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EFFECT OF REPRODUCTIVE EFFICIENCY IN REGARDS TO WORKLOAD ON PERIPHERAL CIRCULATIONS OF BLOOD CORTISOL AND LEPTIN IN THE OPEN MARE

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ABSTRACT

Effect of Reproductive Efficiency in Regards to Workload on Peripheral Circulations of Blood Cortisol and Leptin in the Open Mare

(December, 2016)

Michaelle Kathleen Coker, B.S., Texas A&M University

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Quarter and Paint horse mares (n= 9) were randomly assigned to 1 of 3 groups after being blocked based by expected conception date. Groups consist of mares on no work (NW), light work (LW), and moderate work (MW). Blood was taken weekly via jugular venipuncture prior to first heat cycle from each mare. Additionally, blood was taken at time of ovulation and then weekly until a 30 day (d) heartbeat was reported for the fetus. Each blood sample was assayed for leptin and cortisol concentrations, to see if cardiovascular fitness played a role in these reproductive hormones. There was a statistical difference between body weight (BW) (p-value < 0.0001) between the groups based on a week interaction. When blood leptin concentrations were observed for a successful 30d pregnancy between the groups, a statistical difference was seen between LW and MW (p-value < 0.0001). Statistical significance was seen between LW and NW (p-value $= 0.0069$) for serum cortisol levels for mares with successful 15d pregnancies. This study showed moderate workload could be beneficial on reproductive efficiency, while light work could be harmful to the open mare; however more research is needed.

DEDICATION

To my father,

my mother,

my husband,

my uncle, and

most importantly, to a great horse and my best friend.

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I would like to say thank you to a few special people in my life who have not only helped but offered words of wisdom and encouragement during my quest to obtain a Master of Science degree from Stephen F. Austin State University. Most of these individuals have followed me through this process and were at the beginning when I started this journey at Texas A&M University in 2008. First to my committee, you have offered advice, guidance and criticism, which has not only expanded my knowledge, but also made me into the confident researcher who stands before you now. Each of you took the tough-love approach and instead of giving me answers you gave me tools to excel and, because of this, I am a person and a researcher who has the ability to stand on her own two feet. Second, to Dr. Tom Welsh from TAMU and Dr. Donald Thompson from LSU, for running my assays; without each of you this project would never have been completed; thank you.

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Sir Isaac Newton once said, “If I have been able to see further than others, it was because I stood on the shoulders of giants.” I truly believe I have been able to see farther then my counterparts and it is because of my family, friends and fellow colleagues. Thank You.

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CHAPTER I

Introduction

Overview

It has long been known that nutrition plays an important role in reproduction; however, the current literature is limited and still needs to be expanded concerning cardiovascular fitness and if a correlation exists in regards to reproductive efficiency. Common understanding that maternal nutrition and body condition have an effect on reproductive capability in multiple species has been the focus of much research; nevertheless, further insight into cardiovascular fitness in the broodmare is still of interest. Additionally, maternal nutrition along with cardiovascular fitness and the resulting effect on certain hormones such as leptin and cortisol may be of importance in not only the reproductive efficiency of the mare, but also in the health and well-being of the subsequent offspring as a neonate and later in life.

Body Condition Score (BCS) is a subjective evaluation that ranges from a score of 1 to 9 with 5 being moderate (Henneke et al., 1983). Five is ideal for breeding efficiency in that this level of adiposity is associated with successful pregnancy rates and acceptable intervals between ovulations (Henneke et al., 1984; Cavinder et al., 2007). However, studies performed comparing moderate (5-to-6) and fat (7-to-8) mares showed no difference in days to first or second ovulations and conception rates (Cavinder et al., 2005). Additional information on overfeeding in relation to actual intake will compliment previous research. Body condition and body fat are directly related; therefore, as a mare increases in body condition, the amount of body fat also increases.

In addition to BCS, leptin may also play a reproductive role in the mare. Leptin is an adipocyte hormone that is found in the adipose tissue of animals, and defects in its signaling pathway have been known to result in obesity in animals (Prolo et al., 1998). Furthermore, it has been reported that one-third of non-pregnant mares who were considered to be obese had hyperleptinemia and hyperinsulinaemia, and leptin was elevated in those mares that were on pasture full time as compared to those who were not (Huff et al., 2008). Additionally, leptin levels found in mares milk were highest at day 0 and decreased over time (Berg et al., 2007), thus alluding to a suspected level on importance for leptin intake of the neonate. Therefore, conclusions can be drawn that leptin has a direct positive correlation to BCS and body weight (BW) because it is secreted from the adipocyte; it also plays a defining role in reproductive function of the mare.

Cortisol is produced by the adrenal cortex and is known as the “stress hormone” (Martin and Silberzahn, 1990). It is responsible for increased blood pressure and blood sugar levels in the body (Martin and Silberzahn, 1990). It has been reported that birth weights and cortisol concentration have been correlated with morbidity and mortality in the human (Aucott, 2008). Most importantly, proper fetal cortisol production in-utero is needed to ensure a healthy foal, as it is responsible for maturation of fetal organs (Ousey, 2006). Cebulj-Kadunc (2000) determined in a study focused on ewes during both gestation and postpartum that cortisol levels rose and fell due to seasonal or human influence; however, the most important finding was that cortisol was not affected by reproductive state. Finally, Teixeira et al. (2008) divided mares into 3 groups; pregnant, not pregnant and postpartum. The results were that cortisol possessed a circadian rhythm with no difference in terms of blood concentration among all 3 groups. Therefore, it can be concluded that cortisol plays an important role, especially in terms of reproductive outcome and the unborn fetus.

Limited research has been performed to determine the correlation between cardiovascular fitness and reproductive efficiency, especially in the mare. Wasinski et. al (2015) studied female mice and their offspring to determine if diet-induced obesity as adults can be stopped while still in utero. Maternal exercised offspring had lower birth weights through 2 months of age, along with possessing less body fat, less weight gain, decreased leptin levels and increased insulin sensitivity when fed a high fat diet (HFD) at 4 months of age. In mares, Smith et. al (2015) showed exercise increased ovarian arterial blood flow leading up to ovulation, but decreased embryo recovery rates.

Goals and Objectives

The aim of this study was to determine the hormonal profiles of mares in varying work groups prior to conception and to evaluate if this had an effect on the reproductive efficiency of the mare. Hormone profiles of leptin and cortisol were compared in the mares. Therefore, the objectives of this study were to:

1.) determine if any differences exist in the time between the number of estrus cycles and 30 days pregnant between mares that do not work, light work and moderate work,
2.) evaluate potential changes of blood plasma concentration profiles of cortisol and leptin for mares, and
3.) perform a DNA five-panel test on each mare to determine their genetic makeup for certain diseases.

This study was performed with the hopes that producers and researchers alike can more adequately understand nutritional effects, cardiovascular fitness and hormonal interactions of the mare. Further information detailing how maternal nutrition can impact the endocrinology of the mother and the subsequent foal may lead to more efficient feeding programs, while determining the relationship between breeding efficiency and cardiovascular fitness may lead to more efficient breeding programs.

CHAPTER II

Review of Literature

The equine industry has always been concerned about factors which affect breeding efficiency of mares. Multiple studies on body fat deposition in relation to reproductive efficiency have been conducted in horses (Henneke et al., 1984; Kubiak et al., 1989; Cavinder et al., 2009), cattle (Rutter and Randel, 1984) and sheep (West et al., 1991). Along with body condition, hormones such as leptin, IGF-1, and cortisol may also play an important role in reproduction (Cavinder et al., 2007) and could potentially impact the unborn foal later in life. Numerous studies have been conducted in order to better understand the impact that nutrition has on the mare's reproductive efficiency but limited research has explored the affect maternal work load and cardiovascular fitness could have on the mare's reproductive efficiency.

Body Condition

Body condition score is a numerical indicator as to the level of adiposity a horse possesses. This score ranges from 1 to 9, with 1 being emaciated, 9 being extremely fat, and 5 being moderately fleshy (Henneke et al., 1983). Body condition score is a subjective evaluation that takes into consideration the following areas: neck, withers, behind the shoulder, ribs, tail head, and lumbar spinous (Henneke et al., 1983). Body condition and amount of body fat are directly related; therefore, as a mare increases in body condition, the amount of body fat also increases.

Previous reports have suggested that mares in a higher BCS at the time of mating had increased pregnancy rates along with fleshier mares having decreased intervals between ovulations (Henneke et al., 1984; Cavinder et al., 2007). Furthermore, mares who entered the breeding season with a BCS of 5 or higher and maintained that score throughout pregnancy achieved a higher degree of breeding efficiency (estrus length, teasing score, length of foaling to first and second ovulations) when compared to mares that maintained a BCS below 5 (Kubiak et al., 1989). It has also been suggested that mares possessing a moderate (5 to 6) versus fleshy (7 to 8) condition are not different in terms of days to first ovulation following parturition, days to second ovulation, and conception rates (Cavinder et al., 2009). Additionally, Gentry et al. (2002) observed 2 groups of mares receiving different planes of nutrition, and found a strong variance between the groups. These researchers reported that mares maintained in a higher BCS (7.5 to 8.5 and received *ad libitum* pasture time) throughout the breeding season, and the winter months continued to ovulate, as compared to mares retained in a lower BCS (3.0 to 3.5 and received restricted pasture time) through both the breeding season and winter months. Mares with a lower BCS became reproductively inactive during the winter months.

Studies regarding reproductive efficiency in other species have also been conducted and may be comparable to reproduction in the mare. Rutter and Randel (1984) evaluated nutritional status of cows by feeding 3 diets. The diets were as followed: low (90% National Research Council (NRC) requirements), maintenance (100% NRC requirements), and high (110% % NRC requirements). Cows receiving maintenance and high diets expressed a decrease in the postpartum interval to estrus as opposed to cows kept at 90 % NRC requirements. This was also true between 100 % and 110 % NRC requirement groups. Additionally, West et al. (1991) fed ewes either a low or a high plane of nutrition, with high plane of nutrition being defined as grazed pasture with supplemental alfalfa pellets and low plane of nutrition ewes being placed on sparse, dry pasture. Results showed that ewes in a high plane of nutrition possessed greater ovulation rates and litter size, when compared to the ewes in a lower level of nutrition.

Plane of Nutrition

Although BCS is discussed in length in many previous publications, the level of nutrition the animal receives should be considered in discussing reproductive efficiency. Montgomery et al. (1985) used Angus cows to determine if a correlation existed between season of calving and plane of nutrition. Cows were divided into 2 groups; those calving early in the year and those calving late in the year. Additionally, each group had cows that were either fed a high or medium plane of nutrition for 55 d before and 40 d after calving. Cows in high plane of nutrition for the 55 d prior to parturition received 2 kg of crushed barley and 9.5 kg meadow hay/d and following parturition a pasture allowance of 30 kg of dry matter (DM) was received. Cows in the medium plane of nutrition received1 kg of crushed barely and 5.5 kg of meadow hay/d and after birth 10.5 kg of DM/d was offered. Those groups on a high plane of nutrition during the testing period were on average 35 kg heavier than cows fed a medium plane of nutrition. Furthermore, following birth individuals who were bred to calve earlier in the year were 11 kg heavier than those who calved later in the year. Therefore, conclusions can be made that season of calving interacts with plane of nutrition in cows in terms of reproductive efficiency. However, the same cannot be concluded for sows. Heap et al. (1967) performed a study utilizing 3 groups of 15 sows. Groups were either given 3-, 6-, or 9- lb meal/d until slaughter, mean weight gains were 6-, 40-, and 65- lb, respectively. Adjustments were made for differences in sow BW at service. No difference was seen between treatment groups and number of normal embryos and survival rate; however, mean volume of embryonic fluid and mean weight of embryonic membranes were greater on the low as compared to the medium or high plane of nutrition.

