EFFECTS OF STRENUOUS EXERCISE ON STALLION SPERM QUALITY

A Thesis

by

JENNIFER L. ROSENBERG

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2012

Major Subject: Animal Science

Effects of Strenuous Exercise on Stallion Sperm Quality

EFFECTS OF STRENUOUS EXERCISE ON STALLION SPERM QUALITY

A Thesis

by

JENNIFER L. ROSENBERG

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Approved by:

Chair of Committee,	Clay A Cavinder
Committee Members,	Martha M. Vogelsang
	Charles C. Love
Head of Department,	H. Russell Cross

August 2012

Major Subject: Animal Science

ABSTRACT

Effects of Strenuous Exercise on Stallion Sperm Quality.

(August 2012)

Jennifer L. Rosenberg, B.S.A., California Polytechnic State University, San Luis Obispo

Chair of Advisory Committee: Dr. Clay A. Cavinder

Some stallions are expected to perform athletically and breed contemporarily. Athletic activity has the potential, especially during the summer months, to induce thermal stress to the testes, resulting in reduced reproductive capability due to decreased sperm quality and libido. There is concern in the horse industry about what level of exercise, if any, affects the reproductive capability of a stallion. Thermal stress associated with training and exercise may impact sperm quality and the future reproductive capability of the stallion. The goal of this study was to determine the effect of strenuous exercise on stallion sperm quality. The objectives were to measure changes in body and scrotal temperatures following strenuous exercise and sperm quality following strenuous exercise.

Miniature Horse stallions (n = 7), implanted with subdermal thermosensory devices in the subcutaneous neck and scrotal tissue, were assigned to treatment group based on age and semen quality. Exercising stallions (EX; n = 3) were exercised 4 d/wk for 90 min for 12 wk, while non-exercising stallions (CN) were tied in the shade. Semen was collected from stallions for 5 consecutive days every 4 wk to evaluate semen quality (raw, 24 h and 48 h cooled). Subcutaneous scrotal (SQST), rectal (RT) and neck (NT) temperatures were recorded along with heart rate.

Spermatozoa data were normally distributed; therefore, they were subjected to parametric analysis by repeated measures (wk) using the PROC MIXED procedure (SAS v 9.1; SAS Inst. Inc., Cary, NC). Model included treatment (CN or EX), time (wk 0, 4, 8, or 12), and stallion as the subject of the repeated measures.

Compared to the CN group, EX stallions had elevated temperatures (avg RT 39.27 vs 37.07°, NT 39.77 vs 37.44°C, and SQST 34.90 vs 33.40°C; $P < 0.0001$). There was no difference in sperm quality between treatment groups ($P > 0.05$). In this study, strenuous exercise in Miniature Horse stallions, did not affect sperm quality. This suggests that anecdotal reports of reduced sperm quality in stallions in training may have other causes other than elevated scrotal and body temperature. While previous studies have illustrated that prolonged insulation of the testes reduces semen quality, strenuously exercising stallions for up to 90 min under hot and humid ambient conditions may not be harmful to spermatogenesis.

DEDICATION

This thesis is dedicated to my parents who have been there for me every step of the way. I am so fortunate to have had you guiding and encouraging me to be the best I can possibly be. A person could not ask for better people to be a part of their life. Mom, you have always been that shoulder to cry on and the drill sergeant when needed. You are my not only my mother but, also my best friend. Dad, you have shown me through your great example that no matter what happens if a person works hard and keep faith in oneself he/she will prosper in any endeavor. I love you both very much.

ACKNOWLEDGEMENTS

I would like to show appreciation to my committee chair, Dr. Cavinder, for his continued faith in a California girl, and pushing me to be my best. I am so grateful for the opportunities and experiences that I have had in the past two years.

My gratitude goes out to my committee members, Dr. Love and Dr. Vogelsang, for their guidance and support throughout the course of this research. Also, thanks go to Dr. Brinsko for his quick advice and guidance with statistical analysis.

Thanks also go to my undergraduate and graduate helpers. They were invaluable to this project and I could not have asked for a better group of people. Many thanks go to Sheila Teague for her endless help with semen analysis and Krissy Schroeder for helping with semen collection.

NOMENCLATURE

AMBT	Ambient temperture
AV	Artificial vagina
BCS	Body condition score
bpm	Heartbeat per minute
BW	Body weight
CN	Control (non-exercised) group of stallions
$COMP_{\alpha t}$	Sperm chromatin structure assay cells outside the main population
CONC	Concentration of spermatozoa
DSO	Daily sperm output
EX	Treatment (exercised) group of stallions
HR	Heart rate
HUM	Relative humidity
ITT	Intratesticular temperature
NORMCELL	Morphologically normal spermatozoa
NT	Subcutaneous neck temperature
PMOT	Progressively motile spermatozoa
RT	Rectal temperature
SCSA	Sperm Chromatin Structure Assay
SQST	Subcutaneous scrotal temperature
SST	Scrotal surface temperature
T0	Fresh semen sample, time 0

T24	24-h cooled semen sample, time 24
T48	48-h cooled semen sample, time 48
THI	Temperature heat index
TMOT	Total motility of spermatozoa
TOT	Total number of spermatozoa per ejaculate
VIAB	Viability of spermatozoa
VOL	Gel-free volume of ejaculate

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

In the horse industry, a stallion's reproductive and therefore economic value is derived primarily from his performance as an athlete (e.g. racing, cutting, jumping). A stud fee, or the price a stallion owner receives for breeding their stallion to one mare ranges from $1,000 to hundreds of thousands of dollars. Some racing breeds, including Thoroughbred, Standardbred and Quarterhorse "retire" their stallions following a racing career, to stud, or to a breeding career. In contrast, there are other performance activities, such as cutting and reining where stallions train and compete while breeding. Stallions that are both competing and training are potentially exposed to more stressful conditions, which have the potential to affect their reproductive capability through reduced sperm quality.

Exercise and the resulting increase in body temperature may negatively influence sperm quality and the quality of frozen-thawed sperm, thus interfering with reproductive success of the stallion (Janett et al., 2006). Varied modes of exercise may result in different physical, mechanical and biological stress factors in addition to the hormonal and temperature changes normally associated with human endurance exercise (Gebreegziabher et al., 2004). Some factors that might contribute to damage include changes in flow of blood, lymph and tubular fluid, hypoxia and hypercapnea, tissue metabolic changes, alterations in enzymatic activity, release of lysozymes, changing androgen synthesis, and altered gonadotropin production (Blanchard et al., 1996). Some of the functions that aid in testicular

This thesis follows the style of the Journal of Animal Science.

thermoregulation are muscular contraction of the tunica dartos muscle, a venous network called the pampiniform plexus and numerous sweat glands (Senger, 2003). Exercise and elevated ambient temperature (AMBT) have the potential to overwhelm these thermoregulatory mechanisms, elevating testicular temperature and reducing sperm quality (Freidman et al., 1991; Gebreegziabher et al., 2004). There are anecdotal isolated reports of decreased stallion fertility during periods of training and competition and therefore there is concern within the horse industry that strenuous exercise, such as occurs during training and competing, may reduce sperm quality, thereby reducing the reproductive potential of a stallion. Areas of high AMBT and humidity (HUM), such as those seen in the summers of Texas, may cause added concern for sperm quality. However, it is unclear whether exercise, AMBT during exercise period, length of exercise or other factors that may be stallion specific, lead to reduced sperm quality. It is critical to realize the practical point at which these apparatuses fail to compensate for the workload of the stallion.

Economic loss resulting from testicular dysfunction in the equine breeding industry are substantial and cause lost breeding fees, increased management costs, and destruction of valuable male genetics (Oristaglio Turner, 2007). When addressing the dual role of performance stallions, it is imperative to determine the most efficient and effective way to manage them. Stallions exercised in hot and humid conditions may experience a level of hyperthermia that the scrotum cannot recompense. Increased body temperature and resulting thermal testicular insult could potentially cause stallions to suffer severe reproductive losses. The consequence of exercise and affecting temperature must be further investigated to determine the plausible point where damage to a stallion's reproductive success might occur. Therefore, the objectives of this study, using a model of strenuous exercise were to:

1. Measure rectal, subcutaneous neck and scrotal temperatures following strenuous exercise,
2. Compare sperm quality parameters (viability, motility, morphology, etc.) of strenuously exercised and non-exercised stallions, and
3. Determine the relationship between rectal, subcutaneous neck and scrotal temperatures and semen quality.

2. LITERATURE REVIEW

2.1 Temperature Effects on Spermatogenesis

Increase in body temperature or an increased AMBT may elevate scrotal temperature and therefore negatively affect spermatogenesis (Levine et al., 1990; Freidman et al., 1991; Blanchard et al., 1996; Gebreegziabher et al., 2004; Janett et al., 2006). Elevated temperatures, especially during exercise, can have various effects in mammals including fatigue, profound fluid and electrolyte losses, impaired performance capacity, decreased sweating rate, reduced peripheral perfusion, persistent tachycardia, panting, a decrease in serum total proteins, cortisol, appetite, and semen quality, while increasing urea-N, creatininem glutamic-pyruvate transaminase, glutamic oxalo-acetic transaminase, and alkaline phosphatase (Casady et al., 1953; Hodgson et al., 1994, Marai et al., 2002). Maintenance of scrotal temperature has been shown to be of importance to successful reproduction in boars, bulls, rabbits, rats, humans and stallions (Levine et al., 1990; Desouza et al., 1994; Marai et al., 2002; Gebreegziabher et al., 2004; Kunavongkrit et al., 2005; Janett et al., 2006; Shiraishi et al., 2010).

