately 2 to 4 h before insemination by electroejaculation and was immediately evaluated for motility and spermatozoa concentration before being loaded into straws for insemination. Semen from two other bucks was imported from New Zealand by means of cryopreservation techniques. All does were inseminated with at least 40×10^6 spermatozoa.

Method 1: Approximately 52% (n = 63) of the does were impregnated by laparoscopic insemination through the following steps (Asher et al., 1990b). Does were sedated with an i.m. injection of 1.2 ml medetomidine/ketamine hydrochloride mixture (5 mg and 150 mg per ml, respectively). Once recumbent, the does were placed on their backs in a specially designed laparoscopy trolley. Their posterior abdomen was shaved and sterilized with a povidone-iodine scrub (7.5%), iodine tincture spray (2.5%) and isopropyl alcohol (90%), and one end of the trolley was then raised so that the doe was tilted head down at a 40 to 60-degree angle (Youngquist and Threlfall, 2006). The abdomen was inflated with CO_2 gas, and two trocars, one on either side of the midline, were inserted. The laparoscope and the insemination pipette were inserted through the trocars and semen was injected into the uterine horns. The incisions were closed with cyanoacrylate glue and covered with aerosol aluminum bandage (Ideal AluShield) as a protective barrier against external irritant agents. The sedative was reversed with an intravenous (i.v.) injection of atipamezole (2.5 ml). The animals were monitored during the time immediately following recovery and then periodically over 12 hr.

Method 2: Approximately 10% (*n* = 12) of the does within the study group were impregnated by cervical AI through depositing spermatozoa in the *os cervix* through the following steps (Willard et al., 1998). The does were enclosed in a drop-floor deer cradle without anesthesia or sedation. Each doe was restrained manually by the head and along the topline to minimize any movement of the doe while in the deer cradle. An entry door to the back of the cradle allowed the inseminator to access the hind end of the doe. The AI gun and speculum included a light source when inserted into the vagina of the doe to facilitate visualization of the cervix. A (0.25 ml) bovine AI gun and standard insemination pipette containing a straw of semen was then inserted through the speculum into the *os cervix*. Once the site of semen deposition was reached and the semen was deposited, the speculum and AI gun were slowly withdrawn.

Method 3: Approximately 38% (*n* = 45) of the does were chosen to undergo embryo transfer. The donor does (*n* = 12) were selected because they displayed exceptional physical characteristics and were randomly divided into two groups to be administered either a single injection of eCG or eight injections of follicle stimulating hormone (FSH). The group of does that received eCG were injected 60 h before CIDR removal. Whereas the group of does that received FSH were initially injected at 60 h before CIDR removal and again every 12 h until a total of eight injections were administered. The amount of FSH administered declined after every 2 injections given. The dosage of FSH administered was 1.20 ml for injections 1 and 2, 1.00 ml for 3 and 4, 0.75 ml for 5 and 6, and 0.50 ml for 7 and 8.

The embryos were recovered from the eCG and FSH groups by laparotomy 6 d after laparoscopic insemination through the following steps (Morrow et al., 1994). The does were sedated by i.m. injections of (1.2 ml) medetomidine/ketamine hydrochloride mixture (5 mg and 150 mg per ml, respectively). Once recumbent, the does were placed on their backs in a specially designed laparoscopy trolley and given clenbuterol to reduce swelling within the reproductive tract. Next, their posterior abdomen was shaved and sterilized with a povidone-iodine scrub (7.5%), iodine tincture spray (2.5%) and isopropyl alcohol (90%) and one end of the trolley was raised so that the doe was tilted head down at a 40 to 60-degree angle (Youngquist and Threlfall, 2006). Mid-ventral laparotomy allowed the reproductive tract to be evaluated, and the number of ruptured and unruptured follicles for each ovary was record. Approximately 20 to 25 ml of warm medium flushed through the uterine horn via a 12-gauge catheter to assist in the collection of the embryos and were subsequently examined under a microscope. The morphology of recovered embryos was then evaluated according to developmental stage and structural integrity before they were transferred. The abdominal incisions of the donors were closed using cyanoacrylate glue and covered with an aerosol aluminum bandage (Ideal Alushield). Donors were also treated with a long-acting antibiotic before being returned to the pasture.

Embryos were collected 6 d after insemination and identified as a morula or blastocyst. To be classified as a morula the mass within the embryos was at least 16 cells and occupied most of the perivitelline space. Whereas a blastocyst cell mass is darker and more compact with distinct differentiation of the outer trophoblast layer and inner cell mass. After the embryos were confirmed to either be a morula or blastocyst, the quality of each embryo was carefully examined to determine its viability if transferred to a recipient. Only embryos of excellent or good quality with a symmetrical mass and uniform size, density, and color were eligible for transfer. Research evaluating the pregnancy rates according to the embryo development stage in cattle and did not identify any significant difference between embryos transferred at the morula or blastocyst stage. However, a decrease in pregnancy rate has been previously noted for embryos at a more developed stage (Bó and Mapletoft, 2013). Therefore, the acceptability of an embryo to be transferred was determined by an experienced veterinarian at Advanced Deer Genetics (USA), LLC.