Almeida et al. (2001) performed a study to determine if feed restriction at different time intervals of the estrous cycle affected reproductive performance in gilts. The study used 3 groups of gilts with the following planes of nutrition: group 1 was on a high plane of nutrition throughout the cycle (HH), group 2 was restricted fed from d 1 to 7 (RH), and group 3 was restricted fed from d 8 to 15 (HR). Embryo survival at 28 d of gestation was lower for HR gilts than for those gilts that were HH and RH, but no difference was reported in ovulation rate between all groups. Therefore, it was observed that feed restriction during certain time periods of follicular development can impose consequences on embryo survival. Additionally, Findlay and Cumming (1976) studied ewes fed complete rations of alfalfa hay, which provided either one-third of maintenance requirements, maintenance, or 2 times maintenance requirements. Live weight was taken after 7 wk of treatment with the 2 times maintenance requirement ewes weighing the most and the one-third maintenance ewes weighing the least. Furthermore, the ovulation rate was also the highest for the ewes receiving 2 times the maintenance requirements. There was no difference in follicle stimulating hormone (FSH) concentration between the groups during the estrous cycle, so it can be concluded that there is no correlation between FSH concentration, live weight or ovulation rate when comparing the treatment groups.

Karren et al. (2010) and Thorson et al. (2010) ran concurrent studies on mares who received differing planes of nutrition and/or selenium (Se) supplementation to determine muscle composition, colostrum Se concentrations, placental efficiency and colostral immunoglobulin G (IgG). Mares were divided into 4 groups either fed 100 % or 120 % NRC requirements, and either receiving or not receiving supplemental Se during the last trimester of pregnancy. Karren (2010) reported that mares fed 120 % NRC requirements had increased BW and BCS throughout the experiment. Additionally Se concentrations in plasma, muscle, and colostrum were greater for mares who received supplemental Se. Foals whose dams were fed 120 % NRC requirements had higher plasma and muscle Se concentrations. Thorson (2010) concluded that mares fed supplemental Se had decreased placental cell size, but mares fed at 120 % NRC requirements had lower colostral IgG. This was also seen in those mares offspring as the foals serum IgG levels were also lower than their counterparts whose mothers were fed at 100 % NRC requirements. Conclusions can be drawn that maternal diet and Se supplementation throughout the final trimester of pregnancy does affect reproductive efficiency.

In addition to BCS, maternal diet also plays an important role in reproductive efficiency in many species; however, the effect and extent of the restricted or excess plane of nutrition is species dependent.

Leptin

Leptin is a 167 amino acid peptide considered to be an adipocyte hormone found in the adipose tissue of animals. It is responsible for signaling nutritional status to the central nervous system and peripheral organs (Prolo et al., 1998). Leptin is also synthesized in the placenta; however, leptin's role in-utero is not completely clear. Body Mass Index (BMI) and % body fat are directly related to the levels of plasma leptin that are seen, thus the greater the BMI and % body fat, the greater the leptin levels found in the body (Prolo et al., 1998). Defects in the signaling pathway can occur and result in obesity (Prolo et al., 1998). Sabatier et al. (2008) compared leptin, blood pressure and % body fat in women of varying weight and physical fitness levels and concluded that a positive correlation existed between % body fat and leptin plasma concentrations. According to Kitagawa (2001) the same findings were found in obese cats.

Research has shown that leptin may also play a role in reproductive physiology. Silva (2008) compared cord blood leptin levels between 3 groups of pregnant women. The groups were broken down as follows: normal, induced hypertension, and gestational diabetes. Leptin levels were different across all 3 groups with gestational diabetes showing the highest leptin concentration. Silva (2008) also reasoned that leptin levels of the mother are positively associated with fetal birth weight. Chiesa (2008) performed a study utilizing 153 mothers to track leptin and insulin-like growth factors in both the mother and offspring. Results indicated that birth weight, body length and head circumference are positively correlated to IGF-1 levels and leptin blood levels found in cord blood. Moreover, birth weight was correlated to maternal stature and prepartum weight. It was also shown that an increase in leptin and IGF-1 had a positive effect on growth and development in the offspring. Similarly, Eryavuz (2008) found that leptin plays an important role in BW, body fat and reproductive efficiency in dairy cattle. Leptin levels of the cows were low during the beginning of lactation and remained low up to the seventh month. Leptin then continued to increase through the dry period, due to the fact that lactation requires increased energy requirements, so cows BW will decrease and thus leptin levels will also decrease during that time period. Finally, Gamerz-Vazquez et al. (2008) studied the effects of leptin concentrations with body condition being the determining factor. Criollo goats were divided into1 of 4 groups based on current body condition: excellent, good, regular and poor. As BCS increased, serum leptin concentrations also increased in the goats indicating that leptin and BCS are directly related to one another.

In the mare, Huff et al. (2008) noticed one-third of non-foaling mares considered to be obese had hyperleptinemia and hyperinsulinaemia. Mares on pasture full time had higher leptin concentrations than those not on pasture full time. Hyperleptinemia has a higher occurrence in mares that are not lactating, possibly resulting from the fact that mares in the lactation period have higher energy demands and thus a lower BCS. Berg et al. (2007) evaluated mares and their foals in a study to determine leptin and IGF-1 concentrations in the prepartum and postpartum mare, along with milk samples and blood serum of their subsequent foals. These researchers determined that in the mare IGF-1 concentrations changed over time along with leptin concentrations which decreased and then remained stable. In terms of milk serum, IGF-1 showed the greatest amount on d 0 and decreased until becoming undetectable by d 12. Leptin concentration found in mare's milk was also the highest at d 0 (especially presuckle) and became non-existent by d 61. Furthermore, while mare BW changed (due to foaling) there was no change in BCS. However, the foals showed different results as foal plasma leptin concentration did not change over the study's time period. Insulin-like growth factor-1 concentrations increased until d 19 where a peak occurred and then achieved a plateau.

Therefore, it can be concluded that not only does leptin have a direct positive correlation to BCS and BW, because it is secreted from the adipocyte, it also plays a defining role in reproductive function of the mare, other livestock, companion species and humans.

Cortisol

Cortisol is a hormone that is produced by the adrenal cortex and is known as the "stress hormone" and is responsible for blood pressure and blood sugar level increases in an animal (Martin and Silberzahn, 1990). Cortisol also plays a significant role in the body during pregnancy and parturition. Unlike most mammals, the mare's cortisol level decreases throughout pregnancy instead of increasing (Martin and Silberzahn, 1990). In a recent study mares were divided into 3 groups: pregnant, non-pregnant and postpartum. Cortisol was found to exhibit a circadian rhythm with no difference in terms of blood concentration among all 3 groups (Teixeira et al., 2008).

As in other species, horses have maternal cortisol concentrations that increase due to different stress stimuli (Ousey, 2006). Transplacental transfer of maternal cortisol to the fetus is highly unlikely due to cortisol being converted to cortisone (inactive) by a placental enzyme. However, cortisol levels in the fetus can increase due to different stimuli. Fetal cortisol concentration increases are extremely important in order to ensure a successful delivery and healthy foal as cortisol is imperative for maturation of fetal organs. The rise of fetal cortisol occurs during different times of gestation for different livestock species; however, for the horse it occurs during the last 2 to 3 d prior to birth (Ousey, 2006). Without this rise of fetal cortisol prior to delivery, foals appear premature and typically die from multi-organ failure (Ousey, 2006).

Cebulj-Kadunc (2000) reported findings concerning cortisol in ewes during both gestation and postpartum. Throughout pregnancy cortisol levels rose and fell around seasonal or human influence. Cortisol levels were low during December through March (1-4 mo of pregnancy), due to seasonal influence, and rose in April due to shearing season (human influence). The most important finding was that cortisol levels were not affected by reproductive state. In addition, cortisol levels do not change due to seasonal variation when comparing Cushing diseased horses and non-diseased horses, with the exception of the summer where diseased individuals possessed higher cortisol levels (Haritou, 2008).

Cortisol concentrations and birth weights have been correlated with morbidity and mortality (Aucott, 2008). Aucott (2008) and company wanted to determine if low cortisol levels and low birth weight could be used as an indicator (predictive measure) for adverse short-term outcomes, specifically morbidity or mortality in infants. Surprisingly, infants with higher cortisol levels had a positive correlation with both morbidity and mortality (Aucott, 2008), thus concluding that low cortisol levels is not a determining factor of adverse short-term outcomes in infants who possessed a low birth weight, as once thought.

Therefore, it can be concluded that cortisol plays an important role, especially in terms of reproductive outcome and the unborn fetus. However, the amount of cortisol required for an individual to be born healthy is species dependent.

Cardiovascular Fitness

Limited research has been performed on the benefits or disadvantages cardiovascular fitness has on reproductive efficiency, and the results are conflicting. Wasinski et al. (2015) studied C57BL/6 female mice and their offspring, which were divided into two groups: sedentary and swim-trained, prior to and during pregnancy. Results showed maternal exercised offspring had lower birth weights through 2 months of age, and when fed a high fat diet (HFD) for 4 months offspring showed less body fat, less weight gain, decreased leptin levels and increased insulin sensitivity. Kelly et al. (2015) divided female mice into three groups: absence of wheel running, wheel running prior to gestation and wheel running prior to and throughout gestation. Findings showed no substantial effects of maternal exercise in female mice offspring's body composition throughout development, or the need for the offspring to engage in voluntary exercise. This study had conflicting results with previous findings which showed maternal exercise had the potential to change the phenotypes of the offspring.

In gilts worked 30 minutes, 3 times a week from mid- to late- gestation periods showed umbilical blood flow increased when compared to the non-working gilts. Furthermore, there was no difference in fetal weights, piglet birth weights, placental weight, interval between piglet births and blood lactate of newborn piglets (Harris, et al. 2014). In mares, Smith et al. (2015) used 16 light horses with previous training experience; the horses were divided into three groups: control (no exercise), partial-exercised or full-exercised, with the intent to determine the effects of exercise on embryo recovery rates. Partial-exercised mares were worked 30 minutes daily and rested for 7 ds after ovulation; while full-exercised mares were worked 30 minutes daily throughout the entire reproductive cycle. Results showed exercise increased ovarian arterial blood flow leading up to ovulation, no differences in follicle ovulatory diameter between the groups, and embryo recovery rates decreased in exercised mares.

Genetic Diseases

Genetic diseases affect the equine industry. These genetic diseases are just as important in terms of a mare's reproductive efficiency as any of the previously discussed reproductive parameters. In order for sound breeding decisions to be made knowledge of each genetic disease should be known.

Hyperkalemic Periodic Paralysis (HYPP). Hyperkalemic Periodic Paralysis is a dominant autosomal trait which affects stock type breeds (Spier, 2014). It is considered to be a point mutation which causes a "leaky" sodium (Na) channel, resulting in an increase of potassium (K) in the blood. This increase in K changes the voltage current of muscle cells, thus causing the uncontrollable muscle twitching and muscle weakness, seen in infected horses (Spier, 1998). This disease has been identified in descendants of the American Quarter Horse Association (AQHA) stallion Impressive. During his live Impressive never showed signs of HYPP.