Mammals that have their testes in a pendulous scrotum are dependent upon maintenance of a uniform testicular temperature between 2 and 6°C lower than body temperature, for normal spermatogenesis (Kastelic et al., 1996; Momen et al., 2010). It has been speculated that testicular damage as a result of increased testicular temperature include: (i) a direct effect on germ cells by alteration of their metabolism and non-disjunction of the X-Y divalent in spermatocytes, which could interfere with mitosis; (ii) secondary effects on germ cells resulting from adverse effects on biosynthesis of Sertoli cell proteins or Leydig

cell androgens; and (iii) vascular changes, such as increased shunting through arteriovenous anastomoses and alterations in vasomotion in the testes, that could result in testicular cell hypoxia and nutrient deficiency (Mieusset and Bujan, 1994). The mechanism of thermally induced testicular degeneration remains unknown but has been shown to be temporary following short-term thermal insulation with normal function returning within 60 d after the initial insult (Freidman et al., 1991).

2.1.1 Temperature Measurements

There are many factors to consider in measurement of temperature in relation to the testis. In order to maintain normal spermatogenesis, the testis is required to be regulated at a temperature lower than that of the body; however, the testes themselves have a range of temperatures (Coulter et al., 1988; Kastelic et al., 1996). Several researchers looked at scrotal surface temperature (SST) and found varying differences in temperature increase due to intensity of insulation. These measurements were taken via a probe, infrared thermography or a sensor placed on the skin (Coulter et al., 1988; Friedman et al., 1991; Bonde, 1992; Kastelic et al., 1996). The SST can differentiate over 3°C from the top to the bottom of the scrotum (Kastelic et al., 1996).

Various species have been examined with regard to testicular temperature measurement. In the ram, intratesticular temperature (ITT) was shown to be 4.8°C higher than SST at an AMBT between 24.0 and 26.6°C; this is greater than the difference between rectal temperature (RT) and ITT which is 4.2°C (Coulter et al., 1988). The ITT has been shown to increase throughout the testis with scrotal insulation (Kastelic et al., 1996). Thermistors placed in the testis or thermistor needles were used to take ITT measurements

(Coulter et al., 1988; Kastelic et al., 1996). Subcutaneous scrotal temperature (SQST) has also been compared to SST, using needle thermistor for measurement (Kastelic et al., 1996). A study, using bulls, measured all parameters (SST, ITT, SQST) and found there was a moderate to high correlation between SST and SQST, low to moderate correlation between SQST and ITT, and low correlation between SST and ITT. The temperature gradient from top to bottom was most pronounced for SST (1.6°C), smaller for SQST (0.4°C), and slightly negative for ITT (-0.1°C)(Kastelic et al., 1996). In men, a probe for recording temperature was placed into the space between the tunica vaginalis and the scrotal skin through a small incision in the skin ~10 cm above the testis (Mieusset et al., 1992), but Mawyer et al. (2011) was the first to correlate SQST and stallion sperm quality utilizing a thermal sensory device. Thermal sensor implants showed no adverse effects on testicular function. Ambient temperature increased at 22 and 30 min of exercise ($P < 0.0001$), as did RT and SQST of all stallions ($P < 0.0002$). Mean RT increased with exercise by 1.9°C and differed significantly from that of Non-Exercising stallions. Although not significant, a mean increase in SQST of 0.8°C from 0 min to 22 min was achieved (Mawyer et al., 2011).

2.1.2 Semen Quality

There are several possible indicators that could represent a negative correlation between scrotal temperature and semen quality. When insulating testes of stallions the surface area of the scrotum ranged from 35.9-38.6°C (Freidman et al., 1991; Love and Kenney, 1999; Blanchard et al., 2000). Total motility (TMOT), progressive motility (PMOT), sperm concentration (CONC), daily sperm output (DSO), DNA quality and total sperm per ejaculate (TOT) were significantly reduced in all stallions with insulated testes for

24 or 48 h (Freidman et al., 1991; Love and Kenney, 1999; Blanchard et al., 2000). Also, an increased scrotal temperature has been shown to have several different morphological effects on sperm, in stallions, decreasing the percentage of morphologically normal sperm (NORMCELL), with numbers of primary and late spermatocytes increasing with treatment as well (Freidman et al., 1991; Blanchard et al., 1996; Blanchard et al., 2000). The morphological aspect that did not seem effected by increased temperature to the testes was tail and acrosome abnormalities (Freidman et al., 1991). The percentage of morphologically normal sperm decreased significantly 15-26 d after the onset of insulation (Blanchard et al., 2000). Total ejaculate and gel-free volumes (VOL) along with pH of semen in stallions that were subjected to testicular insulation for either 24 or 48 h had no significant changes (Freidman et al., 1991), suggesting that these factors are not affected by increased scrotal temperature.

Semen quality and output seems to be affected by heat stress throughout most mammal species. Spermatogenesis in bulls has been repeatedly tested and found to be effected by insulating the testis (leading to an increased temperature of 34.8 to 36.5°C) or by prolonged exposure to AMBT above 29°C (Casady et al., 1953; Ross and Entwistle, 1979; Vogler et al., 1993; Barth and Bowman, 1994). The duration of 1 cycle of the seminiferous epithelium and epididymal passage of spermatozoa, typically 13.5 d, was not found to be affected by insulation, so the time at which the effected semen is ejaculated reflects the stage of the sperm in the testes during thermal insult (Ross and Entwistle, 1979). Semen quality decreased in motility (12 to 15 d post-thermal insult) and CONC, increased in abnormal spermatozoa (12 to 30 d), and stallions had a decreased libido (Casady et al., 1953, Ross and Entwistle, 1979; Vogler et al., 1993; Barth and Bowman, 1994). Abnormal spermatozoa

included an increase in head defects, pyriform heads, nuclear vacuoles, microcephalic sperm midpiece defects and droplets (Barth and Bowman, 1994; Kastelic et al., 1996). Spermatozoa that were in the epididymis or acrosome phase during insulation were most affected (Kastelic et al., 1996). Scrotal insulation did not seem to effect elongated spermatids and epididymal spermatozoa (Ross and Entwistle, 1979). Results from conventionally cryopreserved semen on thermo-insulted bulls showed that viability (VIAB), motility and acrosomal integrity of spermatozoa in the epididymis during insult was affected adversely (Vogler et al., 1993). There were contradictory results in sperm CONC, DSO and VOL (Casady et al., 1953; Vogler et al., 1993). One study demonstrated the possible effect of breed in heat tolerance for bulls. Scrotal insulation applied to 2 Holstein-Friesian and 2 Belgian Blue bulls for 48 h decreased motility in both breeds but Belgian Blue PMOT was significantly lower than Holstein-Friesian. The same response was seen in the analysis of VIAB, morphology (primarily being acrosome defects, pyriform-shaped heads, micro- and macro-cephalic heads), and chromatin protamine sufficient spermatozoa (Rahman et al., 2011).

Other means of increasing scrotal temperature have varying effects on semen quality. Reproductive traits in male rabbits were measured in cool (21.1°C and HUM of 0.604) and hot (32.0°C and HUM of 0.635) environments. Hot environments did not show effects on the rabbits reaction time, semen pH, sperm motility, percentages of dead sperm, sperm abnormalities and acrosomal damage due to heat stress. However, there was a significant decrease in the VOL, CONC and TOT in heat stressed rabbits (Marai et al., 2002). Boars have a decrease in sperm quality when AMBT reached 30°C or greater, causing an increase in SST, respiration rate and RT. Motility of spermatozoa was generally found to decline,

with one study concluding a decrease of 74%, but alternately there is one with no significant effect on PMOT. The average time for motility changes post-insulation was 30 d (McNitt and First, 1970; Stone, 1982; Kunavongkrit et al., 2005). The proportion of abnormal sperm was increased 3 to 5 wk post-insulation with increases in sperm with head abnormalities, proximal cytoplasmic droplets, coiled tails, tailless heads, and a decrease in sperm with non-aged acrosome droplets. Increased AMBT was also shown to negatively affect CONC but there are contradicting results in VOL (McNitt and First, 1970; Kunavongkrit et al., 2005). Some boars show higher tolerance for heat, but all boars returned to pretreatment levels 5 wk after thermal insult (Stone, 1982; Kunavongkrit et al., 2005). For normal spermatogenesis in men, the scrotal temperature should be 2.2°C lower than the intra-abdominal temperature (Momen et al., 2010). Submerging testis in a 45°C water bath for 30 min led to a decrease in semen quality in 2 of 5 men. Increasing exposure intensity by repeating the same procedure daily for up to 12 d dramatically compromised semen quality for the following 5 to 12 wk with a return of sperm density back to normal thereafter (Jung and Schuppe, 2007). Mieusset and Bujan (1994) suggested that daily mild increase in testis temperature could be a potential contraceptive method for men. Sperm concentration, motility, serum FSH and testosterone were deteriorated with the increase of scrotal temperature in 32 testicular biopsies of men with left varicoceletrated. There was a distinct correlation between generation of 4-hydroxy-2-nonenal-modified proteins and scrotal temperature, indicating a close relationship between oxidative stress and scrotal temperature (Shiraishi et al., 2010). Raising scrotal temperature by 0.8 to 1.0°C by wearing polyester scrotal supports for 52 wk did not affect spermatogenesis or sperm function in men (Wang et al., 1997). Rams showed a significantly higher mean number of dead spermatozoa when testis were heated than in the control rams

