FACTORS AFFECTING PREGNANCY RATE TO FIXED-TIME ARTIFICIAL INSEMINATION IN PEN-RAISED WHITE-TAILED DEER

A Thesis

Presented to

The Faculty of the Department of Agricultural Sciences

Sam Houston State University

In Partial Fulfillment

of the Requirements for the Degree of

Master of Science

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by

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August, 2018

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DEDICATION

For Adam Frampton, always.

ABSTRACT

Cordova, Jacqueline P., *Factors affecting pregnancy rate to fixed-time artificial insemination in pen-raised white-tailed deer*. Master of Science (Agriculture), August, 2018, Sam Houston State University, Huntsville, Texas.

White-tailed deer farming has experienced significant growth in recent years in the U.S. with the majority of the operations located in Texas. Producers now utilize advanced breeding technologies in deer similar to cattle and other livestock to enhance the genetic merit of their herd. The goal is to produce the best quality bucks to sell as stocker bucks for high priced hunts or breeding. Pen-raised white-tailed does (n=177; 1 to 6 yr; 28.5 to 72.5 kg) from an established breeding program (3-S Whitetails, Bedias, TX) were utilized to evaluate factors affecting pregnancy rate to fixed-time AI (FTAI) across four breeding seasons combined (2013-2016). Does were synchronized using a 14-d controlled internal drug release (CIDR; EAZI-Breed CIDR, Zoetis, Inc., Kalamazoo, MI) device as a progesterone source with an injection (i.m.) of eCG (200 IU) at CIDR removal and inseminated laparoscopically 60 h later. At the time of FTAI, BW, BCS, and disposition score (DISP) were recorded and a blood sample was collected via jugular venipuncture to determine serum cortisol concentration. All does were subsequently placed in a pen with a buck for natural service (NAT) to maximize overall pregnancy rate of the herd. DNA testing of each fawn determined parentage and birth dates based on records from the producer were used to determine method of conception (FTAI or NAT). Serum cortisol concentrations were determined via ELISA. Data were analyzed using the GLM, MIXED, LOGISTIC and CORR procedures of SAS. There were no significant differences (P>0.05) found in BW, age, BCS, DISP, or serum cortisol concentration between does that conceived to FTAI and those that did not. Pregnancy rate to FTAI was

61% and overall pregnancy rate (FTAI and NAT) was 86%. Mean litter size was greater ($P<0.02$) for FTAI (1.79) than NAT (1.48). Does that did not conceive to FTAI had lower ($P<0.05$) BW, BCS and were younger ($P<0.05$) than does that conceived to FTAI. In summary, additional research is needed to determine if any of the physiological factors measured in this study have a significant influence on conception rate to FTAI.

KEY WORDS: White-tailed deer (*Odocoileus virginianus*), Fixed-time artificial insemination (FTAI), Fawning rate, Estrous synchronization, Physiological stress.

ACKNOWLEDGEMENTS

This research would not have been possible without Chris Stewart, his family, and the employees of 3-S Texas Outdoors and 3-S Whitetails in Bedias, Texas for graciously allowing us access to the does used for this study. He and his facility were instrumental in this thesis and I cannot extend my gratitude enough to him for entrusting me and other SHSU graduate students with the safety and well-being of his animals. It is people like him that ensure the future of research in academia. This study would also not have been possible without the hard work and dedication put forth by my fellow student researchers Matlin Sain, Elizabeth Evers, Madison McCluskey, Samantha Finley, Megan Adair, Kelly Carlson, and Hali Behrens-Russell. I would also like to thank the Chair of my committee Dr. Kyle Stutts for seeing potential in me and partnering with me to give new life to this research project. I would also like to extend gratitude to my committee members Dr. Stanley Kelley and Dr. Marcy Beverly for always being nothing but extremely supportive of our research. A special thanks also goes out to Dr. Mark Anderson for his endless words of encouragement and help with the statistical analysis on this project and Dr. Jessica Bedore for her copious amounts of patience, time, and instruction so that I could learn to perform the hormone assays involved in this project. I would also like to thank the Department of Agricultural Sciences for the research funds that afforded the materials necessary to do our own lab work in our brand new animal research laboratory. It was an honor to be one of the inaugural graduate students to perform lab work in it for my thesis.

PREFACE

"The American farmer or rancher is becoming the most endangered species on this continent because they can't make a living in agriculture."

-Dr. James C. Kroll, professor and co-founder of Texas Deer Association

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CHAPTER I

Introduction

Background

In the last decade, there has been a dramatic increase in North America in the number of pen-raised white-tailed deer (*Odocoileus virginianus*) being produced as an agricultural commodity. The goal is to attain the best genetics possible for the purposes of selling hunts at high fenced game ranches to trophy hunters. Bucks with a greater score based on a standardized system that measures the antlers in inches will sell at a higher price. As the white-tailed deer production industry has grown, producers have realized the economic value of raising breeder bucks with large antler sets to market valuable semen. This new market has resulted in intense competition among producers and auction prices for one straw of semen can range from $120 to $20,500 USD (Barnes et al., 2016). Therefore, an entirely new and lucrative industry has emerged, particularly in Texas, where there are more captive-bred white-tailed deer operations than any other state in the U.S. (Frosch et al., 2008). However, there is a gap in the literature regarding the physiology of the white-tailed doe's reproductive cycle as well as factors that may affect pregnancy rate. Further research in this area would benefit not only producers by enhancing their breeding programs, but the information could also be utilized in assisted reproductive technologies for endangered cervid breeding programs worldwide.

Many white-tailed deer breeding facilities have begun to develop fixed-time AI (FTAI) protocols similar to those already shown to be successful in cattle (Kim et al., 2006), sheep (Ainsworth and Downey, 1986), and goats (Motlomelo et al., 2002). While there are some studies on other members of the cervid family such as red deer (*Cervus* *elephus*, Asher et al., 1992) and fallow deer (*Dama dama*; Morrow et al., 1992), there are very few studies that have evaluated FTAI in white-tailed deer (Magyar et al., 1988). The protocol typically involves herding does through a specialized drop-floor chute system where they are sedated and either inseminated transcervically or laparoscopically using frozen-thawed semen. Understandably, this unnatural process involving human handling may elicit a physiological stress response in the doe. Currently, the relationship between stress response and pregnancy rates to FTAI in white-tailed deer is unknown and any research in this area would be highly beneficial to the producer. Producers may be able to utilize this knowledge to increase fawning success rates for their herd and therefore increase the overall profitability of their operation.