As stated early this disease causes sporadic attacks of muscle tremors, but can also cause weakness and/or collapse of an individual during an episode along with death due to a heart attack or paralysis of the respiratory muscles (UC Davis, 2016). While this disease is a dominant autosomal trait, it can be managed and treated. In order to properly manage and treat the disease it is important to realize the causes of a HYPP attack, these include environment factors such as: dietary changes, fasting, general anesthesia, concurrent illness, and exercise restriction (UC Davis, 2016). In order to successfully prevent and control attacks caused by HYPP owners of HYPP affected horses need to avoid high potassium feeds, alfalfa and brome hay, canola and soybean meal oil, and sugar and beet molasses. Owners instead should feed oats, corn, wheat, barley, beet pulp, and Timothy/Bermuda grass. Also, it is vital for owners to remember to successfully avoid attacks; horses should be fed several times a day and exercised regularly (Spier, 1992).

Finally, the genetics of this disease are imperative to understand. There are three possible outcomes. The first being N/N which is a normal individual and does not carry the HYPP gene, the second is N/H, this individual is affected and has one copy of the HYPP allele, and finally H/H, this individual is affected and has two copies of the HYPP gene (UC Davis, 2016). With the number of offspring from Impressive being so large and HYPP being a dominant trait, AQHA has implemented a rule stating any and all descendants of Impressive must be tested for HYPP prior to registration and if found to be H/H they are not allowed to be registered with the association (AQHA Rulebook, 2016).

<u>Hereditary Equine Regional Dermal Asthenia (HERDA).</u> The recessive genetic disorder Hereditary Equine Regional Dermal Asthenia is also known as Hyperelastosis Cutis or HC (Animal Genetics, 2014). Hereditary Equine Regional Dermal Asthenia is considered a skin disease where the infected individual develops severe lacerations, hematomas, and seromas from minor trauma, resulting in disfiguring scars. (UC Davis, 2016). Infected individuals all trace back to the AQHA stallion Poco Bueno (Animal Genetics, 2014).

Hyperelastosis Cutis is considered to be a recessive autosomal trait characterized by hyper extensible skin resulting in scarring and severe lesions along the back. Symptoms for HERDA are rarely seen at birth, but start appearing when the infected individual is between two to three years of age, when the horse is first being broke to saddle. There is no cure for HERDA and diagnosed horses are euthanized because of the inability to be used and the ability to pass on the undesirable trait (Animal Genetics, 2014).

Like HYPP, HERDA also has three possible genetic outcomes. An individual who is N/N is considered to be normal and does not have a copy of the HERDA gene. The second possible genetic outcome is N/HRD; this individual is not affected with the disease, but is considered to be a carrier of the disease, having only one copy of the disease. If two carriers are bred there is a 25% chance the offspring will be positive for HERDA. The final outcome for HERDA is positive or HRD/HRD. These individuals have the disease and two copies of the HERDA gene (Animal Genetics, 2014).

<u>Glycogen Branching Enzyme Deficiency (GBED).</u> This disease is considered fatal since the body lacks the ability to properly store sugar. Horses use sugar as a form of energy by converting glucose into glycogen. Affected horses lack the enzyme needed to perform this crucial function in the body, resulting in the horse's inability to store enough energy to fuel vital organs including the heart and the brain, along with the skeletal muscles (UC Davis, 2016).

This disease is characterized by abortion or still birth of the affected foal; it is speculated 3% of all aborted Quarter Horse foals are homozygous recessive for GBED. If the foal should survive to term, owners will see foals that are weak with low body temperatures. Foals will also possess a high respiratory rate, weakness of respiratory muscles, sudden death, and contracted tendons found in all four legs. Additionally, foals lack the ability to get up from lying down on its side (Animal Genetics, 2016).

GBED is considered an autosomal recessive trait and therefore, there are three possible outcomes genetically. The double negative individual (N/N) is normal and does not have the disease; nor are they considered a carrier. N/G individuals are not affected but are carriers so the possibility exists to pass on the disease if bred to another carrier. Finally, G/G individuals have two copies of the GBED gene and are affected (Animal Genetics, 2014).

Lethal White Overo (LWO). Lethal White Overo or Lethal White was thought in the early years of the disease to only affect color breeds, but in fact affects Paints, Pintos, Thoroughbreds, Quarters, Tennessee Walking Horses, and even miniature horses (APHA, 2016). A frame overo coat color pattern is a carrier of the Ile118Lys EDNRB mutation also known as the Lethal White gene (Animal Genetics, 2014).

Lethal White Overo is medically known as ileocolonic aganglionosis (McClure, 1993). Lethal White is a fatal disease causing the foal to die within five days of birth. It is characterized by complications from intestinal tract abnormalities, causing the foal to lack the ability to pass food. Foals lack the ability to move milk through the gastrointestinal tract, causing a buildup of milk, thus causing the foal to colic and die. Affected foals are all or nearly all white. It is believed in early embryonic development both the skin and hair producing pigment cells and the nerve cells of the intestinal walls are derived from the same tissues; therefore, being the connecting factor in LWO foals (McClure, 1993).

Lethal White Overo is considered to be inherited as incomplete dominance. The first outcome is an N/N individual resulting in a solid foal which is normal and there is no evidence for the altered sequence. The heterozygote is considered a carrier of the disease, has the frame overo coat color pattern, and one copy of the altered sequence, but no adverse health effects are seen in these individuals. Finally, O/O individuals have two copies of the altered sequence and are considered LWO foals (Animal Genetics, 2014).

Polysaccharide Storage Myopathy Type 1 (PSSM1). Polysaccharide Storage Myopathy Type 1 is considered to be a dominant autosomal trait caused by a base pair substitution in the GYS1 gene (APHA, 2016). This disease causes a genetic form of tying up also known as Monday morning sickness. This genetic form of tying up causes muscle damage and the inability to move, due to the accumulation of abnormal complex sugars in the skeletal muscle (Animal Genetics, 2014). Polysaccharide Storage Myopathy Type 1 is found in at least twenty breeds and 50% of all Belgians and 8% of stock type breeds (Animal Genetics, 2014).

This disease has symptoms that usually begin between two to three years of age when a horse’s workload starts to increase, but some individuals can remain subclinical.

Symptoms include skin twitching, stiffness, firm, painful muscles, weakness, excess sweating, gait abnormalities, muscle wasting, mild colic, and reluctance to move with light exercise (Animal Genetics, 2014). Similar to HYPP, PSSM1 can be controlled and managed. PSSM horses can make and store abnormal muscle glycogen; this is caused by the amino acid change of the glycogen synthase enzyme. In order to successfully combat this problem owners should feed a low starch and low sugar diet and maintain infected horses on an exercise program (Animal Genetics, 2014).

As has been seen with the previous genetic diseases there are three possible genetic outcomes for PSSM1. The first possibility is a normal individual who is N/N; this individual does not show signs of the PSSM gene mutation. The second outcome is N/P1. These individuals are affected since PSSM1 is considered a dominant genetic trait and have one copy of the normal allele and one copy of the affected allele. The last possible outcome for PSSM1 is P1/P1. These individuals are also affected and carry two copies of the gene mutation (APHA, 2016).

Malignant Hyperthermia (MH)**.** Malignant Hyperthermia is a relatively new genetic disease. Comparable to PSSM1 and HYPP; MH is also considered to be a dominant autosomal trait that affects the muscles in affected horses. (UC Davis, 2016). This disease can go unnoticed for years since individuals typically do not show any physical signs of the disorder until there is a trigger (exposure to anesthesia, extreme exercise, extreme stress) (Animal Genetics, 2014). Symptoms can develop rapidly upon onset and include a high temperature, increased heart rate, excess sweating, muscle rigidity, high blood pressure, and acidosis. If not treated quickly, this disease can be fatal for the affected individual (UC Davis, 2016).

Testing for MH is recommended since anesthesia is a trigger and most horses do not show outward signs until a trigger is present. If an individual is known to be positive for MH then drugs can be given prior to administering anesthesia to help combat the symptoms that will occur. It has been discovered that MH and PSSM1 can be present together causing the symptoms to be more severe (Animal Genetics, 2014).

Since MH is a dominant autosomal disease there are three possible outcomes. A normal individual will be N/N and lacks the MH gene mutation. The heterozygote is N/MH and is considered to be an affected individual who has one normal allele and one MH mutation allele. Finally, the third possible genetic outcome is MH/MH. These individuals are affected and have two copies of the MH mutation.

CHAPTER III

Materials and Methods

Horses and Treatments

Quarter and Paint Horse mares (n = 9) from the Stephen F. Austin State University Equine Center broodmare herd located in Nacogdoches, Texas were used to determine the effects of varying workload on open mares during the breeding season on the blood plasma concentrations of cortisol and leptin. Mares ranged in age of 10 to 16 yr and weighed 448-571 kg. All mares were blocked based on expected conception dates and were then randomly assigned to 1 of 3 groups, with each group having 3 subjects: no work (NW), light work (LW) and moderate work (MW). Light work mares were worked 1 h 3 times a week, while MW individuals were worked 2 h 3 times a week in a cage walker to maintain speed. Mares in the NW group were maintained on pasture and brought up for feeding with the other two groups, but were not placed in the cage walker. Diets for each group were decided based on NRC requirements. Mares in the NW group were given grain based on NRC maintenance requirements of 0.5 % BW. The LW group based on NRC requirements will have a 25 % increase in energy fed. Those in the MW group will be fed based on NRC requirements of a 50 % energy increase from maintenance. Mares were fed daily at 0800 h and then again at 1730 h in individual feeding stalls to avoid sharing. Refusals were measured and recorded after each feeding period, if present. All mares regardless of group were placed in individual feeding stalls in order to equilibrate pasture time, and all were allowed free access to water and traced mineralized salt blocks, while being maintained on the same pasture.

Dietary monitoring and administration of each treatment groups began after one successful estrous cycle and continued until a 30 d heartbeat was achieved on the fetus. This was done so that each mare, depending on the assigned group, was receiving NRC requirements for horses based on work load. Furthermore, since pasture intake was not measured, total dietary intake was assumed to be 2 % BW (DM basis; Aiken et al., 1989).

<u>Mare Body Composition</u>

Body weight and BCS, were measured weekly until 30 d pregnant and feed intake was adjusted accordingly. Body weight was determined by a digital scale, while BCS was determined by 1 individual appraiser utilizing the body condition scoring method (Henneke et al., 1983).

<u>Pregnancy Parameters</u>

Mares were palpated and had an ultrasound three days a week to correspond with selected stallion collection days. When signs of estrus (squatting, winking, tail raising, general interest in the stallion) were presented, mares were sent to Ward's Animal Hospital located in Nacogdoches, Texas and semen ordered. Upon arrival of semen mares were artificially inseminated (AI), and checked until ovulation was achieved. While at Ward Animal Hospital water was offered *ad libitum.* Bermuda grass hay was also offered; normal feeding times and feed amounts were maintained. Mares in LW or MW groups were not worked while at Ward Animal Hospital being bred. During the trail, conception parameters were recorded and included: BW, BCS, number of estrus cycles and days between first estrus cycle and viable pregnancy. Additionally, a five-panel DNA test was performed on each mare to determine genetic makeup for major equine diseases which could be passed on to subsequent offspring.

Blood Samples and Analysis

Blood samples were taken weekly from each mare approximately 30 d prior to expected onset of the first estrus cycle of the season via jugular venipuncture preceding the 0800 h feeding, at time of ovulation and continued until d 30 of pregnancy. Samples were collected in sterile blood collection tubes and stored for approximately 1 h prior to being centrifuged. Centrifugation was done at 2,700 x G and 4°C for 5 min. The serum was then harvested and stored at -20°C until analysis could be performed which included quantification of peripheral circulating concentrations of leptin and cortisol.