on the same day and in the heated rams before treatment. In the heated group of rams the TOT and the percentages of motile and of rapid spermatozoa were lower at 21 d of heating than in the same rams before treatment (Mieusset et al., 1992). In mice, a 20 min heat exposure at 39°C had no effect on spermatogenesis but shock heat treatment at 42°C for 30 min or greater did have an effect (Shilkina, 1976; Rockett et al., 2001; Pérez-Crespo et al., 2008). Spermatocytes present within the testis at the time of heat stress resulted in a lower CONC with reduced VIAB and low motility. Vacuoles were common in tubules, many germ cell nuclei contained highly condensed or fragmented chromatin, and some nuclei appeared to be breaking up into apoptotic bodies (Rockett et al., 2001; Pérez-Crespo et al., 2008). Spermatocytes in the early stages of meiosis are most sensitive to the action of high temperatures. Intratubular regeneration of the spermatogenic epithelium observed in mice repeatedly subjected to high AMBT, indicating possible adaptation of the sex cells (Shilkina, 1976). Heat shock effect on spermatozoa present in the epididymis at the time of thermal stress resulted in a sex ratio distortion. Sex ratio was only distorted when males were mated with females on the same day of scrotal heat treatment and resulted in fewer males (Pérez-Crespo et al., 2008).

2.2 Exercise Effects on Spermatogenesis

Aerobic exercise has been shown to improve a horse's performance capacity in the arena (Webb et al., 1988). On the other hand, the effect of this exercise on reproductive performance is less understood. In 2-yr-old stallions the libido of non-exercised horses was significantly greater than those that were exercised, although, it was not clear whether this was simply due to an increase in energy from inactivity (Dinger et al., 1986). Horses that

were exercised on a treadmill twice weekly, alternating between a walk and trot until exhaustion showed significant changes in semen parameters including acrosome defects, nuclear vacuoles in fresh semen and motility and VIAB in frozen-thawed semen. Data shows that the quality of fresh and frozen-thawed semen was negatively influenced by repeated exercise sessions over the 4 wk period (Janett et al., 2006). Several weeks after the conclusion of exercise semen quality returned to normal (Janett et al., 2006).

Exercise results in an increase in body temperature. Resting RT averaged 38°C for horses but after only 15 min of exercise that average increased to 41°C for all body conditions (Scott et al., 1992). The horse has a low body surface area and a large metabolic capacity which puts a great demand on the thermoregulatory system during exercise. Heat dispersion mechanisms may be overtaxed by prolonged exercise causing increased risk of thermal stress in the horse (Geor et al., 1995). This is especially prevalent in adverse ambient conditions (Geor et al., 1996, Kohn et al., 1999, Staempfli et al., 2006, Mawyer et al., 2011,). Heat is lost from the skin to the environment via convection, radiation, and sweat evaporation with the rate and extent of heat loss depending on the difference between the environment and skin (Geor et al., 1995). During the summer in central Texas, Mawyer et al. (2011) tested the effect of high AMBT and exercise on reproductive quality of Miniature Horse stallions. Stallions were exercised for 22 min and a mean increase of 1.9°C was seen in the RT, and although not significant an increase of 0.8°C was achieved in mean SQST. For these stallions there were no significant changes in fresh or cooled semen parameters as a result of exercise (Mawyer et al., 2011). In contrast, a study that included a scrotal suspensory in less adverse ambient conditions found a significant increase of 1.1°C in SST

during exercise and a negative influence in semen quality was demonstrated in these stallions (Staempfli et al., 2006).

The same range of effects can be seen in men. Human males under high endurance training were found to have a significant reduction in the TMOT, CONC, NORMCELL and an increased number of immature spermatozoids and round cells compared to moderate to low mileage runners (Desouza et al., 1994, Vaamonde et al., 2006). High mileage runners were also found to have a reduced *in vitro* sperm penetration of standard cervical mucus, suggesting compromised fertility (Desouza et al., 1994). It has been indicated that high endurance exercise did not alter reproductive hormones including gonadotropins and cortisol levels (Lucía et al., 1996). Others show a significant increase in dehydroepiandrosterone sulfate and thyroxine while experiencing a significant decrease in FSH, LH, cortisol, prolactin, with no significant changes found in the levels of testosterone, estradiol, progesterone, and TSH (Vaamonde et al., 2006). Normal sperm morphology also shows contrasting results. It is speculated that AMBT may contribute to the change in normal morphology since studies done at lower ambient temperatures did not show significance (Gebreegziabher et al., 2004).

When the air is hot, the thermal gradient for heat loss is reduced so that the rate of heat accumulation by the body is increased and fatigue, or an inability to exercise voluntarily, occurs sooner than when exercising in cool conditions (Lindinger, 1999). A running horse uses a greater proportion of its body mass for locomotion than does a human who is running or cycling, so horses have a greater rate of heat production per unit of body mass (Lindinger, 1999). Work capacity is reduced in human athletes when introduced to a hot environment but within a few days, the body acclimates to the increased AMBT and has an improved

ability to exercise. Numerous studies have found that exercise trained human subjects are better able to dissipate the thermal load of exercise than their untrained counterpart. In general, the primary adaptive responses to heat acclimation are an improved cardiovascular capacity and an increase in sweat rate (Geor et al., 1996).

2.3 Stress and Temperature

It is not clear whether it is stress or the inability of a male to adequately thermoregulate the testes that is the cause of the specific abnormalities in the testis (Vogler et al., 1993). Bulls treated with dexamethasone or scrotal insulation, appeared to have a greater increase in sperm nuclear abnormalities, including pyriform heads, nuclear vaculation, microcephalic heads, and abnormal DNA condensation when insulated, even though the duration of insulation was shorter than the duration of dexamethasone treatment. The more severe effect of insulation may be due to a combination of tissue anoxia and depression of testosterone secretion, rather than the depression of testosterone secretion alone expected with the dexamethasone treatment. Both anoxia and low testosterone may interfere with the same cellular metabolic processes, leading to similar outcomes in defects of the cells affected during spermatid metamorphosis (Barth and Bowman, 1994). Dexamethasone treatments appeared to cause an earlier and more severe effect on epididymal sperm than did insulation. Although distal midpiece reflexes and cytoplasmic droplet retention occurred 10 to 12 d after insulation, apparently as a result of low testosterone, there also appears to be a direct effect of heat on epididymal spermatozoa resulting in an increased proportion of dead spermatozoa and detached heads by d 8 after insulation. The increase in the eosin stain ("dead sperm"), indicating loss of cell membrane integrity, was coincident with the increase in the numbers of

sperm with detached heads. This suggests that head detachment is directly related to loss of cell membrane integrity leading to cell breakdown (Barth and Bowman, 1994).

2.4 Fertility

Testicular degeneration is a common cause of subfertility and infertility in stallions (Oristaglio Turner, 2007). Evaluations of ejaculates of semen from stallions and records from 2 consecutive breeding seasons were collected and analyzed for sperm characteristics and the correlation with fertility (Jasko et al., 1992). Stallions with lower fertility than the mean overall seasonal fertility had significantly lower mean values for subjective appraisal of the TMOT and PMOT, velocity of motile spermatozoa and NORMCELL which coincided with the computer-aided movement and analysis. On the basis of evaluation of a single ejaculate for each stallion, the variation in these characteristics only account for approximately 20% of the observed variation in fertility rate (Jasko et al., 1992).

Love and Kenney (1998) determined the relationship between fertility and susceptibility of sperm DNA to denaturation in 84 actively breeding, clinically fertile stallions. The 3 measures of sperm chromatin structure analysis (SCSA), meanα_t, SDα_t, and percentage of cells outside the main population (COMPα_t), were negatively correlated, in varying degrees, with fertility variables. The percentage of COMPα_t was the measure most highly correlated (negatively) with fertility, as represented by the percentage pregnancies per cycle and the percentage pregnant mares per first cycle. Meanα_t and SDα_t were not as highly negatively correlated, with meanα_t being the parameter having the lowest significant correlation with any of the fertility variables (Love and Kenney, 1998). Most (74%) of the stallions had a percentage pregnant per first cycle between 35 and 100%, an SDα_t between 60 and 100 and a percent COMPα_t ranging from 5-25. All 6 stallions with a 100% pregnant per

first cycle had a $SD\alpha_t$ of less than 90 and a $COMP\alpha_t$ of less than 15% (Love and Kenney, 1998).