Once the embryos were collected, analyzed, and prepared for transfer, the recipients underwent the same initial steps as the does inseminated by laparoscopy. The fresh embryos were transferred into the recipients by laparotomy. After the midline incisions was performed, the embryos were placed into the uterus. The incisions were closed with cyanoacrylate glue and covered with aerosol aluminum bandage (Ideal AluShield) as a protective barrier against external irritant agents. The recipients were given a long-acting antibiotic before being given an i.v. injection of atipamezole (2.5 ml) to reverse the sedative effects. The animals were monitored during the time immediately following recovery and then periodically over 12 hrs.

Conception rate to AI was determined 45 d after insemination by restraining the does in a drop-floor deer cradle without anesthesia or sedation and using a lubed 7.5-MHz transrectal ultrasonography probe near the interior anal sphincter to evaluate either side of the midline for indications of a fetus (Lenz et al., 1993). Pregnancies at this time were confirmed by the presence of a fetus, fetal heartbeat, and fluid within the uterus.

The does were thoroughly examined for injuries, diseases, and internal or external parasites before commencing with one of the three assisted reproductive techniques previously mentioned, and the same technicians performed each AI attempt.

Data Analysis

Pregnancy rates to AI, ET, and natural service were analyzed by X^2 analysis used the frequency procedure (SAS Institute, Inc., Cary, NC). Logistic regression was preformed to determine the odds of pregnancy after FTAI using the following variables: insemination method, sire, and fresh or frozen semen. Logistic regression was used to determine the odds of production of transferable embryos using the following variables: superovulation method and ovarian status at the time of insemination. The differences for the “clean-up” bucks and weather on various days were analyzed to ensure there were no significant impacts on the research group.

CHAPTER IV

RESULTS AND DISCUSSION

Pregnancy rates to FTAI, ET, and natural service are presented in Table 1. There was no difference (P = 0.29) in conception to fixed-time artificial insemination (FTAI) between the two insemination methods used in the study. Pregnancy rate to FTAI utilizing cervical insemination was 16.7 % and pregnancy rate to FTAI using the laparoscopic method was 30.2%. Additionally, the logistic regression model indicated that there was no main effect of sire ($P = 0.09$) on pregnancy rate to FTAI. However, the does inseminated with fresh semen (42.5%) had a greater ($P < 0.03$) pregnancy rate than the does inseminated with frozen semen (12.2%) within this study. Also, the subsequent pregnancy rates to cover/clean-up bucks were not affected by either of the two previous AI methods ($P > 0.07$), cervical (62.0%) or laparoscopic (54.6%) insemination.

Table 1

Pregnancy rates to fixed-time artificial insemination, embryo transfer, and natural mating of fallow does.

	Number Inseminated	Number Pregnant to Each Method	Number Pregnant to Clean-up Bucks
Laparoscopic intrauterine	63 (52.5%)	19 (30.2%)	26 (59.1%)
Cervical	12 (10%)	2 (16.7%)	6 (60%)
Embryo Transfer	45 (37.5%)	17 (37.8%)	24 (85.7%)

Note: Percentages are based on the total ($n = 120$), and each column after is from the remaining open does.

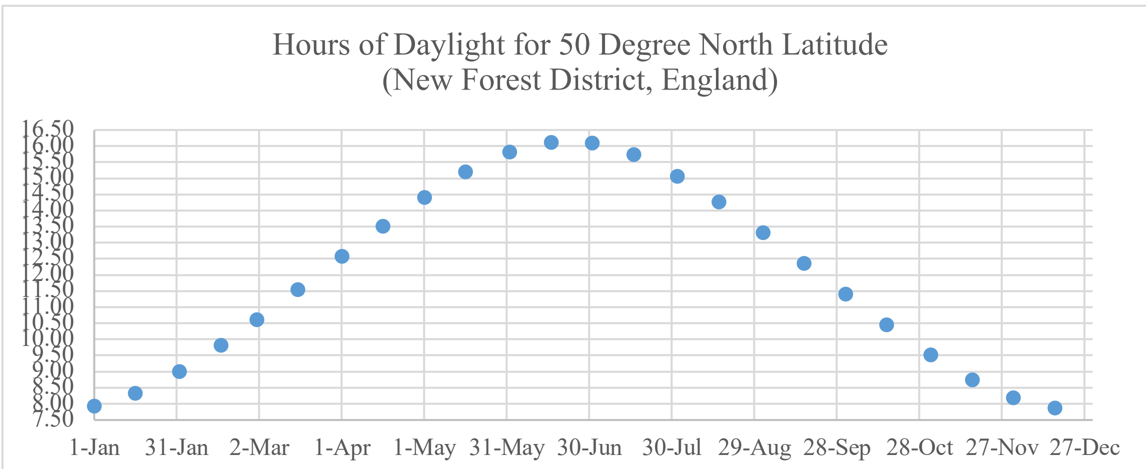
The low conception rate to FTAI in this study is not in agreement with previous studies. Results of previous studies indicate that fallow deer consistently have conception rates between 60% and 80% (Asher, 1986; Asher et al., 1990a; 1990b; 1992). Due to the fact that a large proportion of does returned to estrous following the first AI attempts, it is most likely that the conception attempts failed rather than the occurrence of embryonic loss. Though it is not possible to determine from the data if the does failed to conceive at the first conception attempt or aborted later in the gestation period, it is more likely that absence of a conception is not due to an abortion which tends to prolong the estrous cycle in some cases (Asher et al., 1986).