Radioimmunoassay (RIA) Procedures

Concentrations of leptin and cortisol were evaluated by RIA which has been previously described for horse serum samples (Freestone et al., 1993; Buff et al., 2002; Ropp et al., 2003). Leptin and cortisol were analyzed using a Packard Cobra II gamma counter. All RIA kits contained the required reagents needed for assay completion. Additionally, all samples from a known animal were evaluated in a single assay for each hormone listed above. Each standard and reagent was run in triplicate, while each sample was run in duplicate for each hormone examined.

Leptin. Leptin was measured using a multi-species RIA kit (Linco Research Inc., St. Charles, MO). Each leptin assay required 3 d to perform and a sample volume of 100 μL with a sensitivity of 1.0 ng/mL was used. On d 1, 300 μL of Assay Buffer was pipetted into Non-Specific Binding (NSB) tubes. Additionally, 200 μL of Assay Buffer was pipetted into Reference tubes, and 100 μL in each standard and sample tube. Next, 100 μL of Standards were pipetted in corresponding tubes and 100 μL of each sample in the correct tube. Multi-species leptin antibody was added to each tube excluding the total count, and NSB tubes in an amount that equaled 100 μL. Each tube was then vortexed and incubated while covered, for 20 to 24 h at 4°C. D 2 consisted of adding 100 μL of ^{125}I-Human Leptin to all tubes in the assay. Once again tubes were vortexed, then covered, and incubated for 20 to 24 h at 4°C. Finally, d 3 consisted of adding 1.0 mL of cold (4°C) Precipitating Reagent to all tubes with the exclusion of the total count tubes. Tubes were then vortexed and once again incubated at 4°C for 20 min. All tubes were then centrifuged (with the exception of the total count tubes) at 4°C for 20 min at 2,000 to 3,000 x G. Upon completion of the samples being centrifuged, all tubes were immediately decanted (except the total count tubes). All tubes were then counted using a gamma counter for 1 min.

Cortisol. Plasma cortisol was analyzed using an RIA kit (Coat-A-Count®, Siemens Medical Solutions Diagnostics, Los Angeles, CA). A sample size of 25μL was used with a sensitivity of 0.2 μg/dL. First, 25 μL of calibrators, controls, and samples were pipetted into each correctly labeled tube. Next, 1.0 mL of ^{125}I cortisol was added to every tube and then vortexed. All tubes were then incubated for 45 min in a water bath set to a temperature of 37°C. In conclusion, all tubes (minus the total count tubes) were decanted and placed in a gamma counter for 1 min.

Five-Panel DNA Test

Each mare had a five-panel DNA test ran to test for the major genetic disease which affects their respective breed. All American Paint Horse Association (APHA) mares were tested for, HYPP, HERDA, GBED, PSSM 1, MH, and LWO. Each AQHA mare was tested for HYPP, HERDA, GBED, PSSM 1, and MH. DNA kits were requested from each association the mare was registered. Once kits came via the United States Postal Service (USPS) approximately 50 mane hairs were pulled and placed in each mare's respective kit and sent via USPS to the Veterinary Genetics Laboratory, School of Veterinary Medicine at the University of California, Davis. The University of California, Davis then ran each five-panel DNA test and results for each mare were sent via USPS and placed on file at the respective breed association.

Statistical Analysis

Body measurement parameters and hormonal concentrations were analyzed by 1-way ANOVA (JMP) and the Tukey's HSD was used to separate the means. Time, treatment, and time by treatment interactions were evaluated and means found to be different were subjected to standard means separation with all differences of $P \leq 0.05$ being considered significant and *P 0.05 to 0.10* were considered trending.

CHAPTER IV

Results

Pregnancy Parameters

There was no statistical difference between BCS (p-value 0.7994) (Figure 1) among the groups. However, there was a statistical difference between BW (p-value < 0.0001) (Figure 3) between the groups based on a week interaction. There was no difference between NW and LW groups in terms of body weight. However, a difference was seen when comparing MW to NW and LW in terms of body weight (Figure 2). When work was compared with number of cycles for 15d pregnancies (Figure 4) and 30d pregnancies (Figure 5) there was no statistical difference among the groups (p-value 0.6297 and 0.9231).

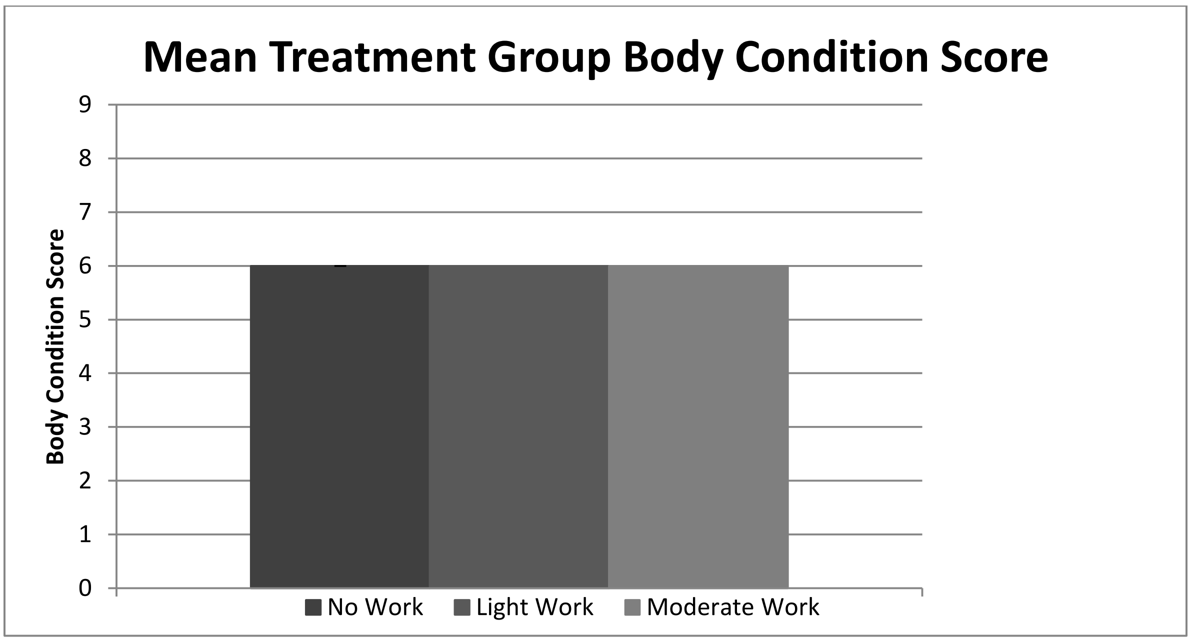

Figure 1. Mean (±SE) body condition score among the groups.

ANOVA for Mean Treatment Body Condition Score

Source	DF	Sums of Squares	Mean Square	F Ratio
Model	1	0.011300	0.011300	0.0648
Error	143	24.926631	0.174312	**P-Value**
C. Total	144	24.937931		0.7994

Table 1. ANOVA for Mean Treatment Body Condition Score

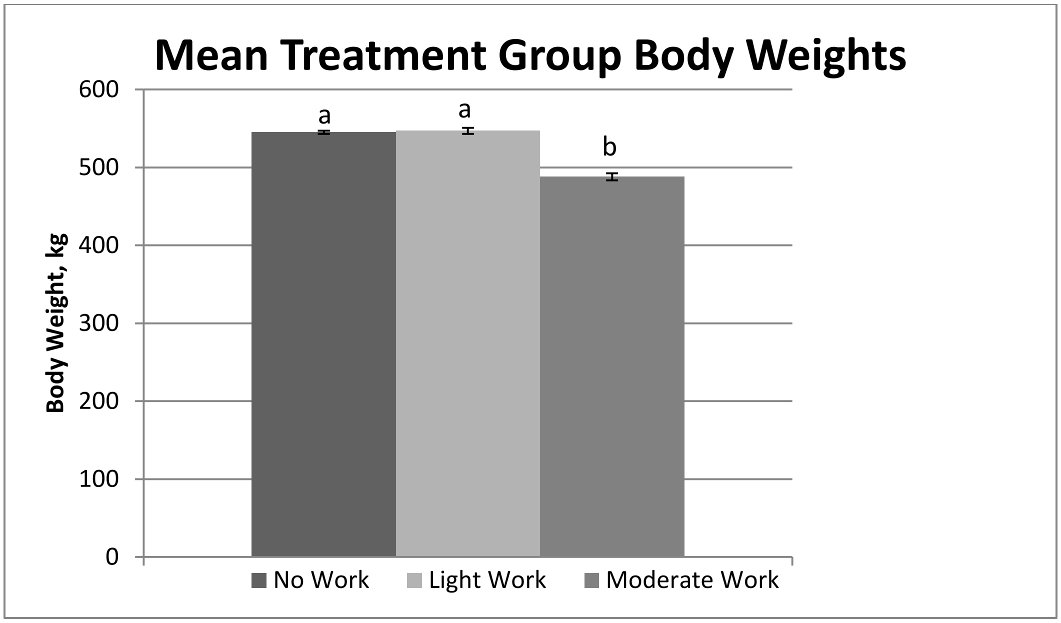

Figure 2. Mean (±SE) body weight among the groups.

ANOVA for Mean Treatment Body Weight

Source	DF	Sums of Squares	Mean Square	F Ratio
Model	1	363316.62	363317	86.1047
Error	143	603385.27	4219	**P-Value**
C. Total	144	966701.89		< 0.0001

Table 2. ANOVA for Mean Treatment Body Weight.

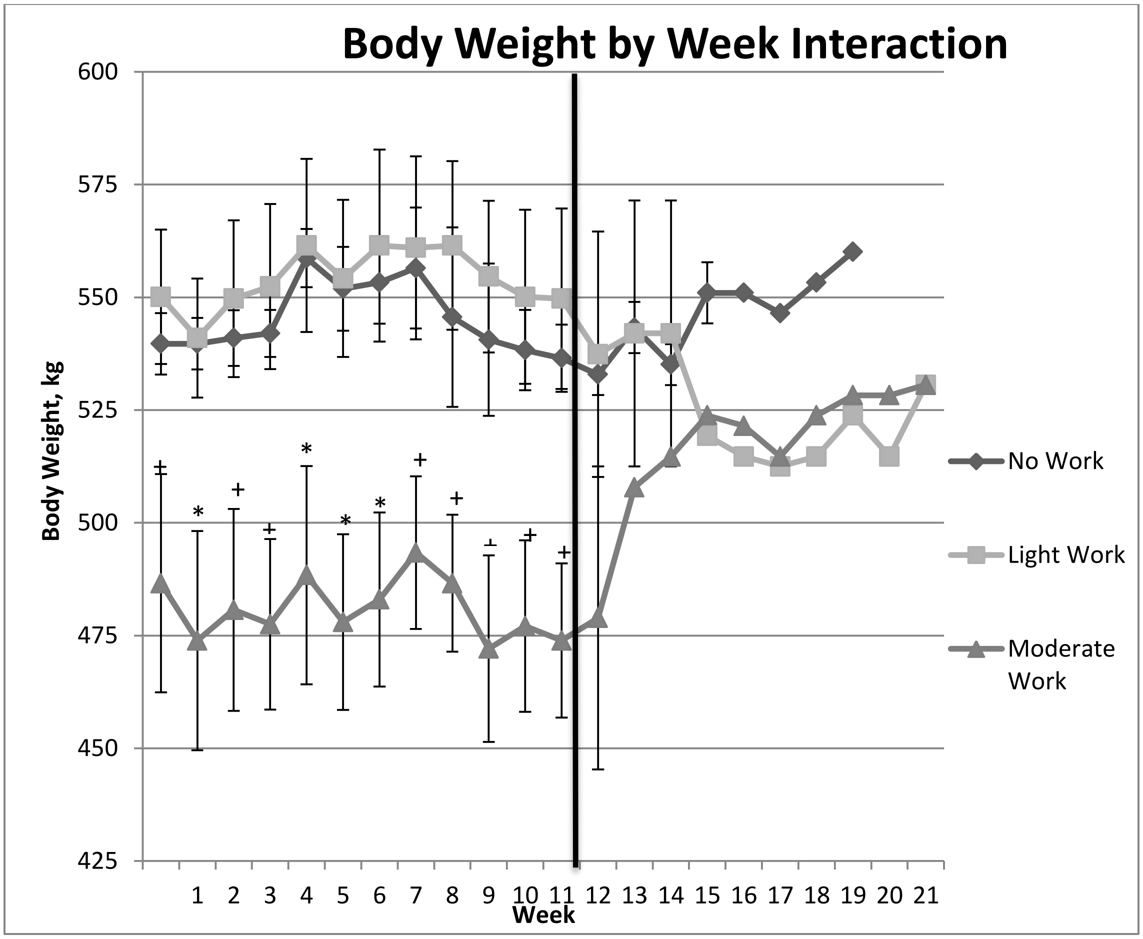

Figure 3. Mean (±SE) weekly body weights during the study in mares in either one of three groups: no work, light work or moderate work. (+) indicates a p-value (0.05-0.10) and (*) indicates means are statistically significant (p-value < 0.05 to 0.0001). The vertical line marks when mares started coming off the project and achieving a successful 30d heartbeat of the fetus.