Research Objectives

The objectives of this study are (1) to evaluate the efficacy of FTAI in white-tailed deer and (2) to analyze the effects of physical and physiological factors on pregnancy rate in white-tailed does subjected to FTAI. These data are expected to offer valuable predictors of successful pregnancy to FTAI for producers as well as establish a standard for physical and physiological factors necessary to facilitate pregnancy in white-tailed does.

CHAPTER II

Literature Review

Reproductive Physiology of the White-tailed Doe

White-tailed does are seasonally polyestrous, capable of exhibiting continuously repeated estrous cycles during certain periods of the year. The time of parturition is important to ensure optimal survivability and growth of the neonate. Breeding typically occurs in November and parturition in late May or early June. The approximate gestation length of the doe is 200 ± 5 d. The exact duration of the normal estrous cycle has been difficult to ascertain due to insufficient research. Ozoga and Verme (1970) first reported that white-tailed does have an approximate estrous cycle of 21 to 28 d, noting it is possible they may ovulate multiple times within a cycle. Plotka et al. (1977) then narrowed that time frame down to 24 to 26 d. Even though relatively little research has been dedicated specifically to estrus in the white-tailed doe, it has been observed in both red deer and fallow deer that the onset of estrus coincides with the onset of the preovulatory LH surge, which itself peaks at about 24 h prior to ovulation (Asher, 2010). Of course, certain environmental factors such as nutritional inadequacy (resulting in starvation) or abundance (resulting in obesity) have been seen to disrupt the onset of estrus (Verme, 1965). While this can be problematic in the wild, environmental factors are much more manageable in a pen-raised setting. Ovulation typically occurs just prior to the day of estrus. The ovarian hormones progesterone and estrogen play a key role in all of the essential events of reproduction such as estrous cycling, ovulation, gestation and parturition (Woolf and Harder, 1979).

An Overview of FTAI in Farmed Deer

FTAI has been successfully incorporated into commercial beef and dairy operations for several decades now, so it was logical to apply the same technique to white-tailed deer when their popularity as an agricultural enterprise began to rise (Haigh, 1984). Does are synchronized using controlled internal drug-releasing devices (CIDR) that are inserted vaginally for 14 d. Laparoscopic or transcervical AI is performed 60 h after CIDR device removal since this is the time frame that is associated with greater instances of pregnancy (Gentry et al., 2012). Does are then typically placed in a pen with a buck to be naturally serviced in the event that the AI was unsuccessful. This buck is often referred to as the "cover buck" or "clean-up buck" in the industry. Producers then have the ability to DNA test the fawn shortly after birth to establish pedigree.

A study conducted by Mellado et al. (2013) compared fawning rates of two FTAI techniques (transcervical and laparoscopic) and natural mating. The does considered non-treated were those only exposed to natural mating. Their results showed that fawning rate did not differ between FTAI methods and overall fawning rate (proportion of all does fawning after FTAI and a subsequent period of buck exposure) did not differ between transcervical, laparoscopic, or natural insemination. They also found that litter size per doe was greater in naturally serviced does rather than in either method of FTAI. Of course, there are several other factors that could affect fawning rates aside from method of conception, most notably among them being semen quality and overall health of the doe.

Embryo transfer (ET) is another popular assisted reproductive technology being utilized in synergy with many FTAI programs. ET involves the process of inseminating genetically superior donor does with equally valuable semen from bucks with high genetic merit. The resulting embryos are then collected from the superior doe and transferred into the less valuable recipient does with proven exceptional mothering abilities. This allows the producer to get multiple offspring in one breeding season from a valuable doe while also not risking her life with the multitude of complications that can be associated with pregnancy and fawning. Economically speaking, the additional cost of hiring a technician to perform ET on multiple does can be rewarding to the producer for the quantity and value of offspring he or she can produce with this technique. Sperm sex sorting by flow cytometry is also starting to become more widely utilized in the growing industry. This is the most practical and effective methodology to produce offspring of the desired sex. It has been applied successfully in Sika and red deer (Anel-Lopez et al., 2018) and now is emerging as the latest trend on white-tailed deer ranches.

Cortisol Concentration as an Indicator of Stress

Stress in farm animals is defined by the inability of an animal to cope with its environment, often resulting in a failure to achieve its genetic potential (such as fertility, growth rate, milk production, etc.; Smith and Dobson, 2002). Plasma cortisol concentration has been measured in beef cows to determine how CIDR device insertion (an environmental stressor) affects stress response. Because there are many acutely stressful situations involved in reproductive management in cattle (palpation, AI, general handling, etc.) researchers wanted to observe how cortisol concentrations compared before, during, and after insertion of the CIDR device. Plasma samples were analyzed and cortisol concentrations were determined by ELISA. The results indicated an increase in plasma cortisol concentration in the treatment group (CIDR insertion group) and no change in the control group (no CIDR device was inserted; Long et al., 2011). Although the exact pathophysiological significance of increased cortisol in beef cows is unknown, this study verifies a relationship between external stressors and a significant increase in cortisol. Another study was conducted by Smith and Dobson (2002) involving the effects of transport stress on plasma cortisol levels in ewes. They observed that plasma cortisol concentrations elevated in response to being loaded, handled, and unloaded from a transport trailer. Elevated levels were observed during the actual periods of transport, however there was a declining response after the initial stressor was over. This again correlates external stressors to an increase in plasma cortisol concentration. The authors also noted while it may seem that stress is reduced over time based on their experiment, much of that reduction could be attributed to the hormone negative feedback. It is important to note the difference between a reduction in hormone concentration due to hormonal control mechanisms and that due to a decrease in the stress stimulus, especially when interpreting data for the purposes of animal welfare.

Stress responses were compared by Hastings et al. (1992) to various handling procedures in both free-ranging and hand-reared captive Chinese water deer (*Hydropotes inermis*) at a wild animal park. Plasma cortisol levels were used as a measurement of acute stress in response to either being anaesthetized by a dart or netted and manually restrained. Blood collected immediately upon routine culling of some of the deer was used as the control group. While they acknowledged that darting appeared subjectively to be less stressful on the deer than manual restraint, there was no significant difference in cortisol levels between the two groups. The hand-reared deer did not react any less violently behaviorally than the free-ranging deer, however they did show significantly lower cortisol levels. The authors suggested that hand-rearing may be beneficial in reducing physiological stress response, in terms of plasma cortisol, to handling.

Physiological Stress Response and Reproduction

Although the literature is significantly lacking regarding the effects of physiological stress response on pregnancy rate in cervids, there has been an abundance of research on the subject in cattle. Cortisol (an adrenal glucocorticoid) is a measurable indicator of stress and has been used to evaluate the relationship between stress and reproduction (Lyiomo et al., 2000). Research conducted by Echternkamp (1984) in beef cows indicated a relationship between handling stress and LH secretion. In that study, cortisol concentrations were measured to determine handling stress. The results suggested that the influence of stress on gonadotropins and subsequent reproductive responses is dependent on both the magnitude of the stressor and the animal's ability to acclimate to that stressor. Dramatic increases in cortisol concentrations associated with intensive stress appeared to suppress tonic LH secretion, especially pulsatile LH release (Echternkamp, 1984).