Reduced fertility in females mated with heat-stressed males can result from (i) failure in fertilization, (ii) a normal fertilization, but an increase in embryonic death, (iii) a failure in fertilization or an increase in embryonic death (Mieusset et al., 1992). In sheep the percentage of ewes still pregnant 65 d after insemination was significantly less in the heated than in the control rams for semen collected at d 4, at d 15 and at d 21 of treatment. An increase of ≈2°C in SQST for 16 h/d does not induce modification in the fertilizing capacity of the spermatozoa after 4, 15, and 21 d of treatment, but did increase in embryonic loss; at least until d 21 of heating, when the quality and, to a lesser extent, the quantity of the spermatozoa are affected. Embryonic loss was significantly increased in ewes inseminated with semen from the heated rams as early as d 4 of treatment, while there was no modification in the fertilization rate. Spermatozoa from the rams with an insulated scrotum might be more sensitive to freezing than sperm from control animals because they have 'suffered' from the effects of increased temperature (Mieusset et al., 1992). This was even more evident in 36 male mice; after a single heat shock of 43°C for 20 min, only one small litter was sired 23 to 28 d after heat shock (Rockett et al., 2001). In fertile men, an induced increase of 1 to 2°C in testicular temperature resulted in a marked depression in spermatogenesis. At least one-third of infertile men were reported to have an increase in scrotal temperature of ~0.5 to 1.5°C and this increase was associated with significant alterations in the exocrine and endocrine functions of the testis (Mieusset et al., 1992).

2.5 Time of Spermatogenesis

The lifespan of primary spermatocytes in a stallion is 19 d, secondary spermatocytes 0.7 d, spermatids with round nuclei 8.7 d and spermatids with elongated nuclei 10.1 d and the mean extra-gonadal transit time is 8 to 11 d (Swietstra et al., 1975). Barth and Bowman (1994) performed a study on bulls claiming that it appears stress, caused by dexamethasone, does not affect the rates of spermatogenesis or epididymal passage. Therefore, spermatozoa appearing in ejaculates up to 2 wk after insulation or dexamethasone treatment would have been present in the epididymis at the time of treatment, and sudden large increases in sperm defects would be attributable to abnormal epididymal function caused by treatment (Barth and Bowman, 1994). A possible explanation for the type, occurrence, and duration of appearance for each specific abnormality may reside in the stage of spermiogenesis that those cells were in at the time of scrotal insulation (Vogler et al., 1993; Barth and Bowman, 1994) . The earliest effect of heat increase seems to be on the epididymal spermatozoa. Epididymal maturation of the spermatozoa could be affected by raised temperature and embryo mortality is known to be increased in fertilization with immature, epididymal spermatozoa (Mieusset et al., 1992). In insulated stallion testes, a decrease in TMOT and PMOT was noticed on d 1 after treatment and continued to decrease until d 15 to 30. Motility values returned to normal within 75 d for all test subjects (Freidman et al., 1991; Blanchard et al., 1996). Concentration reached its low between d 25 and 45, and returned to pre-treatment values around d 70 (Freidman et al., 1991). Total sperm per ejaculate declined by d 10 and was lowest at d 25 to 35. Within 65 d TOT returned to pre-treatment conditions (Freidman et al., 1991).

The time at which abnormal spermatozoa appear in the ejaculate would depend to some degree on the length of storage in the cauda epididymis which, in turn, is dependent on

collection schedule (Barth and Bowman, 1994). Morphological abnormality increases seen by 10 d postinsulation, in scrotal insulated stallions, peaked around d 20 to 30. After this period the morphologies started to decrease until they reached pre-treatment values around d 52-60 (Freidman et al., 1991; Blanchard et al., 1996). The appearance of morphological abnormalities between d 25 and 40 suggests that structural irregularities induced by heat would most likely occur in cells capable of meiosis or mitosis, namely primary and secondary spermatocytes. Cells that reach the spermatid phase are less susceptible to morphological deformation. The increase in LH secretion due to the thermal injury suggests that Leydig cell function is compromised, resulting in a decrease in the negative feedback signal to the brain, which in turn allows a greater release of LH (Blanchard et al., 1996).

In a Miniature Horse, there was approximately a 50% loss in potential DSO with each germ cell type observed 24 d after insulation implying there is a prolonged adverse effect of heat on spermatogenesis, which is evident as reduced yield from both meiosis (requiring 19 d) and spermiogenesis (requiring 18.6 d)(Blanchard et al., 2000). In bulls, the data indicates that nuclear defects would most likely develop during nucleus condensation and shaping, a period of 6-14 d before spermiation and assuming 11 d for epididymal transit, 17-25 d before sperm are in a position to be ejaculated. Mitochondrial sheath defects would develop later, 1-4 d before spermiation, and spermatozoa injured at this time would require 11 to 15 d to be in a position of ejaculation (Barth and Bowman, 1994).

The amount of time to detect a difference in semen quality postinsulation could be due to thermal damage to spermatids and spermatocytes based on the time required for spermatozoa passage through the epididymis and the length of the cycle of the seminiferous epithelium (Blanchard et al., 1996). Spermatogonia, particularly round spermitids are more

resistant to the detrimental effects of heat; whereas, spermatocytes and spermatids are more susceptible (Freidman et al., 1991; Blanchard et al., 2000). Mature spermatozoa also are susceptible to increased temperatures, but are somewhat protected from its effects within the proximal portion of the excurrent duct system (Freidman et al., 1991).

2.6 Mini vs. Full-Sized Stallions

Stallions with smaller body size have lesser VOL but a difference in the CONC based on size of the animal does not exist. Thus, we know that miniature stallions with smaller testicles will have fewer TOT than is commonly accepted as normal in full-sized stallions. The average TOT of miniature stallions in 1 study was $4.94 \pm 0.22 \times 10^9$ cells, with $1.75 \pm 0.09 \times 10^9$ total normal, motile spermatozoa (Paccamonti et al., 1999). Testicular size, both length and width, were greater in Miniature Horse stallions of greater body size and smaller testicular size corresponds to reduced sperm production. Findings in Miniature Horse stallions support the suggestion of Love et al. (1991) that efficiency of sperm production (sperm produced per gram of testicular parenchyma) is consistent, regardless of testicular size or horse breed (Paccamonti et al., 1999).

2.7 Summary

It is necessary for stallions to be accomplished in their discipline to be in high demand for reproductive purposes. One of the ways these stallions achieve an extraordinary value is by successful accomplishment in their equine discipline. Because of the need to perform, the equine athletes may have to endure strenuous exercise during the breeding season (Janett et al., 2006) and there may be many adverse side effects on reproductive

function due to exercise causing increased scrotal heat (Levine et al., 1990; De Souza et al. 1994; Gebreegziabher et al. 2004; Janett et al. 2006). Some of these consequences include changes in the hormonal profile of testosterone, LH, Cortisol, estradiol and prolactin. Semen quality may also be affected and shown through a decrease in TMOT, PMOT, VOL, NORMCELL, VIAB and CONC both fresh and frozen breeding doses (Levine et al., 1990; De Souza et al. 1994; Gebreegziabher et al. 2004; Janett et al. 2006). Stallion semen now has the possibility of being cooled or frozen to allow shipping across the world for artificial insemination. It is imperative that the semen collections are at their peak condition to allow for maximal pregnancy rates. Therefore, it is necessary to further investigate the effects of exercise and temperature on viable semen production and at what point it negatively effects successful reproduction of the stallion.

3. MATERIALS AND METHODS

3.1 Stallions, Housing and Diets

Nine Miniature Horse stallions (5 to 17 y) were used for this study. The study was conducted at Texas A&M University between the months of May and August, for a total of 12 wk (Table A.1). Stallions were housed in individual 1.8 m x 1.8 m stalls and allowed free exercise in adjacent turnouts (7.3 m x 1.8 m) every other day, 30 d before the onset of the study and continuing throughout the study. Project approval was granted by the Texas A&M University Institutional Agricultural Care and Use Committee using guidelines set forth by the Federation of Animal Science Societies (1999).

Body weight (BW) of each stallion was measured at wk 0 and then every other week starting at wk 5. Body condition score (BCS) was measured according to protocol established by Henneke et al. (1983) on each stallion every other week starting at wk 3 (Table 1).

Each stallion was fed 12.5% CP coastal hay (Table B.1) in amounts according to 1.5% of BW daily, and a 16.1% CP concentrate (Table B.2) twice daily in amounts consistent with each stallion's BCS. Each stallion was allowed water *ad libitum*.

TABLE 1. Mean (± SD) body condition score (BCS) and body weight (BW) endpoints for exercised (EX) and non-exercised (CN; control) stallions

	Week 0		Week 3		Week 11	
	CN	EX	CN	EX	CN	EX
BW (kg)	89.9 ± 6.4	91.9 ± 7.2	-	-	91.9 ± 6.4	91.6 ± 7.2
BCS	-	-	5.2 ± 0.4	4.9 ± 0.5	5.7 ± 0.4	5 ± 0.5

3.2 Initial Rest/Acclimation Period

Stallions were stalled 30 d prior to the onset of the study and allowed free exercise in turn out every other d. Stallions not familiar with mounting a phantom and ejaculating into an artificial vagina (AV) were trained to the procedures. All stallions had semen collected during this time in order to familiarize each stallion with new surroundings and procedures before beginning the study.