ANOVA for Body Weight by Week Interaction

Source	DF	Sums of Squares	Mean Square	F Ratio
Model	2	364492.54	182246	42.9734
Error	142	602209.35	4241	**P-Value**
C. Total	144	966701.89		< 0.0001

Table 3. ANOVA for Body Weight by Week Interaction.

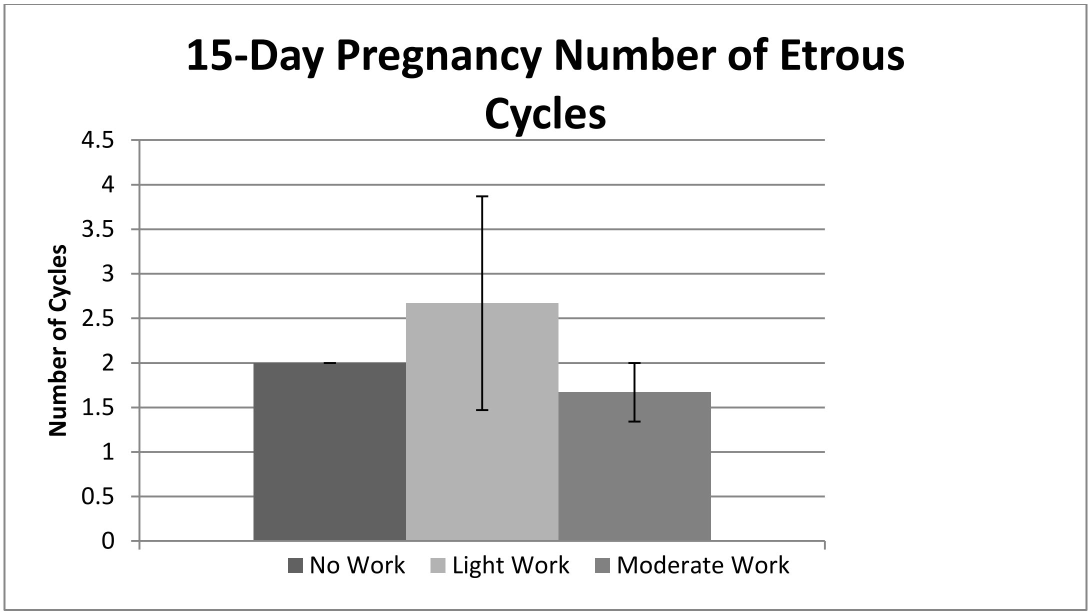

Figure 4. Mean (±SE) of number of cycles for 15d pregnancies among the groups.

ANOVA 15-Day Pregnancy Number of Estrous Cycles

Source	DF	Sums of Squares	Mean Square	F Ratio
Model	2	1.555556	0.77778	0.5000
Error	6	9.333333	1.555556	**P-Value**
C. Total	8	10.888889		0.6297

Table 4. ANOVA 15-Day Pregnancy Number of Estrous Cycles.

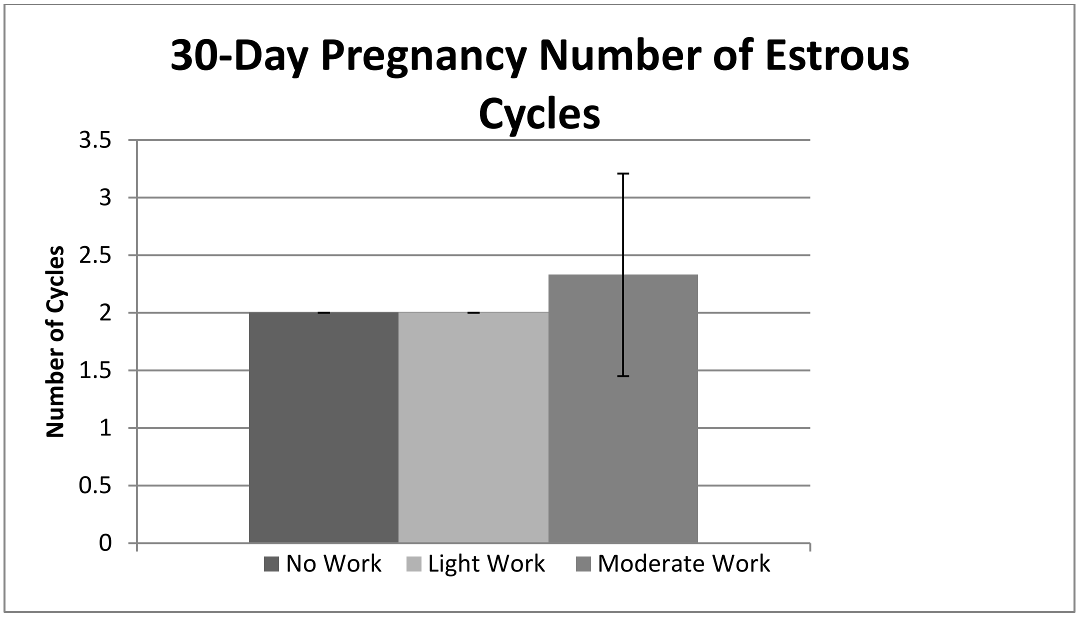

Figure 5. Mean (±SE) of number of cycles for 30d pregnancies among the groups.

ANOVA 30-Day Pregnancy Number of Estrous Cycles

Source	**DF**	**Sums of Squares**	**Mean Square**	**F Ratio**
Model	2	0.1904762	0.09524	0.0816
Error	4	4.6666667	1.16667	**P-Value**
C. Total	6	4.8571429		0.9231

Table 5. ANOVA 30-Day Pregnancy Number of Estrous Cycles.

Endocrine Analyses

Leptin. When blood leptin concentrations were observed between the groups with a successful 15d pregnancy, a statistical difference was seen between LW and MW (p-value 0.0018) (Figure 6). Additionally, when comparing blood leptin concentrations among NW and MW with a successful 15d pregnancy a trend was noticed (p-value 0.0546). No statistical difference was seen between NW and LW with a successful 15d pregnancy (p-value 0.3731). Additionally, no statistical difference was seen for mares for a day interaction with a successful 15d pregnancy (p-value 0.5780) (Figure 7).

When blood leptin concentrations were observed for a successful 30d pregnancy between the groups, a statistical difference was seen between LW and MW (p-value < 0.0001) (Figure 8). Furthermore, a statistical difference was seen between NW and MW for a 30d pregnancy (p-value 0.0018). No statistical difference was seen between NW and LW for 30d pregnancy (p-value 0.2329). Additionally, no statistical difference was seen for day interactions between the groups for a successful 30d pregnancy (p-value 0.4659) (Figure 9).

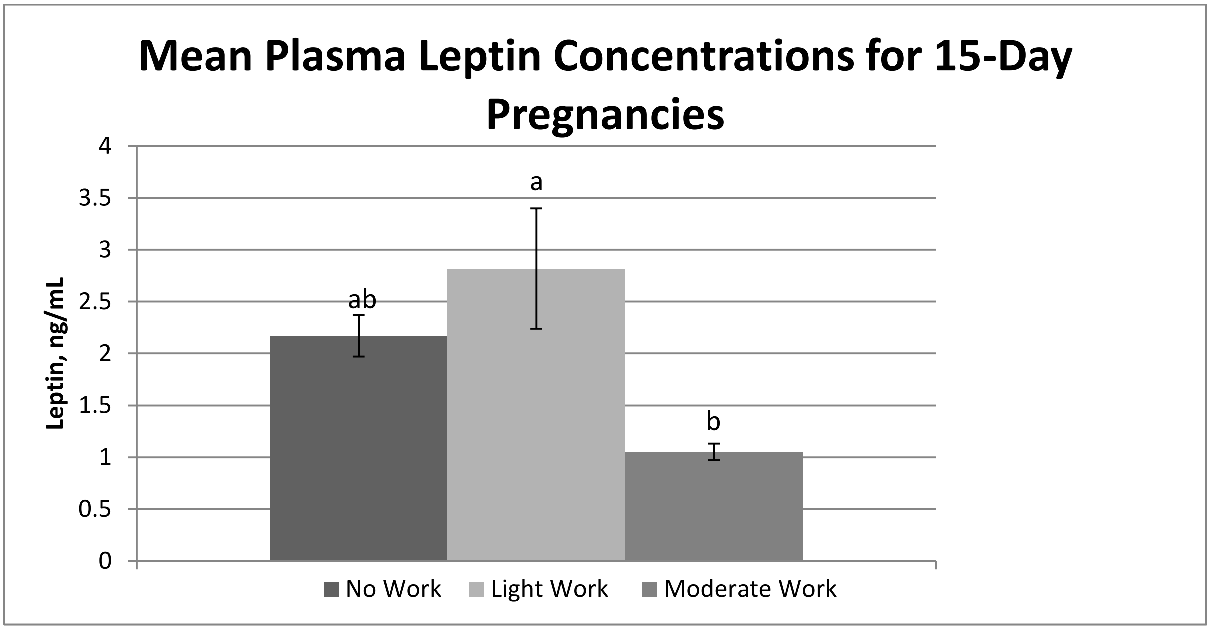

Figure 6. Mean (±SE) plasma leptin concentrations in mares among the groups for 15d pregnancies. Different letters indicate means are statistically significant (p-value < 0.05 to 0.0001).

ANOVA Mean Plasma Leptin Concentrations for 15-Day Pregnancies

Source	DF	Sums of Squares	Mean Square	F Ratio
Model	2	38.19972	19.0999	6.5924
Error	71	205.70646	2.8973	**P-Value**
C. Total	73	243.90618		0.0024

Table 6. ANOVA Mean Plasma Leptin Concentrations for 15-Day Pregnancies.