Additionally, it has been observed in dairy cows that stressors interfere with the precise timing of reproductive hormone release, specifically by reducing the frequency and amplitude of GnRH and LH as well as delaying the onset of the LH surge (Dobson and Smith, 2000). GnRH is produced by the hypothalamus located ventral to the brain. LH is released by the anterior lobe of the pituitary gland. In order for the female to experience an estrous cycle, there must be a pulsatile secretion of GnRH from the tonic center of the hypothalamus causing pulsatile secretion of FSH and LH from the anterior lobe of the pituitary resulting in follicle growth and maturation. The surge of GnRH from the surge center of the hypothalamus is stimulated by estrogen from a large follicle (or many follicles) resulting in a surge of LH to be released by the pituitary causing ovulation. Success of follicular growth and development is dependent upon the proper FSH (also produced by the anterior lobe of the pituitary gland) and LH ratio (Senger, 2003). A physiological stress response may result in an improper ratio and concentration of these hormones. Dobson and Smith (2000) studied the influence stress has on reproduction in cattle. Their results indicated that an excitable temperament or disposition resulting in excess secretion of cortisol in a cow can negatively influence reproductive function by inhibiting the synthesis and release of gonadotropins.

Reproductive Hormones

Many FTAI estrous synchronization protocols use CIDR devices as a progesterone source. For white-tailed deer, sheep and goat CIDR devices are typically used because of their design and size. CIDR devices provide a controlled release of progesterone into the bloodstream of the animal. Progesterone is a gonadal steroid hormone that promotes and maintains pregnancy in-vivo. It also suppresses estrus. Removal of a CIDR device generally induces estrus due to the sudden drop in progesterone concentration and promotes a synchronized estrus for the herd (Senger, 2003).

Equine chorionic gonadotropin (also referred to as pregnant mare's serum gonadotropin or PMSG) is commonly administered at CIDR removal in red deer to improve frequency and synchrony of ovulation (Asher et al., 1987). Equine chorionic gonadotropin originates from the fetal membranes and primarily acts in the development and maintenance of pregnancy (i.e. FSH-like activity that helps form and support the corpus luteum during pregnancy; Senger, 2003). A study by Gentry et al. (2012) evaluated the effect of eCG on pregnancy rate to FTAI of white-tailed deer. Of the 74 does synchronized with CIDR devices and subjected to FTAI 60 hr post-CIDR device removal, 34 were given eCG at removal. Interestingly, their results indicated that administration of eCG at CIDR device removal did not significantly affect pregnancy rate (eCG = 59%; no eCG = 43%). The authors did note that their study had limited statistical power to detect the effect of eCG on pregnancy rate and their sample size may have been a limitation. Therefore while it may be routinely used in red deer, it has not been specifically recommended for other cervidae and may not be as effective in white-tailed deer. However, it is still commonly used in their estrus synchronization protocols.

Estradiol, along with progesterone, is another steroid hormone that controls ovarian follicular development and embryo survival (Sirtotkin et al., 2016). Research conducted on European roe deer (*Capreolus capreolus*) revealed that in cycling females, there was an increased level of plasma progesterone from the time of ovulation up to corpus luteum maintenance. Pregnancy was associated with the second peak of progesterone and an increase in estradiol release (Hoffmann and Karg, 1978).

Factors Affecting Pregnancy Rate to FTAI

Other physical factors that could potentially influence the efficacy of pregnancy to FTAI are age of the doe, BW, and BCS. Although there have been no studies regarding the relationship between these factors and pregnancy rates in white-tailed deer specifically, there have been studies in other species. In a study by Gaskins et al. (2005) about the effect of age on fertility and prolificacy in ewes, it was determined that ewes bred as lambs actually had a greater lifetime production rate compared with those bred as yearlings. However, the overall fertility and prolificacy on ewe lambs was lower than that of adult ewes. In terms of BW, it was found that averaged across the ewes in the study, BW at breeding had a positive effect on fertility. According to Flajsman et al. (2017) yearling European roe deer must reach a critical threshold body mass to attain reproductive maturity, while adult females are fertile even at a low body mass. This suggests that BW and BCS is more important in yearlings than in adult does. Therefore, it is reasonable to assume that yearlings with a greater BW and BCS have a greater chance of fawning than their cohorts with lower BW and BCS.

In FTAI protocols designed for beef cows, there is a substantial amount of research to support that BCS has a direct influence on expression of estrus and conception rates. A study conducted by Richardson et al. (2016) established a threshold regarding this relationship. Cows in a BCS of > 4 (1 = emaciated, 9 = obese) were more likely to express estrus, and therefore more likely to conceive to FTAI. The same is expected to occur in white-tailed deer, however there has been no conclusive research published on the effect BCS has on conception rate in this species. A study observing the effects of body condition on pregnancy status in Rocky Mountain elk by Willard et al. (1994) determined that elk cows that calved had a greater BCS both in early rut and after rut than cows that remained open.

Economic Impact of the Industry

Cervid farming as an agricultural industry has experienced a tremendously rapid growth in popularity in the last three decades, particularly in Texas, with white-tailed deer making up the largest portion of cervids being farmed. These high-fence deer hunting and breeding operations play a significant role in the economies of the rural communities where they are located, as well as the economy of the state in which they reside. The Agricultural Food and Policy Center at Texas A&M University reported in 2007 that an additional $757 million USD was generated from hunting, with hunters being the major consumer of cervid farming products (Anderson et al., 2007). According to a survey conducted by Frosch et al. (2008), as of 2007 there were 7,828 cervid farms nationwide and 1,060 in the state of Texas alone. In that same survey, it was reported that U.S. cervid farming had a total economic impact of $1.1 billion USD and accounted for approximately 29,000 jobs. Since then, there has been an updated survey conducted by Outlaw et al. (2017) which reports that, as of 2015, the total economic impact of the industry to the Texas economy reached $1.6 billion USD annually and the industry supports 16,892 jobs in the state, the majority being in rural areas.