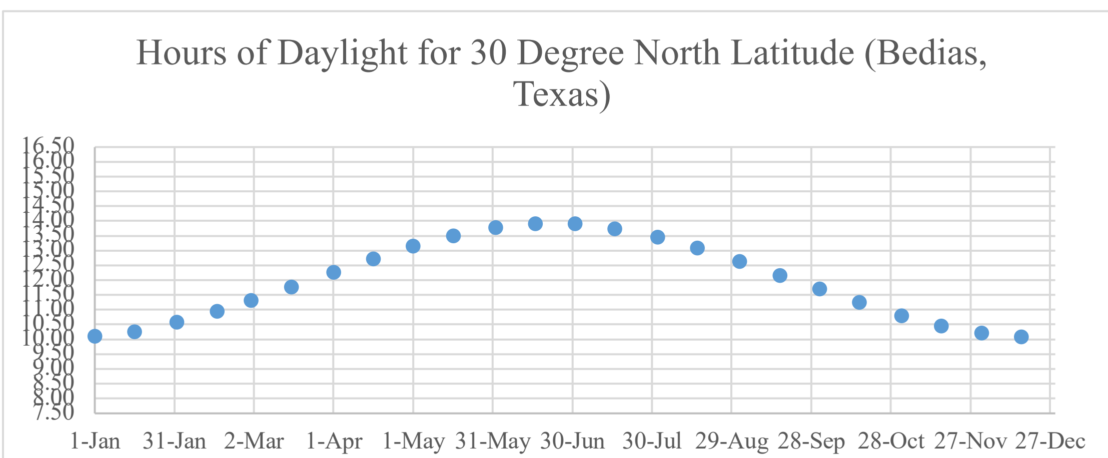
According to Asher (1986), previous treatment of does with exogenous hormones, such as pregnant mare serum gonadotrophin (PMSG), during anestrous is instrumental in causing early cessation of cyclic activity. The study that presented these findings used PMSG to initiate estrous synchronization during the transition phase from anestrous for does that have previously experienced an estrous cycle at least one year earlier. Does synchronized with 500 IU of PMSG yielded an approximate 30% conception rate. Whereas, the does administered 1000 IU of PMSG resulted in formation of multiple corpora lutea (CL), which produce such a high volume of progesterone that once the CL regress, the doe did not cycle again for that breeding season. Although PMSG has been used to induce multiple ovulations or synchronize cycles early within the breeding season, the results indicated that high levels of this treatment are counterproductive. Because of the limited reproductive research regarding this species, there has not been further investigative studies to determine the appropriate dosage to reduce the incidence of multiple ovulations and/or luteinizing follicles to prevent early estrous cessation when administered during the transitional anestrous period.

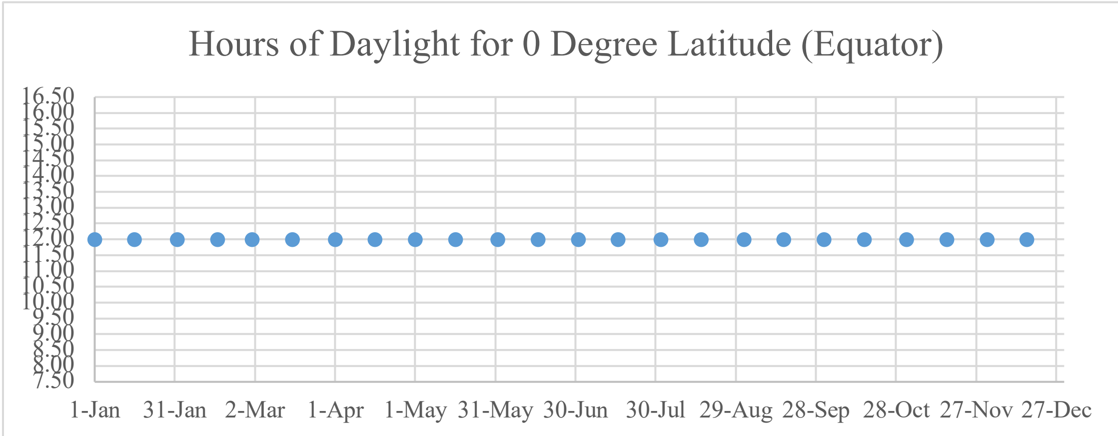
When comparing the conception rates from this study to previously published research, the most similar conception rates were from research regarding estrous synchronization techniques with PMSG during the anestrous phase. Upon further analysis, it was noted that the majority of reproductive fallow deer research has previously been conducted in New Zealand (Asher, 1985; 1986; 2011; Asher et al., 1990a; 1990b; 1992; 1999; Jabbour et al., 1993; 1994) located at 40 degrees south latitude, or in the New Forest District in England (Lascelles, 1886; Taylor Page, 1962; Cadman, 1966; 1971; Chapman and Chapman, 1969a; 1069b; 1975; Chapman et al., 1984;) located at 50 degrees north latitude. The study was conducted in Bedias, Texas located at 30 degrees north latitude (Fig 3). Though previous published works indicate crossing the equator results in an exact 6 mo cycle change, further analysis into the amount of daylight at different geographic locations during the peak of the breeding periods showed approximately 10.5 to 11 h of daylight for the peak of conception within the rut in both New Zealand and the New Forest District, England. Because of earth's natural rotational patterns, the further a location is from the equator, the greater the variance in the hours of daylight presented at various times within the year. Since fallow deer are seasonally polyestrous mammals, future reproductive research might benefit from analyzing blood progesterone levels from does at various life stages to confirm if the peak breeding season in Texas, at 30 degrees north latitude, is at the same time as the peak breeding season for the New Forest, England at 50 degrees north latitude. Conducting further research evaluating blood progesterone levels will indicate if the maximum conception rates within fallow deer in Texas also occur at approximately 10.5 to 11 h of daylight, which would occur between October 28th and November 11th.