3.3 Thermal Sensor Implants

Two subdermal sensory devices (Digital Angel Corp., St. Paul, MN) were surgically implanted subcutaneously into the neck and scrotum of stallions by a veterinary surgeon. These devices were used throughout the study to read subcutaneous temperatures of the stallions. Each device was scanned (Pocket Reader EX^{TM}, Digital Angel Corp., St. Paul, MN) before surgery to confirm proper working condition. Prior to surgery, a 10 x 10 cm area on the left side of the horse's neck was clipped. Each stallion received an intramuscular (IM) tetanus toxoid (1 mL) and an IV flunixin meglumine (1 mg/kg) to control inflammation and analgesia. Xylazine (1 mg.kg, IV) was administered as a sedative followed by ketamine (2 mg/kg, IV) to place the stallions in dorsal recumbency. Once subdued, the neck and scrotum were scrubbed with betadine and alcohol in preparation for surgery. A sterile, prepackaged telemetric probe was preloaded into a syringe with a 12-g needle. The first device was placed subcutaneously in the most ventral part of the scrotum by tenting the skin of the ventral scrotum and inserting the needle past the skin into the vaginal tunic. The plunger was deployed and the skin around the needle was tightly grasped as the needle was removed to ensure that the device did not move. The scrotum was then manually palpated to

verify correct placement. The second device was placed in the subcutaneous tissue above the trapezius muscle on the left side of the neck using a similar technique. The test scan was then repeated on the implanted devices to insure working order had not been disrupted during surgery.

3.4 Treatment Groups

In wk 0 stallion ejaculates were collected for 5 consecutive day. Since it takes 1 wk of daily seminal collections to stabilize the extragonadal reserves of sperm, only d 4 and 5 were used for analysis (Gebauer et al., 1974, Thompson et al., 2004). Stallions were analyzed and blocked by age, total number of spermatozoa per ejaculate, percent normal spermatozoa and percent progressively motile. Once blocked the horses were randomly assigned to either a non-exercised (CN, control, n = 5) or exercised (EX, treatment, n = 4) group (Table 2).

TABLE 2. Mean (± SD) values of blocking criteria used to assign stallions to non-exercising (CN) or exercising (EX) groups

	CN	EX
Age (yr)	9.4 ± 5.30	9.75 ± 3.70
Normal Cells (%)	69.13 ± 7.02	60.17 ± 7.19
PMOT	69.63 ± 13.71	75.0 ± 6.23
Total # of cells (10^9)	1671.97 ± 519.04	1853.17 ± 980.59

3.5 Exercise

Over a 12 wk period stallions were exercised in 3 wk intervals, with wk 0, 4, 8, 12 utilized for sperm collection week. Treatment stallions were exercised as a group in a 14 m

round pen 4 d/wk starting at 1100 h, while CN stallions were tied in the shade. One stallion selected at random at the beginning of each exercise bout was fitted with a Polar Equine WearLink™ heart rate monitor (Polar Electro Oy, HQ, Finland). Target heart rate (HR) during exercise bouts was 145-155 bpm. Rectal temperatures, SQST and NT were recorded in the shade prior to exercise on all stallions and every 10 min throughout exercise. If a stallion reached a RT temperature of 40˚C he was removed from exercise bouts and hand walked until RT decreased to avoid severe hyperthermia and metabolic disorder (Hodgson et al., 1994).

Ambient temperatures were recorded hourly via iButton® temperature loggers (Maxim DS1923 iButton®, Maxim Integrated Products, Inc., Sunnyvale, CA). iButtons® were placed in the shade near the stalls, where the CN stallions were tied and in the sun near the round pen. iButtons® in the sun were housed in copper toilet floats that were coated in flat black paint. This allowed for protection of the iButtons® while still allowing the influence of solar radiation on the temperature. Hourly HUM recordings were obtained and used to calculate temperature heat index (THI)(NOAA, 2011).

Each 3 wk interval had an increased exercise intensity facilitated by additional weight in EX stallions. In wk 1 to 3 of exercise stallions were exercised without weight. In wk 5 to 7 EX stallions were fitted with Targhee Mini Donkey pack saddles® (Get Your Goat Gear®, St. Anthony, ID) weighing 2.7 kg. In wk 9 to 11 of exercise, weight (6.8 to 11.34 kg) was added to the packs at 10% of BW to stimulate the effects of carrying a rider.

TABLE 3. Exercise protocol for exercising stallions (EX) 4 d/wk

Time (min)	Pace	
0	Stand (in shade)	Temperature Recording
10	Extended trot	Temperature Recording
20	Extended trot	Temperature Recording
25	Stand	
30	Extended trot	Temperature Recording
35	Stand	
40	Extended trot	Temperature Recording
45	Stand	
50	Extended trot	Temperature Recording
55	Stand	
60	Extended trot	Temperature Recording
65	Stand	
70	Extended trot	Temperature Recording
75	Stand	
80	Extended trot	Temperature Recording
85	Stand	
90	Extended trot	Temperature Recording
95	Stand	
100	Stand (recovery)	Temperature Recording
110	Stand (recovery)	Temperature Recording
120	Stand (recovery)	Temperature Recording

3.6 Semen Collection

At wk 0, 4, 8, and 12 of the study, semen was collected from each stallion for quality assessment, and wk 0 was used as a baseline for the study. Ejaculates were collected in a 33 cm Missouri-model AV (Nasco, Ft. Atkinson, WI) at the Texas A&M University Horse Center Breeding Farm facilities. Depending on stallion preference, each AV was filled with water between the temperatures of 50.0 to 57.0°C. A sterilized liner (Playtex® Drop-Ins®, Neenah, WI) was placed in the collection bottle and a micromesh semen filter was placed in

the liner to separate the debris and gel fraction from the semen sample. All collection materials were kept in a 37˚C incubator until time of use. Semen samples were immediately taken to the laboratory for processing upon collection.

3.7 Semen Processing

Semen processing took place in the Texas A&M University Horse Center Laboratory. Gel-free volume of each ejaculate was measured using 10-mL graduated cylinder. Two 0.5-mL aliquots of raw semen were prepared, one was placed in a 16°C cooler (Koolatron™ PC3, Brantford, Ontario) until analysis of CONC and VIAB. The cooler served a dual purpose, first to cool the semen since it has been found that maintaining semen at room temperature (20°C) can reduce the metabolic rate of spermatozoa, and second, reduce the rate of decline in VIAB and motility while protecting the spermatozoa from harmful ultraviolet light (Davies Morel, 2008). The second sample was immediately frozen in liquid nitrogen and maintained at -80°C for assessment of DNA quality using the SCSA. Raw semen was diluted in buffered formol saline for morphological analysis. A commercial semen extender (INRA-96, Breeder's Choice, Aubrey, TX) was used to dilute three 1.5-mL aliquots of raw semen in a 1:4 dilution. Two aliquots were stored in separate cooling containers (Equitainer-11, Hamilton-Thorne Biosciences, Beverly, MA); one container contents was analyzed after 24 h and the other after 48 h of storage (T24 and T48 samples, respectively). One of the aliquots was placed in the 16°C cooler for immediate analysis of motion characteristics (T0 sample). All materials used to process ejaculates were kept in an incubator until used. The 16°C cooler was transported to the Theriogenology Laboratory for analysis within 30 min of collection. The equipment for semen analysis was located at the Texas A&M School of

Veterinary Medicine Theriogenology Laboratory approximately 2 km from the Texas A&M Horse Center.

3.8 Semen Analysis

Raw semen was analyzed first for fluorescence-based spermatozoa CONC and VIAB (NucleoCounter SP-100; ChemoMetec, Allerød, Denmark). A computer-assisted spermatozoa motion analysis (CASMA, IVOS version 12.2 L, Hamilton-Thorne Biosciences, Beverly, MA) was used for analysis of TMOT and PMOT spermatozoa. The IVOS system settings were; frames acquired-45; frame rate-60 Hz; minimum contrast-70; minimum cell size-4 pixels; minimum static contrast-30; straightness threshold for progressive motility-50%; average path velocity (VAP) threshold for PMOT-30 μ/sec, VAP threshold for static cells <15 μ/sec; cell intensity-106; static head size-0.06 to 2.00; static head intensity, -0.20 to 2.01; static elongation-40 to 85; LED illumination intensity-2200. Using calculations from CONC, each sample was diluted with INRA-96® extender for a concentration of 25 to 30 x 10^6 spermatozoa mL. Samples were then incubated for 15 min to obtain optimum possible motility (Jasko et al., 1992). Six μL of extended semen were loaded into warmed (37°C) analysis chambers (fixed height of 20 μm) affixed to microscope slides (Leja Standard Count 2 Chamber slides; Leja Products, B.V. Nieuw-Vennep, The Netherlands). Slides were then loaded into the CASMA instrument and 10 microscopic fields or a minimum of 500 spermatozoa were analyzed per sample.

One to 2-μL of previously prepared semen samples in 10% buffered saline were placed on a microscope slide with a coverslip. They were evaluated using wet mount preparation and differential interference contrast microscopy (Olympus BX60, Olympus

America, Inc., Melville, NY, 1250X magnification). One hundred cells were counted from each sample, identifying the NORMCELL along with abnormalities including, abnormal heads, acrosomes, midpieces, detached heads, bent tails, coiled tails, and premature germ cells (Kenney et al., 1983).