Based on economic information regarding operating costs for white-tailed deer farms collected by DeVuyst at Oklahoma State University (2013), AI procedures at a mid-size operation average $710 USD per doe for semen, drugs, and veterinary services. Because the cost can be high, most producers purchase a breeding buck and only AI their most genetically superior does. The average cost of a breeder buck is $8,000 USD and that buck will need to be replaced every seven years. Texas Parks and Wildlife Department reported that the number of permitted breeding facilities in Texas has grown from 946 in 2006 to 1,257 in 2016 (Outlaw et al., 2017). With this enormous growth comes a need for further research on the efficacy of FTAI in white-tailed deer to determine if they require adjustments to improve pregnancy success or if the protocols currently being utilized based on cattle, sheep, and goats and exotic cervids also apply to white-tailed deer without need for adjustment.

CHAPTER III

Materials and Methods

Ethical Statement

This research was approved by the Sam Houston State University Institutional Animal Care and Use Committee (Protocol number: 15-02-24-1019-3-01). All blood samples were collected while animals were anesthetized for a routine procedure under the supervision of a veterinarian in order to minimize pain and discomfort in accordance with USDA Pain/Distress Category C. The researchers were trained in handling deer and blood collection, and were under direct supervision of the ranch manager. All animals were returned to the herd after measurements and samples were collected.

Data Collection

Pen-raised white-tailed does (n = 177) ranging in age from 1 to 6 yr from an established breeding program (3-S Whitetails, Bedias, TX) were utilized to evaluate factors affecting pregnancy rate to fixed-time AI (FTAI) across four breeding seasons combined (2013-2016; mid to late November). Does were synchronized using a 14-d controlled internal drug release (CIDR; EAZI-Breed CIDR, Zoetis, Inc., Kalamazoo, MI) as a progesterone source with an injection (i.m.) of eCG (200 IU) at CIDR removal and inseminated laparoscopically 60 h later. For laparoscopic insemination, the does were sedated and then restrained in dorsal recumbency on an inclined laparotomy gurney. Using an endoscope to locate the correct site, a single trained professional performed the inseminations by inserting half the total frozen-thawed semen dose per uterine horn using an insemination pipette through incisions made through the ventral abdominal wall and peritoneum.

Immediately following AI, while the does were still sedated, blood samples were collected via jugular venipuncture into both non-additive and K_2EDTA vacutainer tubes (Monoject, VWR, Radnor, PA, USA) by a trained individual. Vacutainers of blood that did not contain an anticoagulate were kept on heating pads to prevent clotting. Blood serum was then harvested following centrifugation at 2000 rpm for 20 min. Serum samples were then stored at -80º C. Serum samples were analyzed in a laboratory to determine cortisol concentrations. Cortisol concentrations were quantified using ELISA (Cortisol ELISA Kit, Cayman Chemical, Ann Arbor, MI). The kit was validated and the proper dilution (1:16; serum to buffer) established for deer serum prior to quantification of samples. This high dilution ratio was necessary due to the hypersensitivity of the kit and its standards.

At the time of insemination, BW (kg) was recorded and a BCS ranging from 1 to 9 (1 = emaciated and 9 = obese) was assigned to each doe. BCS was determined based on palpation of the fat and non-fat tissue surrounding the spine and ribs by a single trained individual, similar in practice to BCS typically assigned in beef cattle (Herd and Sprott, 1998). A disposition score (DISP) ranging from 1 to 5 (1 = calm with no movement, 2 = calm with some kicking, 3 = moderate kicking, 4 = frequent kicking, and 5 = vocalization and kicking) was also assigned by a single trained individual based on a scale previously established in white-tailed deer research (Gentry et al., 2012). DISP were used as a means to evaluate temperament as an indicator of stress while being handled by ranch personnel and herded with rattle paddles through a solid sided drop-floor chute specially designed for white-tailed deer.

Number of offspring and sire identity were determined from pedigree information recorded in the North American Deer Registry (NADR) database inputted by the producer. Birth dates were determined based on written records provided by the producer. Pregnancy rates were determined based on fawning dates and parentage of fawns. Parentage of fawns was determined by hair follicle DNA analysis ordered by the producer (using hair follicles extracted from the base of the tail) which identified whether a fawn or fawns (multiples are commonplace in white-tailed deer) were a result of FTAI or natural service (NAT) with a cover buck. Fawning records provided by the producer were then used to establish the number of offspring born from each doe the following spring or if the doe remained open for that breeding season. All does were exposed to both FTAI and NAT in order to maximize overall pregnancy rate of the herd. In some cases, the same buck was used for both AI and natural service following AI. In this case, the birth date of the fawn was used to determine whether its conception was a result of FTAI or NAT based on the dam's FTAI date and a specific herd average gestation length of 196 ± 10 d. Fawns born outside of this range were determined to be a result of NAT from later mating with a cover buck. The producer reported putting one cover buck in a pen per 20 to 25 does.

Data Analysis

Data were analyzed using the GLM and MIXED procedures of SAS v. 9.4 (SAS Institute, Inc., Cary, NC) to determine differences in age, BW, BCS, DISP, and serum cortisol concentration between does that conceived to FTAI and those that did not. A significant year effect existed, therefore year was included as a covariate in the analysis. The treatments analyzed were AI, NAT, and OPEN with the previously mentioned factors as the dependent variables. The LOGISTIC procedure was used to determine if BW, BCS, DISP, or serum cortisol concentration had a significant effect on pregnancy rate to FTAI. The CORR procedure was used to determine a correlation between DISP and serum cortisol concentration to assess the validity of its use as an on-farm measurement of stress.

CHAPTER IV

Results and Discussion

Results

Of the 177 does in the study, each had a BCS (1-9) assigned to them and age was available in records for every doe. However, only 145 does had their BW (kg) recorded and a disposition score (DISP) ranging from 1 to 5 assigned to each of them. This was attributed to the producer restricting time in the chute for these measurements for his temperamental and higher value does in an effort to reduce any excess stress on them prior to FTAI. Since BCS was evaluated by palpation after the doe was already sedated, those measurements were completely non-invasive for the doe and were allowed in every instance. Serum cortisol concentration was analyzed for 154 of the 177 does (2013, n = 55; 2014 n = 34; 2015, n = 38; 2016, n = 27), using a subset for the 2014 and 2015 breeding years.

Table 1 categorizes FTAI outcome by either success (pregnancy to FTAI) or failure (pregnancy to NAT or OPEN). There were no significant differences in BW, age, BCS, DISP, or serum cortisol concentration between does that conceived to FTAI and those that did not. In addition, results of the LOGISTIC procedure indicate that none of the variables measured have a significant influence on pregnancy rate to FTAI. However, this may have been because the number of observations used in this analysis was reduced to 52, giving a smaller n, since that was the number of does with complete data sets of all variables. When conducting the LOGISTIC procedure, a significance level of 0.3 was required to for a variable to enter the model and a significance level of 0.35 was required for a variable to remain in the model. Using pregnancy to FTAI as the modeled probability, year ($P = 0.2031$) and DISP ($P = 0.2218$) were the variables, although not significant, that remained in the model. When logistic regression was used to evaluate overall pregnancy rate (FTAI and NAT), year ($P < 0.01$), BCS ($P < 0.01$), and age ($P < 0.04$) were identified as having a significant influence on pregnancy rate. Due to year being neither a physical nor physiological factor measured, it should not be considered an influencing factor.