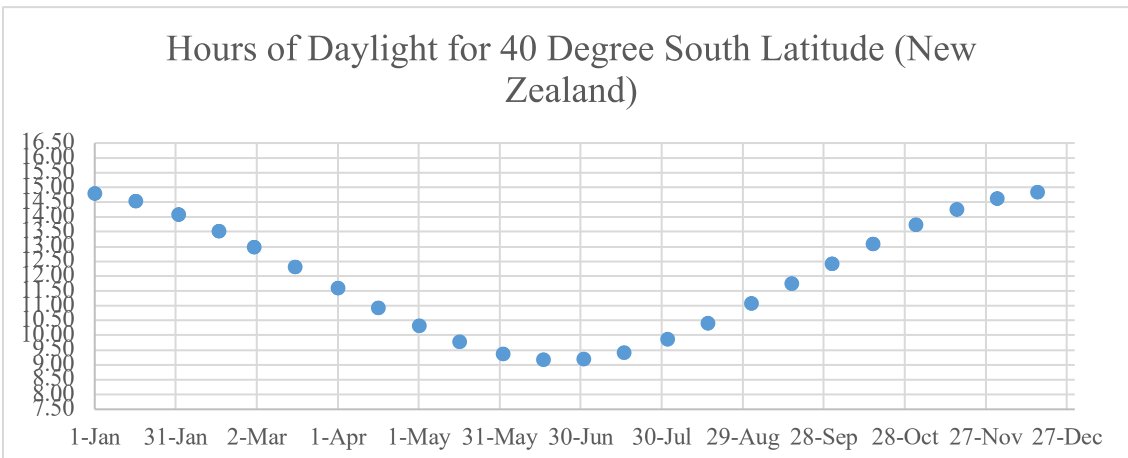
Figure 3

Biweekly hours of daylight for a) The New Forest District, England, b) Bedias, Texas, c) the equator, and d) New Zealand.









Note: Calculations obtained from Astronomical algorithms, 2009, written by J. Meeus.

It is unclear if the discrepancy in conception rates is due to where the animal is located on varying latitudes, the weather conditions at the time of the rut, or the hormonal differences within deer of different geographic locations. Some of the variation may even result from different definitions of when the rut occurs. This may be when the bucks start rutting behaviors like groaning and fighting over rutting grounds, when the peak of conception occurs, or when the silent ovulations occur during the does’ transition from anestrous to becoming reproductively receptive.

The results of the logistic regression analysis on ET indicated there was no effect ($P < 0.14$) of the superovulation protocol (eCG or FSH) on the production of transferable embryos. Also, the ovarian status at the time of insemination of the donor female (presence of follicles, corpora hemorrhagica, or corpora lutea) did not affect the production of transferable embryos ($P < 0.43$). Previously conducted ET research in New Zealand (Morrow et al., 1994) discussed conception rate inconsistencies for fallow deer, therefore the conception rates within this study appeared normal. However, further research should focus on constructing sound protocols that produce consistent ET results for this species.

CHAPTER V

CONCLUSION

This research conducted on estrous synchronization and AI provided more insight on comparing the methods of insemination in fallow deer when utilizing fixed-time insemination methods. Research regarding estrous synchronization procedures often lead to the improvement of time management for producers who use AI by shortening both the breeding and fawning periods. This allows ranch managers to spend more time over a condensed period observing births and, in some cases, reduce the number of fawn losses because of improved management procedures during the fawning periods.

As previously mentioned, AI remains effective for disseminating genes from bucks of high genetic merit more widely and more rapidly than through natural mating, which can also assist in avoiding inbreeding depression. Producers can select breeding stock based upon their specific program objectives and cull stock that appear to digress from those objectives.