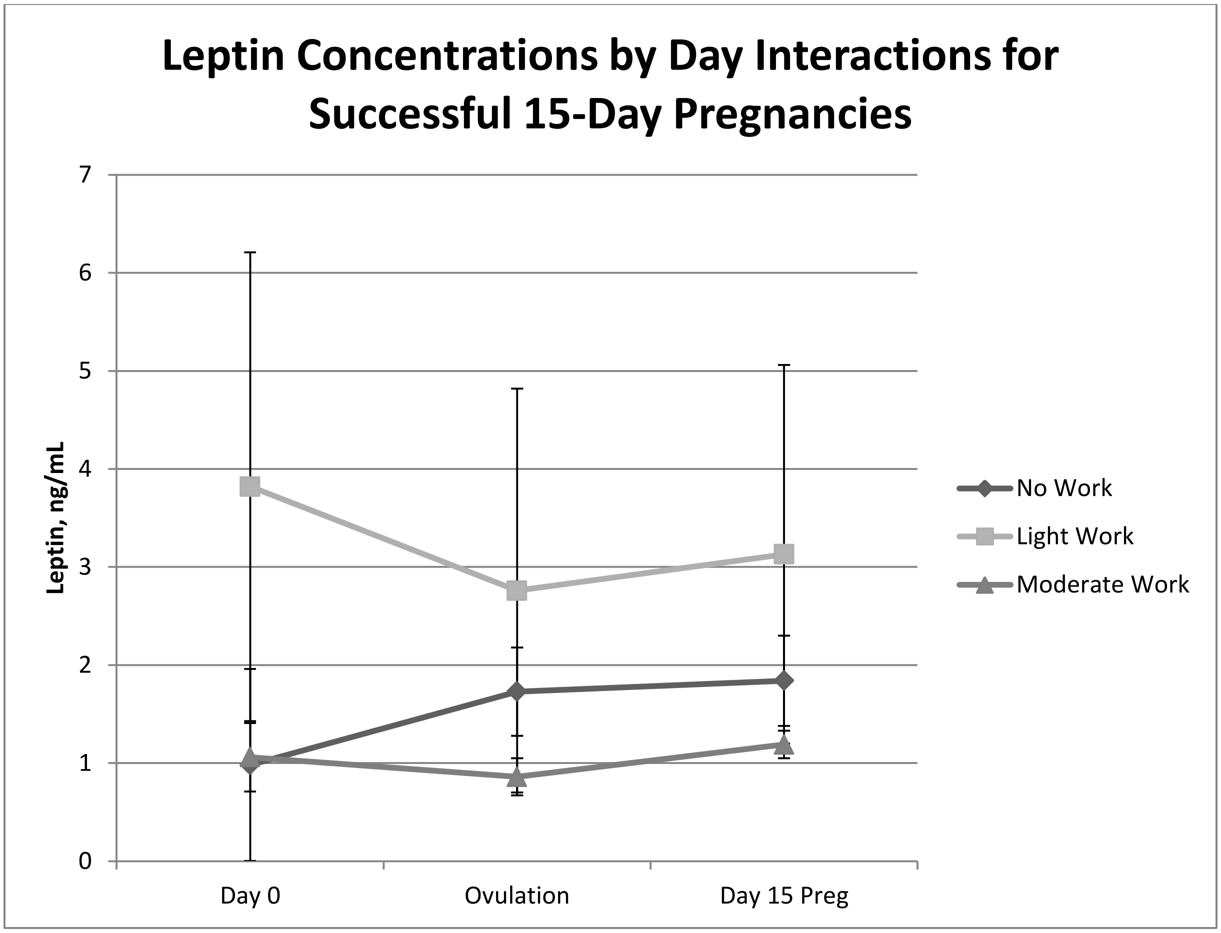

Figure 7. Mean (±SE) daily leptin concentrations during the study in mares successfully 15d pregnant in either one of three groups: no work, light work or moderate work.

ANOVA Leptin Concentrations by Day Interactions for Successful 15-Day Pregnancies

	Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
0 d	Model	2	11.400822	5.70041	0.8387
	Error	6	40.781467	6.79691	**P-Value**
	C. Total	8	52.182289		0.4773
Ovulation	**Source**	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
	Model	2	5.389867	2.69493	0.6014
	Error	6	26.884533	4.48076	**P-Value**
	C. Total	8	32.2744		0.578
15 d Pregnant	**Source**	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
	Model	2	5.871756	2.93588	0.7443
	Error	6	23.667133	3.94452	**P-Value**
	C. Total	8	29.538889		0.5143

Table 7. ANOVA Leptin Concentrations by Day Interactions for Successful 15-Day Pregnancies.

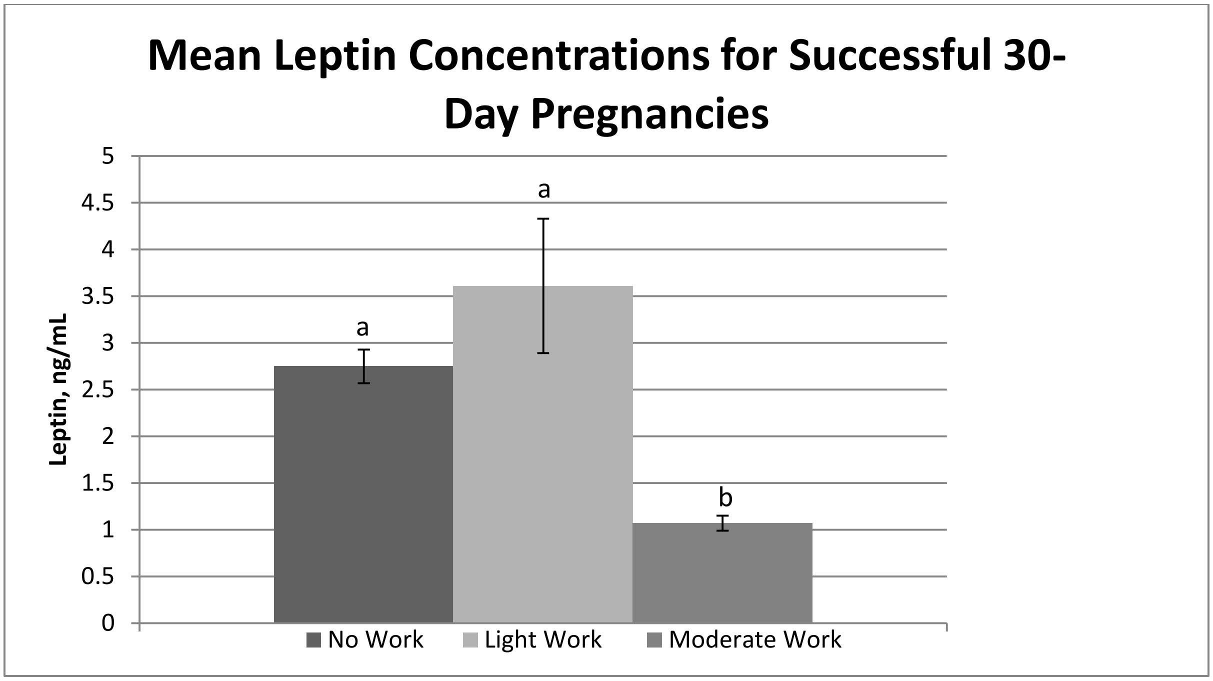

Figure 8. Mean (±SE) plasma leptin concentrations in mares among the groups for 30d pregnancies. Different letters indicate means are statistically significant (p-value < 0.05 to 0.0001).

ANOVA Mean Plasma Leptin Concentrations for Successful 30-Day Pregnancies

Source	DF	Sums of Squares	Mean Square	F Ratio
Model	2	66.96396	33.4820	14.9411
Error	55	123.25151	2.2409	**P-Value**
C. Total	57	190.21546		< 0.0001

Table 8. ANOVA Mean Plasma Leptin Concentrations for Successful 30-Day Pregnancies.

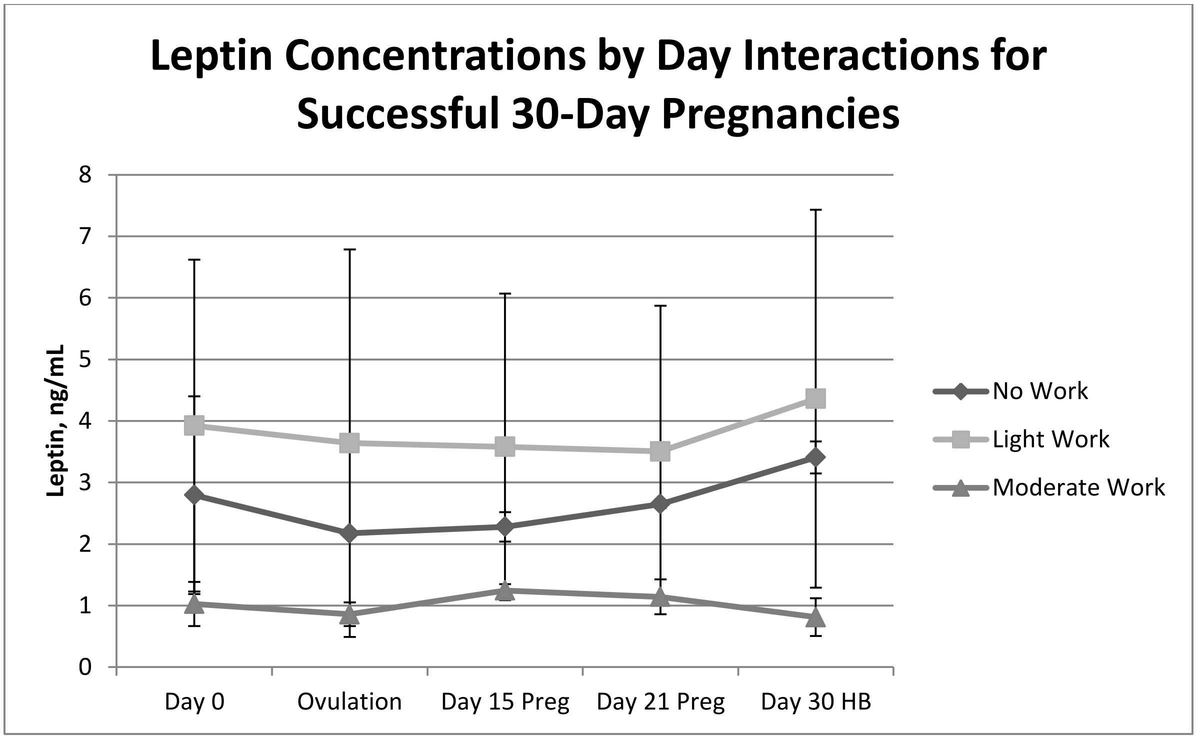

Figure 9. Mean (±SE) daily leptin concentrations during the study for mares successfully 30d pregnant in either one of three groups: no work, light work or moderate work.

ANOVA Leptin Concentrations by Day Interactions for Successful 30-Day Pregnancies

	Source	DF	Sum of Squares	Mean Square	F Ratio
0 d	Model	2	10.610233	5.30512	1.0371
	Error	4	20.461367	5.11534	**P-Value**
	C. Total	6	31.0716		0.4337
Ovulation	**Source**	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
	Model	2	9.33295	4.6647	0.93
	Error	4	20.07085	5.01771	**P-Value**
	C. Total	6	29.4038		0.4659
15 d Pregnant	**Source**	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
	Model	2	6.547619	3.27381	1.0411
	Error	4	12.578667	3.14467	**P-Value**
	C. Total	6	19.126286		0.4325
21 d Pregnant	**Source**	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
	Model	2	7.104919	3.55246	1.2175
	Error	4	11.670967	2.91774	**P-Value**
	C. Total	6	18.775886		0.3864
30 d HB	**Source**	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
	Model	2	17.084041	8.54202	1.7473
	Error	4	19.554923	4.88873	**P-Value**
	C. Total	6	36.638963		0.2849

Table 9. ANOVA Leptin Concentrations by Day Interactions for Successful 30-Day Pregnancies.