Table 1. Least squares means (± SE) for BW, age, BCS, DISP, and serum cortisol concentration for 177 pen-raised white-tailed does that successfully conceived to FTAI and those that failed to conceive to FTAI

	FTAI[1] Outcome		
Factor	Success	Failure	*P*-value
BW, kg	52.5 ± 1.1	50.3 ± 1.2	0.1595
Age, yr	3.3 ± 0.2	3.1 ± 0.2	0.3732
BCS (1-9)	5.9 ± 0.1	5.6 ± 0.1	0.1176
DISP[2] (1-5)	2.5 ± 0.2	2.6 ± 0.2	0.5637
Serum cortisol, ng/mL	13.1 ± 1.1	12.6 ± 1.1	0.7363

[1]FTAI, fixed-time AI.
[2]DISP, disposition score.

The fawning rate to FTAI and overall fawning rate (FTAI and NAT combined) from all four years combined are shown in Fig. 1 below. Sixty-one percent of the does in the study were impregnated successfully by FTAI based on fawning rate. An additional 44 does became pregnant to natural mating with a buck, resulting in an overall fawning rate of 85.88%. Twenty-five does in the study did not produce a fawn the following fawning season.

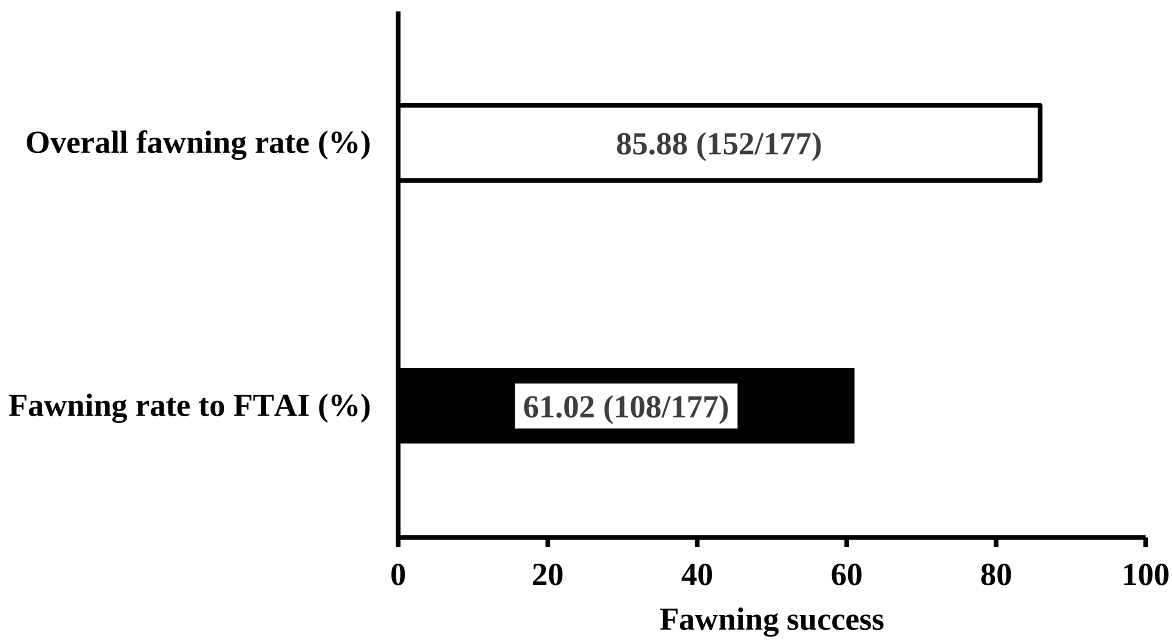

Figure 1. Pregnancy rate to fixed-time AI (FTAI) and overall pregnancy rate (FTAI and natural service) of white-tailed does over four years combined. Numbers are derived from actual numbers of does from which percentage was derived.

As shown in Table 2, the mean BW of the does that conceived to FTAI was 52.5 kg and the mean BW of the does that conceived to natural breeding (NAT) was 51.9 kg. Mean BW of the does that did not produce any fawns (OPEN) was 47.5 kg which was lower ($P < 0.05$) than does that conceived to FTAI and NAT. For BCS, the mean for FTAI and NAT had the same mean of 5.9, and the mean BCS for OPEN was 5.4 which was less ($P < 0.05$) than FTAI and NAT. These results are consistent with previous research indicating that greater BW and optimal BCS (5 to 7) is associated with increased reproduction (Herd and Sprott, 1998). It should be noted that all the does in this study were very well-fed and all had a BCS between 4 and 7. The does with lower BCS also tended to be yearlings or 2-yr-olds.

The mean age of does conceiving to FTAI was 3.3 yr, NAT was 3.5 yr, and OPEN was 2.5 yr, which was younger ($P < 0.05$) than FTAI and NAT. There was no difference ($P > 0.05$) found in serum cortisol or DISP among conception methods (AI, NAT, or OPEN). Mean serum cortisol concentration for FTAI, NAT, and OPEN was 13.1, 13.2, and 11.7, respectively, with no difference ($P > 0.05$) between the two methods or lack of conception. A complete representation of serum cortisol concentration (ng/mL) of does for all years combined can be found in Appendix B.

Table 2. Least squares means (± SE) for BW, age, BCS, disposition score, and serum cortisol concentration collected from 177 white-tailed does during the 2013 to 2016 breeding seasons to evaluate factors affecting pregnancy rate to fixed-time AI

Factor	FTAI[1]	NAT[2]	OPEN[3]
BW, kg	52.5 ± 2.4[a]	51.9 ± 3.3[ab]	47.5 ± 4.1[b]
Age, yr	3.3 ± 0.2[a]	3.5 ± 0.2[a]	2.5 ± 0.3[b]
BCS (1-9)	5.9 ± 0.1[a]	5.87 ± 0.2[a]	5.3 ± 0.2[b]
DISP[4] (1-5)	2.5 ± 0.2	2.7 ± 0.2	2.5 ± 0.3
Serum cortisol, ng/mL	13.1 ± 1.1	13.2 ± 1.5	11.7 ± 1.8

[ab]Means within a row without a common superscript differ, $P < 0.05$.
[1]Does conceived to FTAI, fixed-time AI.
[2]Does conceived to natural service.
[3]Does failed to conceive.
[4]DISP, disposition score.