In summary, this study has shown that fallow deer have the same likelihood of conception through either cervical or laparoscopic insemination. Newly emerging biological developments (Parker, 2020) have indicated sperm competition goes beyond the competition of individual sperm cells to fertilize a specific ovum. Within the last year, seminal fluid proteins have been identified to variously modify the female reproductive tract towards subsequent matings which reduces the probability of secondary mating attempts. Though this concept could explain the conception rate difference in clean-up buck mating from AI when compared to ET, this concept has not been studied in fallow deer.

In addition, there are conflicts between the present results and other data on conception rates to FTAI in European fallow deer (*Dama dama*). Laparoscopic intrauterine or cervical insemination of adult (at least 16 mo) fallow does during the rut in New Zealand (Mid-April to start of May) produced conception rates of 60-80% (Asher, 1986; Asher et al., 1990a; 1990b; 1992; Jabbour et al., 1993), and results in Europe (Mid-October to start of November) produced conception rates of 70-75% (Chapman and Chapman, 1975). Observations at various latitudes provide further evidence of the importance of light in determining the breeding season of fallow deer which are seasonal breeders that are reproductively receptive in the fall and give birth in the early summer. However, research has not been conducted on analyzing latitude in comparison with blood progesterone or other increasing hormonal elements that correspond with an increase in receptivity to determine if the change in hours of daylight presented at different geographic locations effects the optimal time of conception. And, if so, how different the changes would be for various latitudes from the 6 mo time change estimation currently used when crossing the equator.

Though fallow deer are similar to sheep and goats, which are also seasonal breeders that are reproductively receptive in the fall and give birth in the early summer, they lack precedented research to establish body conditioning scores (BCS). Typically, fallow deer breeders list their breeding stock as one of three categories: 1) thin, 2) ideal, or 3) fat. But, as previously stated in other domestic livestock species, judging animals without a predetermined scale will lead to inconsistencies. Therefore, research should be conducted across multiple farms to establish a fallow deer BCS for future breeding efforts.

Because of the limited data within this study on fallow deer, future work will need to be undertaken to determine if the results from this study are consistent with other fallow deer reproductive results in Texas. In addition, fallow deer breeders may benefit from research creating a financial analysis for the current expenses to compare the AI methods. This can benefit fallow deer breeding farms within the United States by enabling ranch owners to make a more educated decision when determining which method to use on their farm. This would potentially decrease their expenses associated with AI attempts to achieve a viable pregnancy.

By conducting more research to determine the optimal time of conception to breed fallow deer within the United States, further understanding of the fallow doe's reproductive cycle can contribute to the success of other reproductive-related fallow deer projects, thus encouraging the utilization of selection methods to increase the quantity and genetic merit within controlled populations in the United States. It is our hope that this work will encourage further research to evaluate the protocols commonly used on fallow deer and studies including a broader range of reproductive knowledge within this species in the United States.

REFERENCES

Asher, G. W. 1985. Oestrous cycle and breeding season of farmed fallow deer, Dama dama. Journal of Reproduction and Fertility 75:521-529. doi:10.1530/jrf.0.0750521

Asher, G. W. 1986. Studies on the reproduction of farmed fallow deer (Dama dama). PhD Diss. Univ. of Canterbury, New Zealand.

Asher, G. W. 2011. Reproductive cycles of deer. Animal Reproduction Science 124:170-175. doi:10.1016/j.anireprosci.2010.08.026

Asher, G. W., M. W. Fisher, J. F. Smith, H. N. Jabbour, and C. J. Morrow. 1990a. Temporal relationship between the onset of oestrus, the preovulatory LH surge and ovulation in farmed fallow deer (Dama dama) Journals of Reproduction and Fertility 89:761-767. doi:10.1530/jrf.0.0890761

Asher, G. W., D. C. Kraemer, S. J. Magyar, M. Brunner, R. Moerbe, and M. Giaquinto. 1990b. Intrauterine insemination of farmed fallow deer (Dama dama) with frozen-thawed semen via laparoscopy. Theriogenology 34:569-577. doi:10.1016/0093-691x(90)90012-i

Asher, G. W., S. L. Monfort, and C. Wemmer. 1999. Comparative reproductive function in cervids: Implications for management of farm and zoo populations. Journal of Reproduction and Fertility Supplement 54:143-156. doi:10.1530/biosciprocs.4.011

Asher, G. W., C. J. Morrow, H. N. Jabbour, R. C. Mulley, F. A. Veldhuizen, and M. Langridge. 1992. Laparoscopic intra-uterine insemination of fallow deer with frozen-thawed or fresh semen after synchronization with CIDR devices. New Zealand Veterinary Journal 40:8-14. doi:10.1080/00480169.1992.35689

Baker, J. R., D. G. Ashton, D. M. Jones, and B. A. Noodle. 1979. Four cases of chondrodystrophy in fallow deer. Veterinary Record 104:450-453. doi:10.1136/vr.104.20.450

Bériot, M., A. F. Tchimbou, O. Barbato, J. F. Beckers, and N. M. Sousa. 2014. Identification of pregnancy-associated glycoproteins and alpha-fetoprotein in fallow deer (Dama dama) placenta. Acta Veterinaria Scandinavica 56:4. doi:10.1186/1751-0147-56-4

Bó, G. A. and R. J. Mapletoft. 2013. Evaluation and classification of bovine embryos. Animal Reproduction 10:344-348.