Quality of DNA was assessed using the SCSA as previously described (Love and Kenney, 1998). Semen samples were thawed in a 37°C water bath and aliquots (about 5 µL) were mixed with 195 µL of a TNE buffer solution (500 mL mixture of 0.79 g Trizma HCl, 4.38 g NaCl, and 0.186 g EDTA), which was then combined with 400 µL of low pH (~1.2) Triton X-100 detergent solution (500 mL mixture of 4.38 g NaCl, 20 mL 2N HCl, and 0.5 mL triton-X degetergent) for 30 sec. A solution of the heterochromatic dye, acridine orange, was added (1.2 mL at 4.0 µg/mL) to the sample and processed within 30 sec on a flow cytometer (FACScan, Becton Dickinson, Mountain View, CA). Quantification of DNA denaturation in each cell was determined by the term alpha-t (αt), defined by the ratio of red/(red + green fluorescence). Spermatozoa containing more normal double-stranded configuration of their DNA will emit more green than red fluorescence. The percentage of cells outside the main population (COMPα_t) was determined by selecting those cells to the right of the main population.

Samples stored for 24 h (T24) and 48 h (T48) were evaluated for CONC, VIAB, PMOT, TMOT, and DNA quality at their respective times.

3.9 Statistical Analysis

Of the 9 stallions that were used in the study, data shows 7 stallions used for statistical analysis due to complications. Stallion 1(Bluey) of CN group, originally part of

CN group, was excluded due to an infection and resulting fever for several days. Stallion 2 (Peppy), of EX group, was excluded due to developing anhidrosis during the study and inability to stay in the exercise protocol due to overheating.

Semenal data were normally distributed; therefore, they were subjected to parametric analysis by repeated measures in time using PROC MIXED (SAS v 9.1; SAS Inst. Inc., Cary, NC). The model included groups (CN or Ex), wk (0, 4, 8 or 12), semen collection sample (T0, T24, and T48), and all interactions, with stallion as the subject of the repeated measures.

Temperature data were analyzed using a nonparametric test, 2 way repeated measures ANOVA with all pairwise multiple comparison procedures using Tukey Test. No assumptions based on distribution of the data were made, as the data were not normally distributed. A Spearman Rank Correlation test was performed to determine the relationship between RT, NT, SQST, and THI during exercise. Values with a P-value of ≥ 0.05 were considered significantly different.

4. RESULTS

4.1 Ambient Temperature, Humidity and Temperature Heat Index

Mean AMBT, in the sun and shade and THI in the shade showed a significant increase ($P < 0.001$) during each h of exercise, for the entirety of the study (11:00, 12:00, 13:00)(Figure 1). Mean THI in the sun significantly increased from 11:00 to 12:00 ($P < 0.012$) but did not show a significant increase from 12:00 to 13:00. A significant difference ($P < 0.001$) in AMBT between wk was observed with wk 3 being significantly less than most wk, and wk 9 being significantly higher than most weeks (Figure 2). However, there was no significant difference in THI between wk (Figure 2). Mean AMBT, HUM, and THI during each h of exercise can be viewed in Table 4.

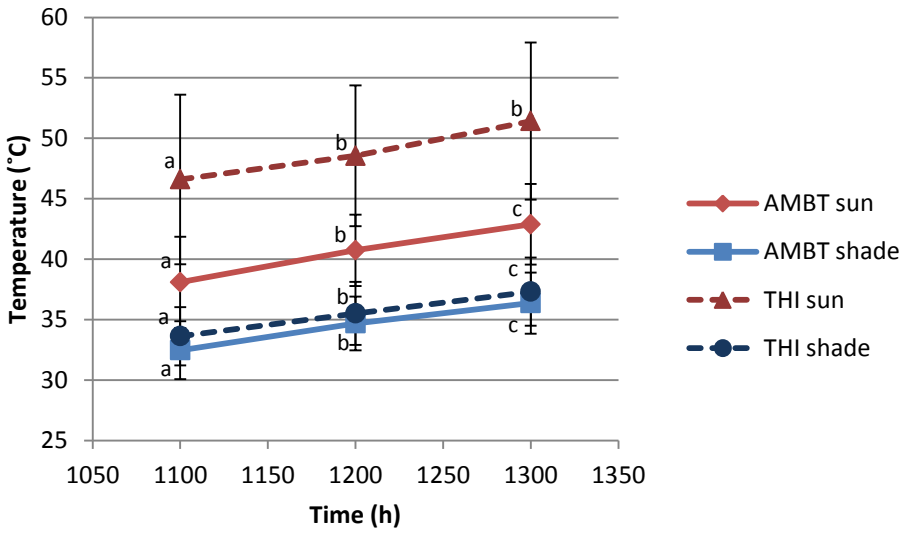


Figure 1. Mean (± SD) ambient temperature (AMBT) and temperature heat index (THI) in the shade and in the sun during exercise

[a,b,c]Means not containing the same superscript indicates a significant increase from other time points along the same line

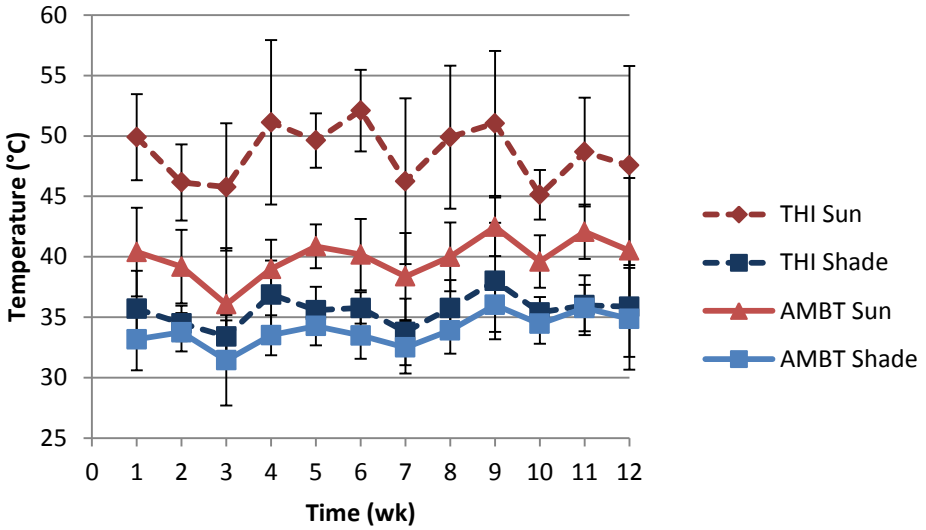


Figure 2. Mean (± SD) ambient temperature (AMBT) and temperature heat index (THI) in the shade and in the sun during exercise over the 12 wk of the exercise

TABLE 4. Ambient temperature (AMBT), humidity (HUM) and temperature heat index (THI) in the sun and in the shade during exercise

	AMBT (˚C)		THI (˚C)		HUM (%)
Time (h)	Sun	Shade	Sun	Shade	
1100	38.08 ± 3.77	32.46 ± 2.39	46.60 ± 7.02	33.62 ± 2.40	47.78 ± 11.01
1200	40.73 ± 2.96*	34.68 ± 2.22*	48.55 ± 5.82*	35.51 ± 2.60*	41.83 ± 10.21
1300	42.88 ± 3.33*	36.36 ± 2.52*	51.42 ± 6.49	37.31 ± 2.82*	38.45 ± 9.25

* indicates a significant increase from previous time point ($P < 0.013$)

4.2 Stallion Temperature and Heart Rate During Exercise

Heart rate for EX stallions, was significantly increased form 0 to 90 min (54 bpm vs. 128 bpm, respectively; $P < 0.0001$). The highest mean value was 141 bpm. Mean HR of individual stallions are shown in Appendix Figure C.1.

Mean SQST was significantly increased ($P < 0.0001$) by exercise with the mean temperature of EX being 34.90°C ± 0.48 and CN being 33.40°C ± 0.42 (Table 5). The mean increase in SQST is therefore 1.5°C. Rectal temperature for EX and CN was significantly increased (39.27°C ± 0.27 vs. 37.07°C ± 0.25, respectively; $P < 0.0001$; Table 6). The mean difference in RT was 2.20°C. Mean NT were also found to be significantly increased (39.72 ± 0.17 vs. 37.43 ± 0.27 for EX and CN, respectively; $P < 0.001$; Table 7). Temperature parameters for EX and CN stallions during exercise are summarized in Figure 3 and 4. Individual stallion variations in temperature can be seen in Appendix Figures D.1 and D.2.