Cortisol. When comparing serum cortisol levels among the groups for mares with successful 15d pregnancies there was no statistical difference seen between MW and NW (p-value 0.6730) (Figure 10). A trend was seen between LW and MW (p-value 0.0638) when analyzing mean cortisol levels. Additionally, LW and NW were statistically significant (p-value 0.0069) for serum cortisol levels for mares with successful 15d pregnancies. Additionally, no statistical difference was seen when comparing groups by a day interaction (p-value 0.9520) (Figure 11).

When blood cortisol levels were compared for successful 30d pregnancies, no statistical difference was seen among the groups (p-value 0.4307) (Figure 12). However, comparing group to day interaction (Figure 13) cycle 0d was statistically significant for all three groups; NW and LW (p-value 0.0349), NW and MW (p-value 0.0238), and LW to MW (p-value 0.0087). Furthermore, a trend was noticed on 15d pregnant between NW and MW (p-value 0.0897) and LW and MW (p-value 0.0933). All other days were not statistically significant (p-value 0.8080).

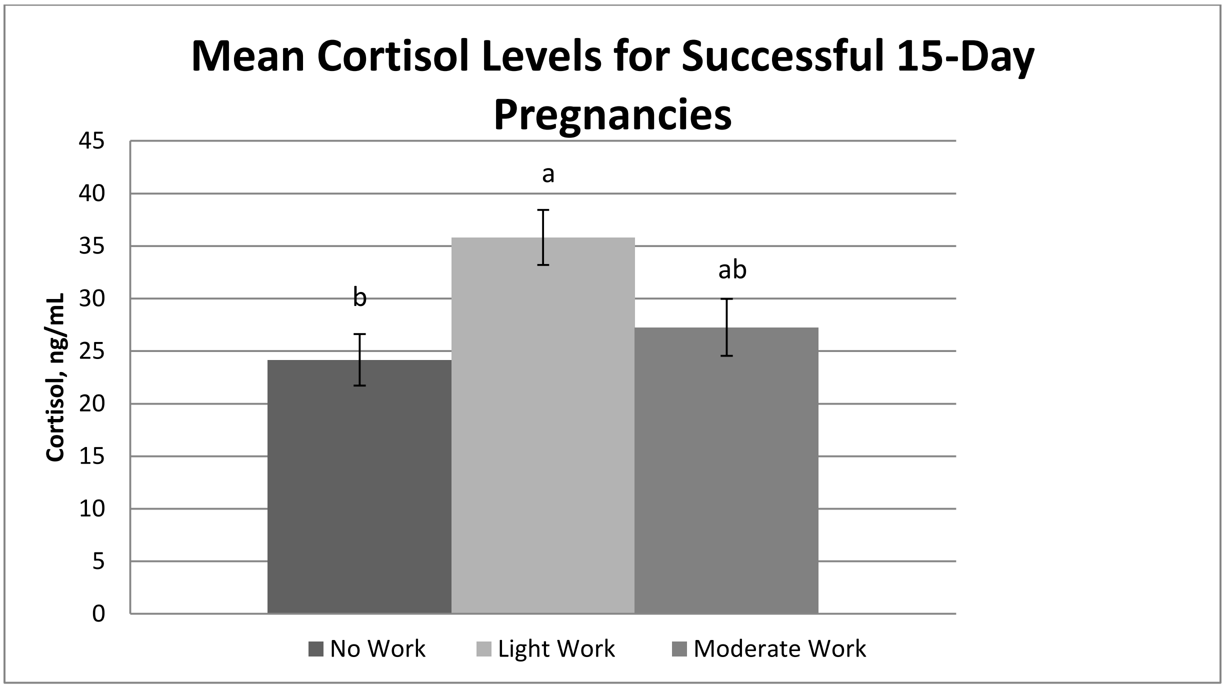

Figure 10. Mean (±SE) plasma cortisol concentrations in mares among the groups for 15d pregnancies. Different letters indicate means are statistically significant (p-value < 0.05 to 0.0001).

ANOVA Mean Plasma Cortisol Levels for Successful 15-Day Pregnancies

Source	**DF**	**Sums of Squares**	**Mean Square**	**F Ratio**
Model	2	1435.5422	717.771	5.3127
Error	57	7700.9599	135.105	**P-Value**
C. Total	59	9136.5021		0.0077

Table 10. ANOVA Mean Plasma Cortisol Levels for Successful 15-Day Pregnancies.

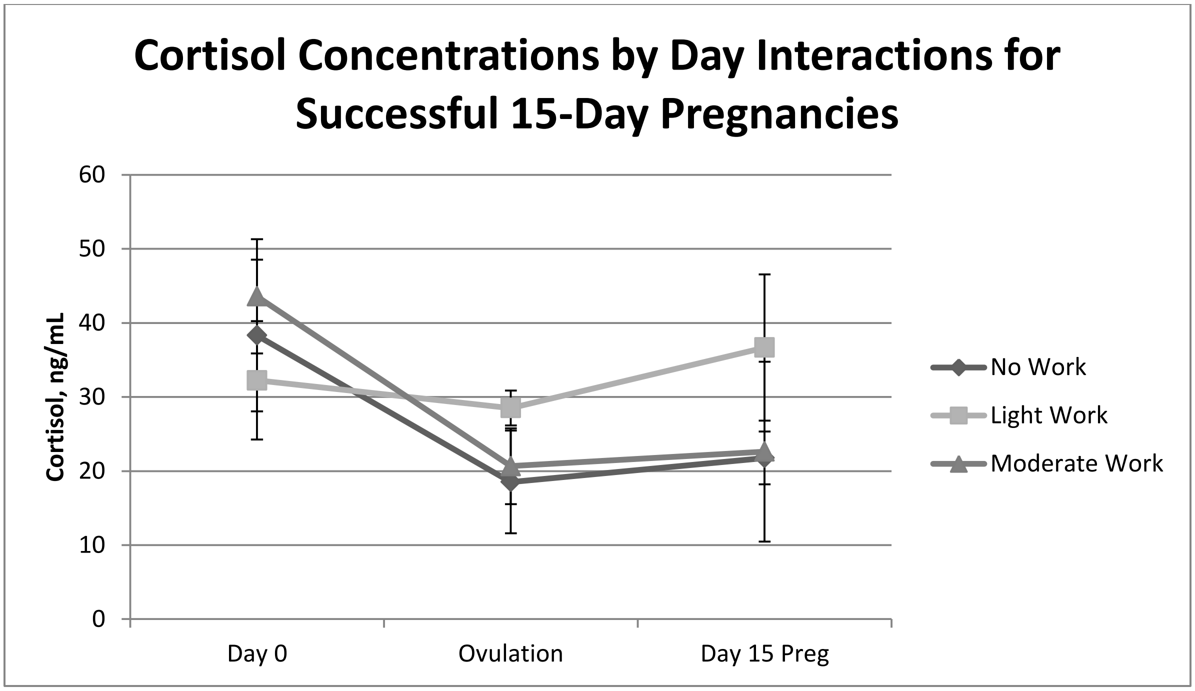

Figure 11. Mean (±SE) daily cortisol concentrations during the study for mares successfully 15d pregnant in either one of three groups: no work, light work or moderate work.

ANOVA Cortisol Concentrations by Day Interactions for Successful 15-Day Pregnancies

	Source	DF	Sum of Squares	Mean Square	F Ratio
0 d	Model	1	41.5014	41.501	0.1907
	Error	7	1523.1814	217.597	**P-Value**
	C. Total	8	1564.6828		0.6755
Ovulation	**Source**	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
	Model	1	6.89082	6.8908	0.757
	Error	7	637.10381	91.0148	**P-Value**
	C. Total	8	643.99462		0.7911
15 d Pregnant	**Source**	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
	Model	1	1.0923	1.092	0.0039
	Error	7	1962.0926	280.299	**P-Value**
	C. Total	8	1963.1849		0.952

Table 11. ANOVA Cortisol Concentrations by Day Interactions for Successful 15-Day Pregnancies.

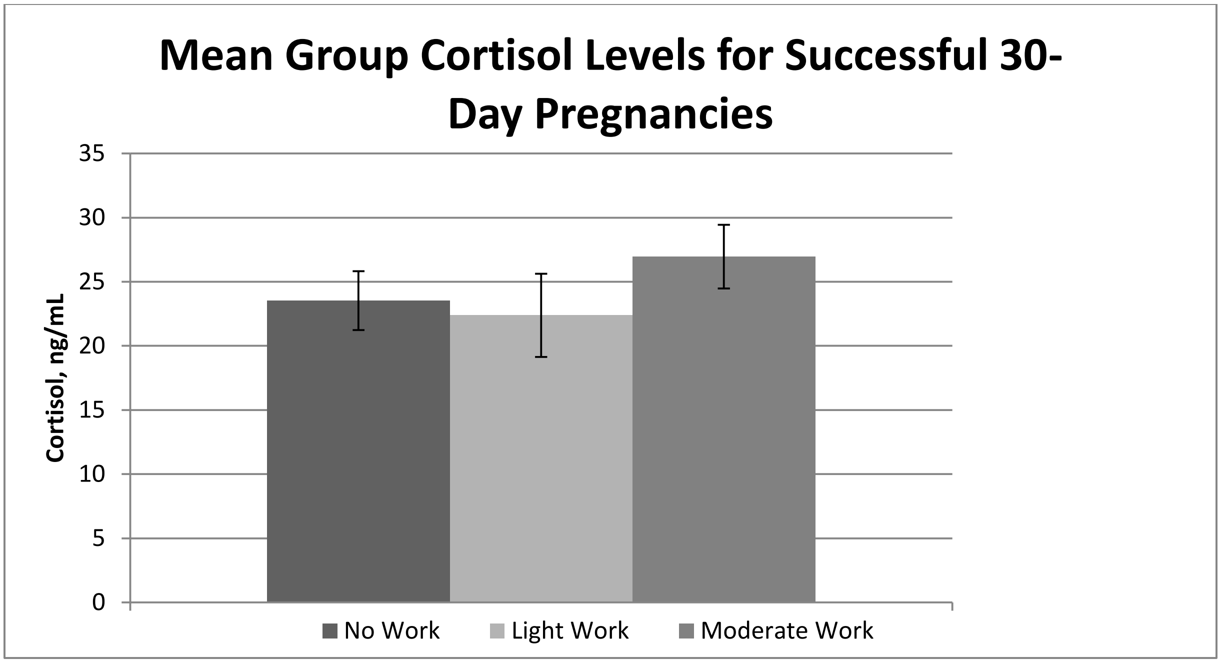

Figure 12. Mean (±SE) plasma cortisol concentrations in mares among the groups for 30d pregnancies.

ANOVA Mean Treatment Group Cortisol Levels for Successful 30-Day Pregnancies

Source	**DF**	**Sums of Squares**	**Mean Square**	**F Ratio**
Model	2	223.3625	111.681	0.8559
Error	53	6915.9341	130.489	**P-Value**
C. Total	55	7139.2965		0.4307

Table 12. ANOVA Mean Treatment Group Cortisol Levels for Successful 30-Day Pregnancies.