Cadman, A. 1966. Dawn, dusk, and deer. Country Life Limited.

Cadman, A. 1971. The fallow deer. Forestry Commission Leaflet 52:1-40.

Chapman, D. I. and N. Chapman. 1969a. Observations on the biology of fallow deer (Dama dama) in Epping Forest, Essex, England. Biological Conservation 2:55-60. doi:10.1016/0006-3207(69)90118-9

Chapman, D. I. and N. Chapman. 1969b. The incidence of congenital abnormalities in the mandibular dentition of fallow deer (Dama dama L.). Research in Veterinarian Science 10:485-487. doi:10.1016/s0034-5288(18)34419-9

Chapman, D. I. and N. Chapman. 1975. Fallow deer: Their history, distribution, and biology. Machynlleth: Coch-y-bonddu Books.

Chapman, D. I., N. Chapman, and L. B. Jeffcott. 1984. Deformities of the metacarpus and metatarsus in fallow deer (Dama dama L.). Journal of Comparative Pathology 94:77-91. doi:10.1016/0021-9975(84)90010-0

Cole, D. J. A., and G. R. Foxcroft. 1982. Pregnancy diagnosis. In: Control of pig reproduction. Butterworth Scientific, London England. p. 293-307.

Dugovich, B. S., L. L. Crane, B. B. Alcantar, B. R. Beechler, B. P. Dolan, and A. E. Jolles. 2019. Multiple innate antibacterial immune defense elements are correlated in diverse ungulate species. PLoS ONE 14. doi:10.1371/journal.pone.0225579

Dziuk, P. J. and R. A. Bellows. 1983. Management of reproduction of beef cattle, sheep and pigs. Journal of Animal Science 57:355-379.

Frosch, B. J., D. P. Anderson, and J. L. Outlaw. 2008. Economic impact of deer breeding operations in Texas. Thesis. Texas A&M University, College Station, Texas.

Geisel, O., E. Betzl, E. Dahme, W. Schmahl, and W. Hermanns. 1997. Enzootic spinal ataxia in fallow and wild red deer in Upper Bavaria. Tierarztliche Praxis Ausgabe G Grosstiere Nutztiere 25:598-604.

Hughes, K. L., R. J. Bildfell, and B. Alcantar. 2017. Pigmented tumors in fallow deer, Dama dama: 11 cases. Journal of Veterinary Diagnostic Investigation 29:483-488. doi:10.1177/1040638717707789

Jackson, R. B., D. J. Kennedy, and E. S. G. Sergeant. 2020. Investigation of Jone's disease in Tasmanian fallow deer (Dama dama). Australian Veterinary Journal 99:44-45. doi:10.1111/avj.13030

Jabbour, H. N., V. S. Marshall, C. M. Argo, J. Hooton, and A. S. Loudon. 1994. Successful embryo transfer following artificial insemination of superovulated fallow deer (Dama dama). Reproduction, Fertility and Development 6:181-185. doi:10.1071/rd9940181

Jabbour, H. N., F. A. Veldhuizen, G. Green, and G. W. Asher. 1993. Endocrine responses and conception rates in fallow deer (Dama dama) following oestrous synchronization and cervical insemination with fresh or frozen-thawed spermatozoa. Society for Reproduction and Fertility 98:495-502. doi:10.1530/jrf.0.0980495

Jaffee, C. C. 1982. Medical Imaging: Many different techniques compete for attention, but each has unique strengths and applications. American Scientist 70:576-585.

Jenkins, D. J., A. Baker, M. Porter, S. Shamsi, and D. P. Barton. 2020. Wild fallow deer (Dama dama) as definitive hosts of Fasciola hepatica (liver fluke) in alpine New South Wales. Australian Veterinary Journal 98:546-549. doi:10.1111/avj.13001

Kidd, G. N. and R. E. Reuter. 1989. Rhabdomyosarcoma in a fallow deer (Dama dama). Veterinary Record 124:466. doi:10.1136/vr.124.17.466

Kleinschmidt, S., M. Peters, and P. Wohlsein. 2012. Presumptive primary central nervous system T cell lymphoma in a deer (Capreolus capreolus). Research in Veterinary Science 93:1334-1336. doi:10.1016/j.rvsc.2012.03.008

Lane S. F. and P. E. Lewis. 1981. Detection of pregnancy in ewes with the ultrasonic scanopreg. Journal of Animal Science 52:463-467. doi:10.2527/jas1981.523463x

Langbein, J. and N. Chapman. 2003. Fallow deer. London: The Mammal Society & The British Deer Society.