Table 3 contains means of pregnancy rate to FTAI and the physical and physiological factors measured by year. Differences existed in the characteristics measured among years due to different does used in the study each year as well as various uncontrollable environmental factors. The pregnancy rate (PR) to FTAI was greater ($P < 0.05$) in 2013 (PR = 68.2 %) and 2014 (PR = 72.7%) than in 2015 (30.8%) and 2016 (37.0%). In the latter two years of the study, the producer used more yearling does for FTAI, which may explain the lower FTAI pregnancy rate for those years. For instance, the year 2013 included seven yearlings, 2014 included eight yearlings, 2015 included 11 yearlings, and 2016 included 14 yearlings. However, the year 2015 had a greater ($P < 0.05$) mean age (4.2 yr) than all the other years. This may be explained by the raw data since the producer also used 15 five-yr-old does in 2015.

The mean BW for the does in 2016 (44.5 kg) was lower ($P < 0.05$) than the does in all the other years, however the mean BCS of 6.0 for that same year indicated their nutritional needs were met if not exceeded. This may relate back to age, as the yearling does have not had as much time to increase in body size and gain as much BW as the older does, though they still had adequate fat and body energy reserves. Mean BCS for the does in 2014 was lower ($P < 0.05$) than the does in all the other years.

Mean DISP was lower ($P < 0.05$) in years 2013 and 2016 than in years 2014 and 2015. Based on the raw data, years 2013 and 2016 had the most does that scored a one (1 = calm with no movement) on the disposition scale (2013 = 21; 2016 = 18). For serum cortisol, only years 2014 and 2016 were similar ($P > 0.05$). To determine if serum cortisol had an influence on DISP, the MIXED procedure was utilized. There were no significant differences in cortisol concentration by DISP with year as a covariate. When

DISP and cortisol were analyzed as two variables across all years using the CORR procedure, there was a weak, positive ($r^2 = 0.2612$ and $P < 0.01$) correlation found.

Table 3. Least squares means (± SE) for fixed-time AI (FTAI) pregnancy rate, age, BW, disposition score (DISP), BCS, and serum cortisol concentration of white-tailed does by year

	2013 (n = 55)	2014 (n = 45)	2015 (n = 48)	2016 (n = 29)
PR to FTAI[1], %	68.2 ± 7.1^{a}	72.7 ± 8.2^{a}	30.8 ± 13.1^{b}	37.0 ± 9.1^{b}
Age, yr	3.1 ± 0.2^{a}	3.7 ± 0.2^{ab}	4.2 ± 1.9^{c}	1.9 ± 0.3^{c}
BW, kg	53.1 ± 2.7^{a}	53.7 ± 3.1^{a}	54.4 ± 4.9^{a}	44.5 ± 3.5^{b}
DISP (1-5)	2.1 ± 0.2^{a}	3.3 ± 0.2^{b}	3.0 ± 0.3^{b}	1.7 ± 0.2^{a}
BCS (1-9)	5.9 ± 0.1^{a}	5.1 ± 0.1^{b}	6.2 ± 0.2^{a}	6.0 ± 0.2^{a}
Serum cortisol, ng/mL	5.5 ± 1.2^{a}	13.1 ± 1.3^{b}	20.3 ± 2.2^{c}	12.9 ± 1.5^{b}

$^{a-c}$Means within a row without a common superscript differ, $P < 0.05$

[1]PR to FTAI, pregnancy rate to FTAI.

Many of the does gave birth to multiple offspring as expected. The ultimate goal of the producer at this ranch and in the white-tailed deer breeding industry as a whole is production of two healthy fawns for each mature doe per year. Figure 2 illustrates mean litter size for each method of conception. The mean litter size for FTAI was 1.79 offspring per doe which was greater ($P < 0.02$) than mean litter size for does that conceived to NAT (1.48 offspring per doe). Fifty-four percent (82/152) of does that fawned had two or more offspring.

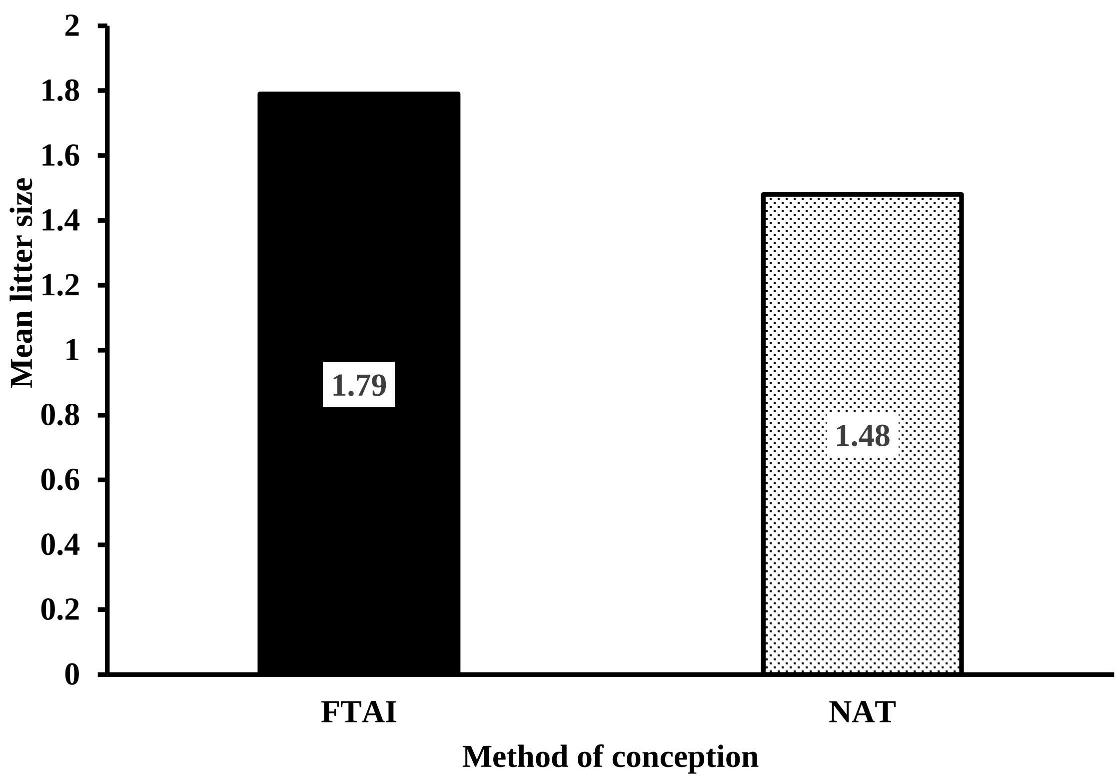

Figure 2. Mean litter size born to white-tailed does that became pregnant to either fixed-time AI (FTAI) or natural service over four years combined (2013-2016).