TABLE 5. Mean (± SD) subcutaneous scrotal temperature (SQST) for non-exercising (CN) and exercising (EX) stallions during exercise bouts

	SQST	
Time	CN	EX
0	33.24 ± 0.51[ad]	32.95 ± 0.60[ad]
10	33.39 ± 0.54[ad]	34.62 ± 1.03[be]
20	33.37 ± 0.56[ad]	35.32 ± 0.84[ce]
30	33.36 ± 0.71[ad]	34.99 ± 0.97[ce]
40	33.44 ± 0.64[ad]	35.27 ± 0.95[ce]
50	33.39 ± 0.63[ad]	35.16 ± 0.86[ce]
60	33.40 ± 0.64[ad]	35.33 ± 0.87[ce]
70	33.37 ± 0.58[ad]	35.35 ± 0.84[ce]
80	33.37 ± 0.63[ad]	35.38 ± 0.91[ce]
90	33.51 ± 0.55[ad]	35.38 ± 0.83[ce]
100	33.48 ± 0.55[ad]	34.80 ± 0.91[ce]
110	33.46 ± 0.60[ad]	34.59 ± 0.90[be]
120	33.45 ± 0.56[ad]	34.66 ± 0.87[be]

[a,b,c]Means not sharing the same superscript within a column are different ($P < 0.001$)
[d,e]Means not sharing the same superscript with a row are different ($P < 0.001$)

TABLE 6. Mean (± SD) rectal temperature (RT) for non-exercising (CN) and exercising (EX) stallions during exercise bouts

	RT	
Time	CN	EX
0	37.21 ± 0.38[ad]	37.27 ± 0.39[ad]
10	37.16 ± 0.42[ad]	38.22 ± 0.47[ce]
20	37.15 ± 0.46[ad]	38.89 ± 0.55[be]
30	37.15 ± 0.42[ad]	39.11 ± 0.57[be]
40	37.11 ± 0.49[ad]	39.24 ± 0.55[be]
50	37.16 ± 0.39[ad]	39.28 ± 0.54[be]
60	37.15 ± 0.37[ad]	39.36 ± 0.48[be]
70	37.12 ± 0.40[ad]	39.41 ± 0.46[be]
80	37.15 ± 0.42[ad]	39.45 ± 0.48[be]
90	37.15 ± 0.41[ad]	39.49 ± 0.50[be]
100	37.19 ± 0.38[ad]	39.25 ± 0.52[be]
110	37.19 ± 0.38[ad]	38.99 ± 0.47[be]
120	37.18 ± 0.39[ad]	38.70 ± 0.51[ce]

[a,b,c]Means not sharing the same superscript within a column are different ($P < 0.001$)
[d,e,]Means not sharing the same superscript with a row are different ($P < 0.001$)

TABLE 7. Mean (± SD) subcutaneous neck temperature (NT) for non-exercising (CN) and exercising (EX) stallions during exercise bouts

	NT	
Time	CN	EX
0	37.21 ± 0.38[ad]	37.27 ± 0.39[ad]
10	37.16 ± 0.42[ad]	38.22 ± 0.47[be]
20	37.15 ± 0.46[ad]	38.89 ± 0.55[be]
30	37.16 ± 0.42[ad]	39.11 ± 0.57[be]
40	37.11 ± 0.49[ad]	39.24 ± 0.55[be]
50	37.16 ± 0.39[ad]	39.28 ± 0.54[be]
60	37.15 ± 0.37[ad]	39.35 ± 0.48[be]
70	37.12 ± 0.40[ad]	39.41 ± 0.46[be]
80	37.15 ± 0.42[ad]	39.45 ± 0.48[be]
90	37.15 ± 0.41[ad]	39.50 ± 0.50[be]
100	37.19 ± 0.38[ad]	39.25 ± 0.52[be]
110	37.19 ± 0.38[ad]	38.98 ± 0.47[be]
120	37.18 ± 0.39[ad]	38.70 ± 0.51[be]

[a,b,c]Means not sharing the same superscript within a column are different ($P < 0.001$)
[d,e]Means not sharing the same superscript with a row are different ($P < 0.001$)

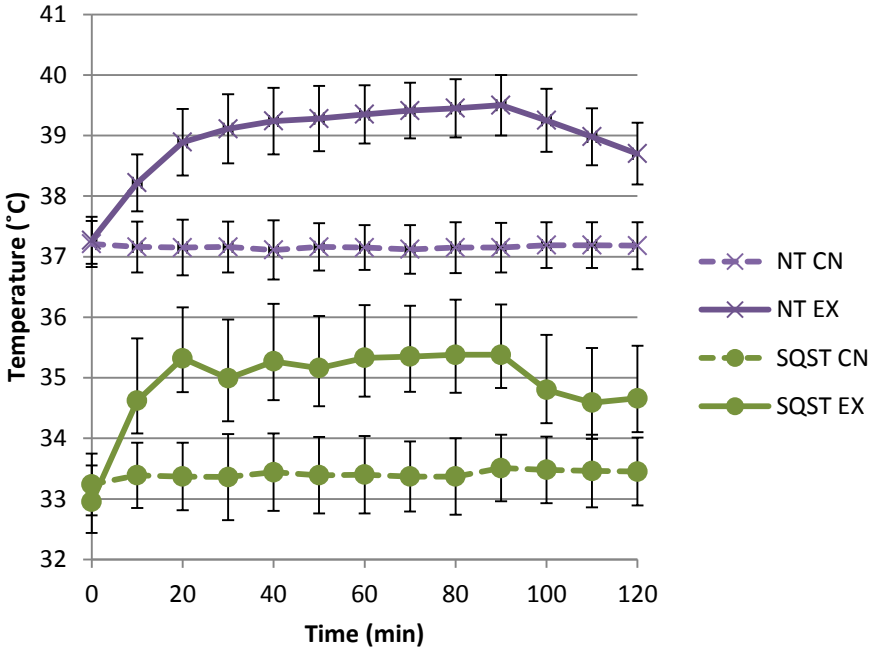


FIGURE 3. Mean (± SD) neck (NT) and subcutaneous scrotal (SQST) temperatures for exercising (EX) and non-exercising (CN) stallions during exercise period

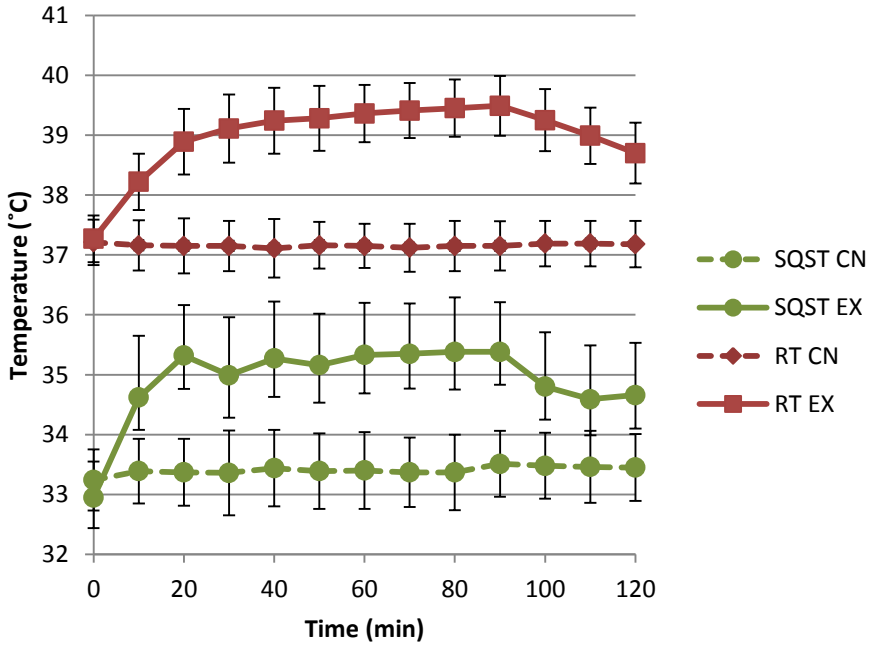


FIGURE 4. Mean (± SD) rectal (RT) and subcutaneous scrotal (SQST) temperatures for exercising (EX) and non-exercising (CN) stallions during exercise period

4.3 Temperature Correlations

Within EX stallions significant, positive correlations existed between NT, SQST, and RT ($P < 0.0001$)(Table 8 and 9). Scatterplots of EX and CN group correlations can be seen in Figure 5 and 6, respectively.

TABLE 8. Mean correlations (r_s) of subcutaneous scrotal temperature (SQST), subcutaneous neck temperature (NT), rectal temperature (RT) of exercising stallions (EX)

	EX NT	EX SQST
EX RT	0.965*	0.931*
EX NT		0.891*
SQST		

* indicates $P < 0.0001$

TABLE 9. Mean correlations (r_s) of subcutaneous scrotal temperature (SQST), subcutaneous neck temperature (NT), rectal temperature (RT) of control stallions (CN)

	CN NT	CN SQST
CN RT	0.543*	0.448*
CN NT		0.222
CN SQST		

* indicates $P < 0.007$

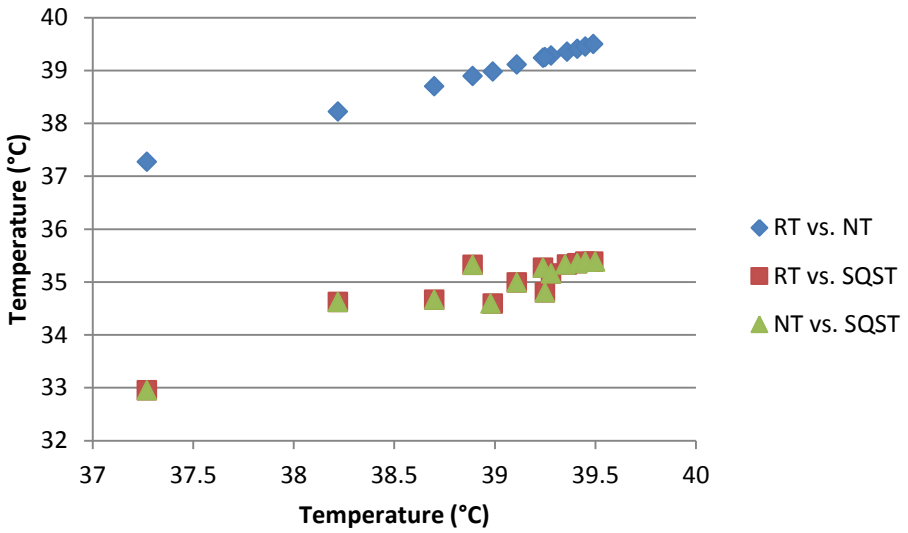


Figure 5. Mean temperature correlations of rectal (RT), subcutaneous neck (NT) and subcutaneous scrotal (SQST) for exercising stallions (EX) during exercise