Lascelles, G. 1886. The chase of the wild fallow deer. The Nineteenth Century Magazine 10:503-514.

Lenz, M. F., A. W. English, and A. Dradjat. 1993. Real-time ultrasonography for pregnancy diagnosis and foetal ageing in fallow deer. Australian Veterinary Journal 70:373-375. doi:10.1111/j.1751-0813.1993.tb00813.x

Madson, D. M. and T. Opressnig. 2009. Multicentric T-cell Lymphosarcoma in a white-tailed deer (Odocoileus virginianus). Journal of Wildlife Diseases 45:791-794. doi:10.7589/0090-3558-45.3.791

Markson L. M. and N. Giles. 1973. Cerebrocortical necrosis in fallow deer (Dama dama). Veterinary Record 93:243-246. doi:10.1136/vr.93.9.243

Meeus, J. 2009. Astronomical algorithms. William-Bell, Richmond, Virg.

Miller, H. and N. Sykes. 2016. Zootherapy in archaeology: The case of the fallow deer (Dama dama dama). Journal of Ethnobiology 36:257-276. doi:10.2993/0278-0771-36.2.257

Monniaux, D., C. Huet, N. Besnard, F. Clement, M. Bosc, C. Pisselet, P. Monget, and J. C. Mariana. 1997. Follicular growth and ovarian dynamics in mammals. Journal of Reproduction and Fertility Supplement 51:3-23.

Morrow, C. J., G. W. Asher, D. K. Berg, D. K., H. R. Tervit, P. A. Pugh, W. H. McMillan, S. Beaumont, D. R. H. Hall, and A. C. S. Bell. 1994. Embryo transfer in fallow deer (Dama dama): Superovulation, embryo recovery and laparoscopic transfer of fresh and cryopreserved embryos. Theriogenology 42:579-590. doi:10.1016/0093-691x(94)90375-s

Morrow, C. J., L. M. Penfold, and B. A. Wolfe. 2009. Artificial insemination in deer and non-domestic bovids. Theriogenology 71:149-165. doi:10.1016/j.theriogenology.2008.09.001

Mulley, R. C., A. W. English, R. J. Rawlinson, and R. S. Chapple. 1987. Pregnancy diagnosis of fallow deer by ultrasonography. Australian Veterinary Journal 64: 257-258. doi:10.1111/j.1751-0813.1987.tb09699.x

Mulley, R. C., N. W. Moore, and A. W. English. 1988. Successful uterine insemination of fallow deer with fresh and frozen semen. Theriogenology 29:1149-1152. doi:10.1016/s0093-691x(88)80040-2

Mungall, E. C., and W. J. Sheffield. 1994. Established Exotics: Fallow Deer. In: Exotics on the range: The Texas example. Texas A&M University Press, College Station. p. 49-53.

Mylrea, G. E., G. Evans, and A. W. English. 1991. Conception rates in European fallow does (Dama dama dama) following intrauterine insemination with frozen-thawed semen from Mesopotamian fallow (Dama dama mesopotamica) and crossbred (Dama dama dama x Dama dama mesopotamica) bucks. Australian Veterinary Journal 68:294-294. doi:10.1111/j.1751-0813.1991.tb03258.x

Patterson, D. J., J. M. Thomas, N. T. Martin, J. M. Nash, and M. F. Smith. 2013. Control of estrus and ovulation in beef heifers. The Veterinary Clinics of North America, Food Animal Practice 29:591-617. doi:10.1016/j.cvfa.2013.07.009

Parker, G. A. 2020. Conceptual developments in sperm competition: a very brief synopsis. Philosophical Transactions of the Royal Society B 375. doi:10.1098/rstb.2020.0061

Polegato, B. F., E. S. Zanetti, and J. M. B. Duarte. 2018. Monitoring ovarian cycles, pregnancy and post-partum in captive marsh deer (Blastocerus dichotomus) by measuring fecal steroids. Society for Experimental Biology 6:1-10. doi:10.1093/conphys/cox073

Sakai, H., T. Yanai, S. Kawakami, and T. Masegi. 2001. Malignant melanoma of the palpebral conjunctiva in a captive fallow deer. Journal of Wildlife Diseases 37:816-819. doi:10.7589/0090-3558-37.4.816

Suedmeyer, K., B. G. Stockingger, M. M. Garner, D. S. Bradway, C. Hollinger, and M. Kiupel. 2015. T-cell lymphocytic leukemia in a fallow deer (Dama dama). Journal of Zoo and Wildlife Medicine 46:580-582. doi:10.1638/2014-0207.1

Taylor Page, F. J. 1962. Fallow Deer. London: Sunday Times Publication.