Discussion

In the present study, the results do not support any of the variables measured as having a significant influence on pregnancy rate to FTAI in white-tailed does. However, the results do support that age, BW and BCS can make a difference between a doe conceiving to FTAI or remaining open. The results also indicate that there was no difference in stress levels, using DISP and cortisol concentration as measurements, between does that conceived or failed to conceive to FTAI. This means that the does were not stressed to the point of their reproduction being negatively impacted. Given that the information based on these results used different sample sizes over different years, it is important to take into account the limitations of this study when interpreting the validity of the results and any conclusions that may be drawn from them. FTAI in captive white-tailed deer is difficult to study because ranch procedures often interfere with obtaining precise quantitative information. There are very few reproductive studies on pen-raised white-tailed deer available in the literature so there are also limited comparisons to be made between protocols.

The fawning rate to FTAI of 62% observed in the present study was greater than the rate of 45% reported by Mellado et al. (2013) utilizing 251 white-tailed does. The overall fawning rate in the present study was also greater, 86% compared to their 80.9%. Mellado et al. (2013) did note that poor semen quality may have contributed to the low FTAI fawning rate they experienced, as the production of frozen deer semen is currently unstandardized. Because semen quality can certainly affect pregnancy rate, FTAI pregnancy rate by sire was considered for the present study but proved to be problematic. There were 18 different bucks that sired offspring, however, there was not an even distribution of the sires across or within years which made it impossible to analyze effect of sire.

The mean litter size of does in the present study contradicted the litter size reported by Mellado et al. (2013). The authors of that study reported a greater litter size associated with natural service (1.65 vs 1.48 for FTAI) while the mean litter size in the present study was greater ($P < 0.05$) for FTAI (1.79 vs 1.48 for NAT) as the method of conception. Another study conducted by Gentry et al. (2012) found that litter size of does pregnant to FTAI was not different compared to does pregnant to natural mating (1.7 for both conception methods). FTAI resulting in a greater litter size seems valid because of the precise placement of semen directly into each uterine horn during the insemination, so it is unclear why there are differences between these previous studies and the present study. Further research is needed to understand which breeding method results in greater litter size. Although these numbers are very similar, even a few additional fawns per fawning season can add substantial value to the herd for the producer. The mean litter size in the present study was comparable to the mean litter size of 1.74 in 27 well-fed, captive white-tailed does in Michigan described by Verme (1965) and the mean litter size of 1.9 reported by Green et al. (2017) in 3,884 wild white-tailed does in Illinois.

There were differences in BW, BCS, and age observed between does that either conceived to FTAI or NAT, or does that remained open. It is reasonable to assume the younger (yearling) does may not have reached full sexual maturity by the time they were inseminated. They also may not have had enough time to gain ample weight and develop the optimal BCS required for successful reproduction. This supports a need for nutrition programs specifically formulated for breeding does as an essential part of every breeding operation. Pregnancy has a high metabolic cost, so females with greater body condition (more fat) are better suited to tolerate body fat loss compared to females with lesser body condition (Green et al., 2017).

Serum cortisol concentrations were compared to the few previous studies that exist in white-tailed deer and other cervids. Hastings et al. (1992) reported plasma cortisol levels in Chinese water deer exposed to varying degrees of handling stress. The authors used free-ranging culled deer (n = 36) as a control and found the mean cortisol concentration to be 4.5 ng/mL. The mean cortisol concentration for hand-reared deer that were manually restrained (n = 7) was 18.9 ng/mL. Mean cortisol concentration for free-ranging deer that were netted and manually restrained, anaesthetized by dart, and both anaesthetized and diseased were 48.7, 59.5, and 71.9 ng/mL, respectively. The mean determined in the present study ranging from 5.5 to 20.3 ng/mL depending on year (2013 = 5.5 ng/mL, 2014 = 13.1 ng/mL, 2015 = 20.3 ng/mL, and 2016 = 12.9 ng/mL) is most similar to the group of manually restrained, hand-reared deer observed by Hastings et al. (1992). Bubenik et al. (1975) analyzed cortisol in seven penned, male white-tailed deer (4 partially tamed and 3 untamed) to study its effect on antler growth. The authors reported cortisol concentration ranging from 10 to 30 ng/mL. Seal et al. (1983) reported greater cortisol levels ranging from 53 to 77 ng/mL in high fenced, free-ranging does that were manually restrained and not anesthetized.

The act of hand-rearing or pen-raising may lead to better acclimation to stressful situations around humans and overall lower cortisol levels when being handled. However, it is still reasonable to expect that handling will increase stress and elevate serum cortisol. The does in this study were familiarized with the handling facility and restraint systems prior to the breeding season. This facility conditioning seems to be beneficial based on the lack of difference in serum cortisol between successful and unsuccessful conception to FTAI. More research is necessary to determine with certainty if concentrations in the present study were normal for white-tailed deer and if there is a significant difference between cortisol concentration in does that conceive to FTAI and those that do not. Studies on larger numbers of deer may yield clearer results in the area of cortisol concentration and whether or not it can affect pregnancy rate to FTAI.

CHAPTER V

Conclusion

A successful breeding management program is essential for the prosperity of any animal agriculture business. That prosperity ultimately impacts the entire economy of the state or region in which that business resides. The objectives of this research were to provide more information on the efficacy of fixed-time AI (FTAI) in white-tailed deer and to identify factors that have a significant effect on pregnancy rate to FTAI. Though none of the factors measured had a significant influence on pregnancy rate to FTAI, does that conceived to FTAI had a greater BW, BCS and were older than does that failed to conceive. Litter size was greater for does that became pregnant to FTAI than natural service, and as expected, pregnancy rates to FTAI varied between years. Stress levels, measured by disposition score (DISP) and serum cortisol concentration had no effect on pregnancy rate to FTAI. Additional research is needed to better understand if any of the physiological factors measured in this study or any others can be used by producers as reliable predictors of conception to FTAI.

It is the hope of the author that this information will benefit the white-tailed deer industry as it continues to grow in the coming years. The information gleaned from this research should be expanded upon in future studies to further improve fawning rates and increase revenues among producers. This research was intended to fill a gap in the literature and expand the current knowledge of white-tailed deer reproductive physiology. Ideally, the present study will serve as a stepping stone for further research pertaining to the white-tailed doe estrous cycle, estrous synchronization in white-tailed deer, and FTAI protocols tailored specifically for white-tailed deer.