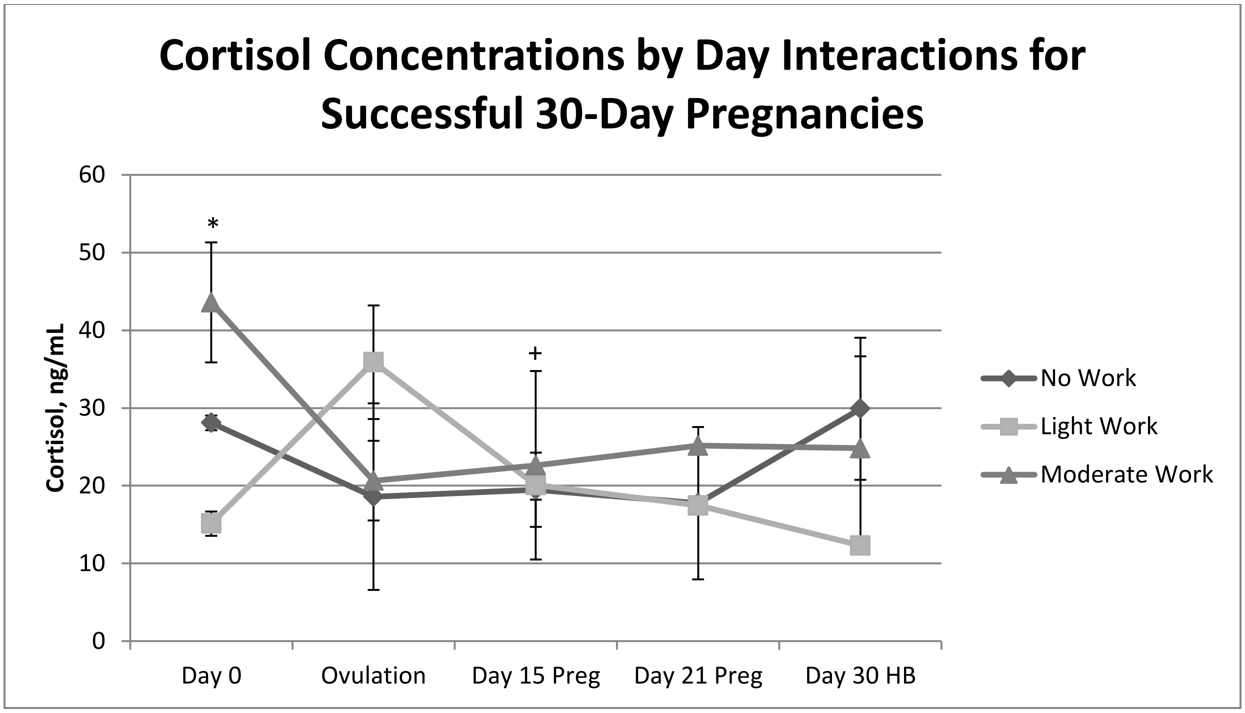

Figure 13. Mean (±SE) daily cortisol concentrations during the study for mares successfully 30d pregnant in either one of three groups: no work, light work or moderate work. (+) indicates a p-value (0.05-0.10) and (*) indicates means are statistically significant (p-value < 0.05 to 0.0001).

ANOVA Plasma Cortisol Concentrations by Day Interactions for Successful 30-Day Pregnancies

	Source	DF	Sum of Squares	Mean Square	F Ratio
0 d	Model	2	701.14578	350.573	105.2756
	Error	2	6.6601	3.33	**P-Value**
	C. Total	4	707.80588		0.0094
Ovulation	**Source**	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
	Model	2	308.08163	154.041	0.7818
	Error	2	394.04825	197.024	**P-Value**
	C. Total	4	702.12988		0.5612
15 d Pregnant	**Source**	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
	Model	2	573.32735	286.664	10.8853
	Error	2	52.67005	26.335	**P-Value**
	C. Total	4	625.9974		0.0841
21 d Pregnant	**Source**	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
	Model	2	45.71923	22.8596	0.2377
	Error	2	192.34485	96.1724	**P-Value**
	C. Total	4	238.06408		0.808
30 d HB	**Source**	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
	Model	2	503.202	251.601	3.093
	Error	2	167.2144	83.607	**P-Value**
	C. Total	4	670.416		0.294

Table 13. ANOVA Plasma Cortisol Concentrations by Day Interactions for Successful 30-Day Pregnancies.

Five-Panel DNA

A five-panel DNA test was run using approximately 50 mane hairs with roots attached and sent to the respective breed association. The results of the DNA test can be seen in Table 14. It should be noted the American Quarter Horse Association (AQHA) does not include Lethal White results when performing the test; therefore this is donated by an X on the table for horses Jazz, Holly, and Penny. Dot was a carrier for GBED; while Annie and Tracey are LWO carriers. All other results were N/N.

Results of Five Panel DNA Test

Horse	HYPP	HERDA	GBED	PSSM1	MH	LWO
Jazz	N/N	N/N	N/N	N/N	N/N	X
Dot	N/N	N/N	G/N	N/N	N/N	N/N
Annie	N/N	N/N	N/N	N/N	N/N	L/O
Tracey	N/N	N/N	N/N	N/N	N/N	L/O
Fancy	N/N	N/N	N/N	N/N	N/N	N/N
Tina	N/N	N/N	N/N	N/N	N/N	N/N
Holly	N/N	N/N	N/N	N/N	N/N	X
Freckles	N/N	N/N	N/N	N/N	N/N	N/N
Penny	N/N	N/N	N/N	N/N	N/N	X

Table 14. Results of all mares for the five panel DNA test.

CHAPTER V

Discussion

Pregnancy Parameters

This study utilized a n=9 sample for data concerning 15d pregnancies; however it should be noted not all mares were able to maintain a 30d pregnancy; therefore, data for 30 day pregnancies used a sample set of 7. Mares in all three work groups showed no statistical difference between BCS (p-value 0.7994). Even though mares came into the project with a BCS range of 5 to 8; BCS was meant to be a control and this can be seen by Figure 1 showing a BCS mean of 6 for all groups and a ±SE of 0. Figure 2 correlates an increase in BW with an increase in leptin concentration in the body; furthermore it should be noted BW is also affected by frame size and the MW group had the smallest framed mares. A BW by week interaction can be seen through 12wk among the groups (p-value <0.0001) (Figure 3). A lack of BW by week interaction from 13wk to the end of the project can be justified by mares achieving successful 30d fetal heartbeats and taken off the trial. To determine if a BW by week interaction exists further into the trial, more research is recommended with a larger sample size.

There is a lack of research done in the equid to determine if exercise affects reproductive efficiency, particularly the number of cycles per conception. When comparing group means on a successful 15d pregnancy (Figure 4), while not significant (p-value 0.6297) LW had the highest number of cycles per conception, followed by NW, and MW showing the fewest number of cycles per conception. More research is recommended to understand the relationship between exercise and reproductive efficiency, but this outcome suggest a moderate level of exercise is beneficial to reproductive efficiency in the equid while the inconsistency of light exercise could be detrimental.

The outcome for successful 30d pregnancy while not significant (p-value 0.9231) was different than successful 15d pregnancy; MW had the highest number of cycles per conception and NW and LW were equal. This can be explained by the mare in MW who was not genetically compatible with the first stallion of choice. Furthermore, it should be noted when the stallion was changed for this particular mare, she was successfully bred on the first estrous cycle. Additionally, the mare from NW and LW that was able to conceive a viable 15d pregnancy, but unable to maintain a 30d pregnancy was not included in their group's mean for 30d pregnancy and thus decreased the mean for both groups. Because of these anomalies across all three groups more research with a larger sample size is suggested to determine if exercise is directly correlated to reproductive efficiency.

Endocrine Analysis:

Leptin. Mares in the LW group possessed higher leptin levels when compared to their counterparts in both NW and MW groups at both 15d (p-value 0.0546 and 0.0018) and 30d pregnancies (p-value 0.2329 and < 0.0001). This outcome further concludes and expands the findings of Henneke et al. (1983) and Cavinder et al. (2007), in that a positive association exists between BW and leptin concentrations in the body.

Furthermore, in humans leptin has shown to increase with age especially in females when compared to male counterparts (King, 2016). While the average age of the mares on this project was 13yr; when looking at the average age of each group oldest to youngest was LW (14yr), NW (12.67yr), and MW (12.3yr). This suggests the equine model follows the human model; that leptin is directly proportional to age. It also suggests and expands the correlation from the pregnancy parameters that moderate exercise is beneficial to the mare; while the inconsistency of light exercise decreases reproductive efficiency, by increasing BW and leptin concentrations in the body. It should be noted more research is required to determine the amount of beneficial exercise which is needed to increase reproductive efficiency in the mare.

While no statistical difference was seen among the groups for a day interaction in either 15d pregnancy (p-value 0.5780) or 30d pregnancy (p-value 0.4659) it should be noted at ovulation leptin levels decreased across all three groups. This suggests a decrease in leptin levels is needed for a successful ovulation and end of the estrus stage of the cycle. Furthermore, when comparing day interactions among the groups NW and LW continued to increase in leptin levels while MW decreased leptin levels at 21d pregnant and continued this trend at 30d pregnant. This finding suggests moderate work helps maintain BW and leptin concentrations during early pregnancy in the mare, but more research is suggested to discover the association between exercise and reproductive efficiency.

Cortisol. When comparing mean cortisol levels among groups LW and NW were statistically significant (p-value 0.0069) and a trend was noticed between MW and LW (p-value 0.0638) for successful 15d pregnancies. This further enhances the findings of Kelley, et al. (2011) which stated cortisol levels rose in exercised mares as compared to their non-exercised counterparts. When comparing day to group interactions cycle 0d was statistically significant for all three groups; NW and LW (p-value 0.0349), NW and MW (p-value 0.0238), and LW to MW (p-value 0.0087). Furthermore, a trend was noticed on 15d pregnant between NW and MW (p-value 0.0897) and LW and MW (p-value 0.0933). Additionally, levels of cortisol at ovulation dropped across all three groups. This finding at ovulation enriches the findings of Asa et al. (1983) which discovered a decline in cortisol values during oestrus is needed to enhance full follicular growth along with ovulation. However, more research is needed to determine the significance of cycle 0d and 15d pregnant in the equid.

Five-Panel DNA Test

The five panel DNA test (Table 14) shows the importance of genetic testing for broodmares owners. It shows heterozygotes exist among the herd and appear phenotypically the same as their homozygous negative counterparts. These finding encourage broodmare owners to perform DNA testing and make informed breeding decisions.

CHAPTER VI

Conclusion

This study had results which were in agreement with other studies, showing the positive correlation between BW and leptin concentrations in the body. Furthermore this study concluded MW is beneficial to reproductive efficiency in the mare while the inconsistency of LW could possibly be detrimental during the breeding season. More research is needed to understand the importance of workload and its effects on reproductive efficiency. Additionally, this study suggest the equid is similar to the human model in that as age increases, leptin levels in the body also increase; however, more research is needed with a larger sample size to determine the impact age, workload, and leptin concentration have on reproductive efficiency.

Leptin levels at ovulation decreased in the mare across all 3 groups. This suggests a decrease in leptin is needed in order for proper follicular growth and ovulation at the end of estrus to occur; but more research is needed in the future to understand the impact leptin has on the mare’s reproductive cycle. Additionally, results showed a continued decrease in leptin levels for mares in the MW group. This shows MW to be beneficial in the mare’s ability to maintain BW during early pregnancy. However; more research is needed to understand the interaction between cardiovascular fitness, reproductive efficiency, BW, and leptin levels in the body.

Previous studies showed cortisol levels will rise as work load increases, this research concluded the same. Furthermore cortisol levels decreased at ovulation across 3 groups, which enhance previous findings, which stated a decrease in cortisol at ovulation is needed in the mare in order for full follicular growth and ovulation to occur during the estrous cycle.

The five-panel DNA test performed on each mare was important to determine if any carriers exist in the herd. These findings are important to enrich the herd at a later point in time and to make sound breeding choices in the future when making stallion selections.

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