Thomas, J. M., B. E. Bishop, J. M. Abel, M. R. Ellersieck, M. F. Smith, and D. J. Patterson. 2016. The 9-day CIDR-PG protocol: Incorporation of PGF2a pretreatment into a long-term progestin-based estrus synchronization protocol for postpartum beef cows. Theriogenology 85:1555-1561. doi:10.1016/j.theriogenology.2016.01.010

Uccheddu, S., E. Pintus, J. J. Garde, L. Fleba, M. Muzzeddu, F. Pudda, L. Bogliolo, A. Strina, S. Nieddu, and S. Ledda. 2020. Post-mortem recovery, in vitro maturation and fertilization of fallow deer (Dama dama, Linnaeus 1758) oocytes collected during reproductive and no reproductive season. Reproduction in Domestic Animals 55:1294-1302. doi:10.1111/rda.13773

Willard, S. T., G. Flores-Foxworth, S. Chapman, M. L. Drew, D. M. Hughes, D. A. Neuendorff, and R. D. Randel. 1998. Hybridization between wapiti (Cervus elephus manitobensis) and sika deer (Cervus nippon): A comparison of two artificial insemination techniques. Journal of Zoo and Wildlife Medicine 29:295-299.

Youngquist R. S., and W. R. Threlfall. 2006. Estrous synchronization and artificial insemination in domesticated cervids. In: Current therapy in large animal theriogenology. 2nd ed. Saunders/Elsevier, St. Louis. p. 925-960.

Zhu, H., X. Chen, X. Shao, H. Ba, F. Wang, H. Wang, Y. Yang, N. Sun, J. Ren, S. Cheng, and Y. Wen. 2015. Characterization of a virulent dog-originated rabies virus affecting more than twenty fallow deer (Dama dama) in Inner Mongolia, China. Infection, Genetics and Evolution 31:127-134. doi:10.1016/j.meegid.2014.

VITA

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Education

M.S. in Agriculture, with a focus in Exotic Wildlife Reproduction.
Sam Houston State University – Expected Graduation May 2021

B.S. in Equine Industry and Business (Magna Cum Laude), Animal Science Concentration.
West Texas A&M University - Dec 2017

Academic Courses Facilitated as Teaching Assistant

Sam Houston State University

AGBU 2317: Principles of Agricultural Economics
AGBU 3385: Quantitative Methods for Agribusiness
AGBU 4365: Legal Issues in Agribusiness

Professional Experience

Agribusiness Teaching Assistant, Sam Houston State University, Huntsville, Texas, 2020-Present

Facilitated agribusiness courses as needed with grading course work and individual tutoring for six professors. Maintained correspondence with students for tutoring and advice. Managed the National Agri-Marketing Association (NAMA) team throughout competitions.

Agribusiness Research Assistant, Sam Houston State University, Huntsville, Texas, 2020-Present

Collected grades and personality results for the previous six years of SHSU undergraduate students enrolled in AGBU 4363, to determine the correlation between how successful students will be within the course based upon their personality type.

Engineering Administrative Assistant, Aurora Technical Services, LLC, Houston, Texas, 2018-Present

Assisted with invoicing and financial cost tracking for projects, drafted professional services agreements, reviewed technical reports and proposals for grammatical errors before submission. Recruited and pre-screened potential new hires before formal interview process.

Overseas Professional Experience

Financial Manager, St. Francis de Sales School for the Deaf, Uganda, 2017-2019

Monitored students' health and escorted to hospitals in medical emergencies, lead sign language interpreter for visitors, managed the financial records, supervised day-to-day operations, tutored students to pass placement exams for secondary school and high-level admissions.

Caretaker, Divine Mercy House of Love for the Poorest of the Poor, Uganda, 2018-2019
Promoted fundraisers within the local communities and taught the orphaned children basic hygiene skills, first aid, manners, and acts of kindness.

Financial Advisor, The Agriculture Rehabilitation and Recovery Support Project, Democratic Republic of Congo, 2018-2019
Assisted in the rehabilitation of war stricken agricultural lands by implementing harvest management procedures that utilized crop rotation techniques.

Curriculum Advisor, St. Aloysius Boys College of Nyapea, Uganda, 2019
Analyzed classroom material for math and science subjects to assist in cultivating new course curriculum plans.

Financial Consultant, Uganda People's Defense Forces (UPDF) Prison, Uganda, 2019
Met with the prison officials to develop rehabilitation workplans to contract the inmates for jobs within the community under tight supervision.

Volunteer Service Activities

Horse Trainer / Lesson Instructor for Ages 4-8, Henry's Home Horse & Human Sanctuary, Conroe, Texas, 2015-Present

Holiday Assistant, Tomball Retirement Center, Tomball, Texas, 2019-Present

Equine Graduate Research Assistant, West Texas A&M University, Canyon, Texas, 2017

Holiday Assistant, Shriners Hospitals for Children – Galveston, Galveston, Texas, 2017

Afterschool Caretaker, Eastridge Mission Center, Amarillo, Texas, 2016-2017

Holiday Assistant, Star of Hope Women's Shelter, Houston, Texas, 2012-2015

Awards and Certificates

Graduate Studies Scholarship, Sam Houston State University, 2020

Entry Level Field Security Training (Hazardous Zones), Sanctuary International, 2018

Magna Cum Laude, West Texas A&M University, 2017

Animal Science Scholarship, West Texas A&M University, 2017

Presidents List, West Texas A&M University, 2015-2016