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APPENDIX A: IACUC FORM

Sam Houston State University

A Member of the Texas State University

Institutional Animal Care and Use Committee

Committee Members

Regular Members
Marcy Beverly, Ph.D.
James Harper, Ph.D.
Mark Anderson, Ph.D.
Autumn Smith-Herron, Ph. D.
T.C. Sim, Ph.D.
Gerald Etheredge, D.V.M.
Frank Adams, Ph.D., Community Member

Alternate Members
Kyle Stutts, Ph.D.
Todd Primm, Ph.D.
Ilona Petrikovics, Ph.D.
Jeff Wozniak, Ph.D.
Michael Moore, D.V.M.
Vernette Porter, Community Member

Date: September 19, 2017

To: Jacqueline Cordova (formerly, Kyle Stutts) [Faculty Supervisor: Dr. Kyle Stutts]

ASET
Box 2088
On-Campus

From: Dr. Marcy Beverly, IACUC Chair

Re: Form G: Amendment

ID #15-02-24-1019-3-01

Project Title*: Factors affecting pregnancy rates to fixed time insemination of pen-raised white-tailed deer [Student Thesis]*

Species: *Cervid (white-tailed deer)*

Start: September 19, 2017 **End:** February 24, 2018

Your **IACUC Amendment** submission was reviewed and approved under **Designated Member Review** (DMR) procedures on **September 19, 2017** with the following result:

Approved

Annual Review Form Deadline: N/A—not required for amendment submissions

APPENDIX B: Additional Figure

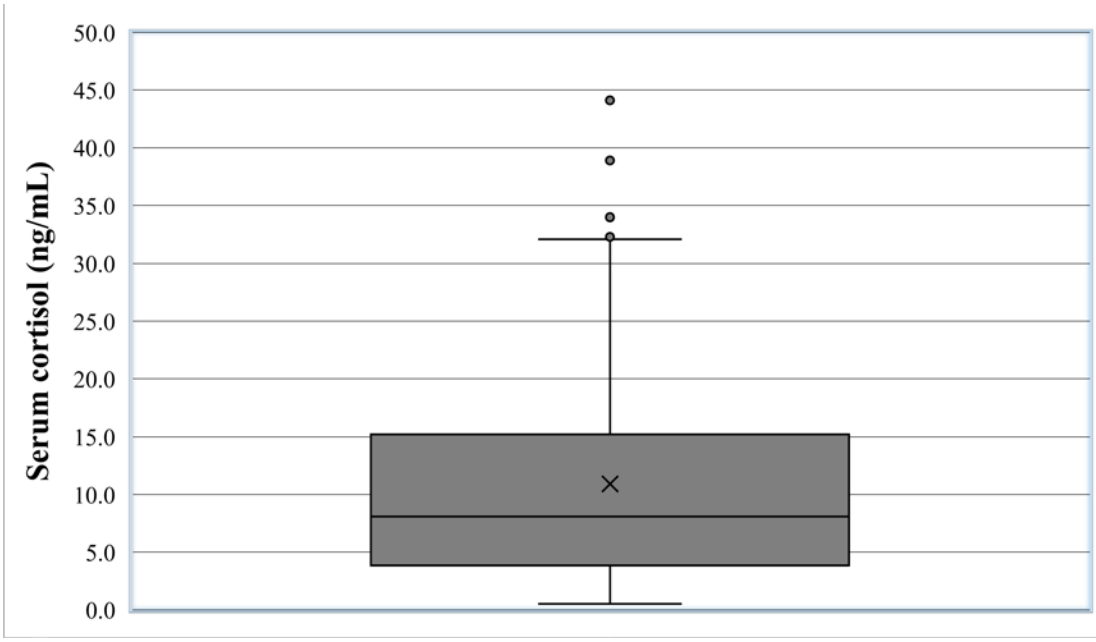

Figure 3. Actual mean serum cortisol concentration (ng/mL) as determined by enzyme-linked immunosorbent assay (ELISA) for 154 of the 177 white-tailed does collected during the breeding seasons of 2013 to 2016.

VITA

Jacqueline P. Cordova

EDUCATION

Master of Science (August 2018) in Agriculture at Sam Houston State University, Huntsville, TX, August 2016 - Present. Thesis title: Factors affecting pregnancy rate to fixed-time artificial insemination of pen-raised white-tailed deer.

Bachelor of Science (May 2016) in Animal Science, Biology Minor at Tarleton State University, Stephenville, TX, August 2012 - May 2016.

PROFESSIONAL EXPERIENCE

Animal Science Teaching Assistant/Laboratory Coordinator, Sam Houston State University, 2017-2018

> Facilitated animal science courses as needed in regards to developing course work, classroom instruction, and grading. Updated curriculum of courses and worked in conjunction with other Teaching Assistants to collaborate on lessons and complete tasks both inside and outside the classroom for the benefit of enhancing the learning environment for students.

<u>Academic Courses Facilitated as Instructor</u>
ANSC 1119: Animal Science Laboratory

<u>Academic Courses Facilitated as Teaching Assistant</u>
ANSC 1319: Introductory Animal Science
ANSC 3376: Meat Science
ANSC 4310: Animal Growth and Performance
ANSC 4394: Feeds and Feeding

Graduate Research Assistant, Sam Houston State University, 2016-2017

> Assisted with thesis research for three graduate student projects at 3-S Whitetails in Bedias, TX. Kept records, collected blood samples, centrifuged and labeled blood, took body measurements, and bottle-fed milk replacer for white-tailed deer.

PEER REVIEW PRESENTATIONS

Beverly, M.M., Kelley, S.F., Anderson, M.J., **Cordova, J.P.** (2018). Shock value: media's momentary influence on perceptions of animal agricultural issues. *North American Colleges and Teachers of Agriculture*. Oral Presentation.

PROFESSIONAL MEMBERSHIPS

- American Society of Animal Science (ASAS)

PROFESSIONAL CERTIFICATIONS

- CITI Program – “Working with the IACUC” Certified (2016)
- EDEN Animal Agrosecurity and Emergency Management (2016)
- Beef Quality Assurance Certified (2015)

HONORS AND AWARDS

Sam Houston State University

- Ag Workers Insurance Graduate Leadership and Service Award Recipient (2018)
- Office of Graduate Studies 3 Minute Thesis ™ Competition Finalist (2018)
- Office of Graduate Studies Outstanding Teaching Assistant Award Nominee (Spring 2018)
- College of Science Special Graduate Scholarship Recipient (Spring 2018)
- College of Science Special Graduate Scholarship Recipient (Fall 2017)
- College of Science Special Graduate Scholarship Recipient (Spring 2017)

Tarleton State University

- Honors College Program Graduate (2016)