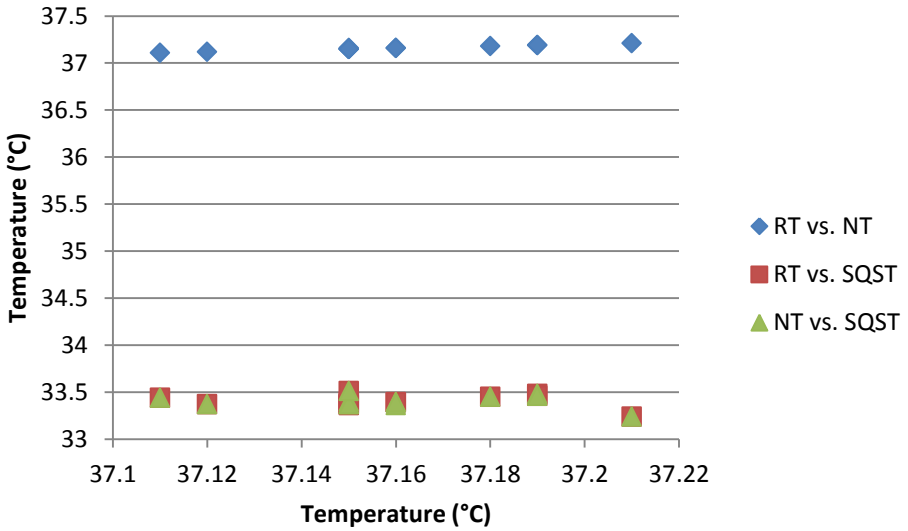


Figure 5. Mean temperature correlations of rectal (RT), subcutaneous neck (NT) and subcutaneous scrotal (SQST) for non-exercising stallions (EX) during exercise

4.4 Semen Characteristics

As previously described, the mean of the last 2 d of each week of semen collections were used to evaluate each of the semen parameters. Exercise was not found to be a

determent to semen quality ($P > 0.05$) over the time period of this study for T0, T24, and T48 (Table 8).

Exercise did not show a time by treatment effect on VOL, CONC, TOT, and NORMCELL for T0 (Appendix Figure E.1, E.2, E.3 and E.4, respectively). Both VOL and CONC were effected by week ($P = 0.0027$, $P = 0.0048$ respectively). NORMCELL decreased in both groups over the duration of the study ($P = 0.03$) with the EX group being consistently lower ($P = 0.04$) than CN, but no time by treatment effect was observed. Due to no statistical difference in NORMCELL no analysis was run on individual abnormalities. Respective morphological abnormalities between groups can be viewed in Appendix Figure G.1 to G.11. A time by treatment effect was not found on any of the parameters measured for T0, T24, T48, which included VIAB, TMOT, PMOT and $COMP_{\alpha t}$ (Appendix Figure E.5 to E.8, respectively). Sperm chromatin structure analysis $COMP_{\alpha t}$ showed a wk effect for EX and CN ($P = 0.02$) but there was no observed treatment by time effect. Variations in individual stallion semen parameters can be seen in Appendix Figures F.1 to F.8.

TABLE 10. Mean (± SD) semen parameters for all samples (fresh (T0), 24 h cooled (T24), 48 h cooled (T48)) for non-exercising (CN) and exercising (EX) stallions

	Wk 0		Wk 4		Wk 8		Wk 12	
	CN	EX	CN	EX	CN	EX	CN	EX
Volume (mL)								
T0	10.76 ± 5.85	13.22 ± 6.25	22.08 ± 10.59	22.60 ± 12.12	14.53 ± 9.65	21.80 ± 9.02	11.95 ± 1.35	22.23 ± 8.33
Concentration (million/mL)								
T0	183.93 ± 77.32	137.57 ± 32.16	106.50 ± 36.36	98.65 ± 32.31	128.59 ± 38.08	105.28 ± 40.46	147.66 ± 59.25	84.15 ± 35.63
Total Cells per Ejaculate (million)								
T0	1671.97 ± 519.04	1953.17 ± 980.59	2040.32 ± 485.36	2066.83 ± 818.24	1600.04 ± 590.84	2276.12 ± 1095.36	1743.97 ± 670.67	2035.41 ± 1462.54
Normal Morphology (%)								
T0	69.13 ± 7.02	60.17 ± 7.19	65.38 ± 4.17	56.33 ± 8.36	64.88 ± 9.23	53.67 ± 10.54	62.38 ± 5.76	50.00 ± 8.79
Viability (%)								
T0	71.00 ± 7.63	64.00 ± 6.32	70.50 ± 2.27	71.67 ± 11.54	70.38 ± 3.58	63.17 ± 11.02	74.13 ± 5.96	73.17 ± 6.08
T24	65.75 ± 9.87	71.33 ± 4.32	80.38 ± 4.21	69.50 ± 18.21	66.02 ± 22.07	76.67 ± 9.42	79.25 ± 7.34	81.67 ± 4.59
T48	71.50 ± 6.23	60.33 ± 14.31	79.88 ± 3.56	72.83 ± 13.23	71.25 ± 7.32	67.00 ± 15.77	75.38 ± 5.92	73.17 ± 2.78
Total Motility (%)								
T0	83.88 ± 7.41	83.50 ± 4.37	86.38 ± 5.53	85.50 ± 3.73	82.38 ± 5.97	84.50 ± 4.32	78.63 ± 8.68	84.33 ± 4.08
T24	77.50 ± 13.45	81.50 ± 3.51	79.63 ± 7.05	75.83 ± 9.28	76.75 ± 6.27	79.83 ± 6.34	70.25 ± 9.04	78.33 ± 5.01
T48	59.63 ± 25.43	60.17 ± 15.99	73.25 ± 6.88	66.50 ± 10.13	52.63 ± 22.33	59.67 ± 9.09	56.50 ± 19.54	56.00 ± 8.58
Progressive Motility (%)								
T0	69.63 ± 13.71	75.00 ± 6.23	71.00 ± 13.73	73.17 ± 7.86	66.25 ± 9.68	68.00 ± 7.75	68.25 ± 9.66	68.67 ± 9.37
T24	60.75 ± 17.85	71.17 ± 5.78	59.50 ± 12.26	58.00 ± 5.93	58.25 ± 10.66	55.67 ± 10.78	57.91 ± 10.91	57.83 ± 12.67
T48	42.25 ± 18.32	43.5 ± 14.32	51.25 ± 8.45	45.33 ± 5.65	33.25 ± 17.50	39.67 ± 9.61	44.75 ± 16.24	41.17 ± 10.42
SCSAcomp								
T0	6.45 ± 1.57	5.26 ± 1.40	7.96 ± 1.48	3.76 ± 0.71	6.65 ± 1.41	6.02 ± 1.54	4.29 ± 1.37	3.03 ± 0.63
T24	6.40 ± 1.93	6.10 ± 1.04	6.77 ± 1.50	6.56 ± 1.08	6.36 ± 3.01	5.61 ± 1.18	10.52 ± 2.99	9.16 ± 1.60
T48	6.62 ± 1.73	6.98 ± 2.54	5.07 ± 1.49	4.47 ± 1.86	7.09 ± 2.30	6.58 ± 0.514	8.59 ± 2.09	6.42 1.52

*No significance was observed ($P > 0.05$)

TABLE 11. Mean (± SD) of semen morphology for non-exercising (CN) and exercising (EX) stallions

	Wk 0		Wk 4		Wk 8		Wk 12	
	CN	EX	CN	EX	CN	EX	CN	EX
Normal Cell	69.13 ± 7.02	60.17 ± 7.19	65.38 ± 4.17	56.33 ± 8.36	64.88 ± 9.23	53.67 ± 10.54	62.38 ± 5.76	50.00 ± 5.79
Abnormal Head	6.00 ± 3.89	11.17 ± 2.99	18.25 ± 2.82	20.50 ± 5.28	11.88 ± 3.56	12.67 ± 6.22	16.63 ± 4.57	15.83 ± 3.66
Abnormal Acrosome	1.13 ± 1.89	0.67 ± 1.03	0.875 ± 0.64	0.83 ± 0.41	0.38 ± 0.52	0.67 ± 1.17	0.38 ± 0.74	0.33 ± 0.52
Detached Heads	2.00 ± 1.31	1.50 ± 1.38	1.00 ± 1.07	0.50 ± 0.55	1.00 ± 1.69	16.67 ± 5.20	0.75 ± 1.04	1.00 ± 0.89
Proximal Droplet	9.00 ± 6.76	10.17 ± 3.06	8.00 ± 5.66	12.5 ± 5.09	8.38 ± 6.48	13.5 ± 8.02	11.50 ± 5.18	1.00 ± 0.89
Distal Droplet	7.63 ± 7.63	10.17 ± 6.74	5.00 ± 5.32	6.00 ± 4.69	10.75 ± 9.57	5.17 ± 5.64	10.13 ± 7.83	20.00 ± 5.40
Abnormal Midpiece	4.63 ± 2.72	5.00 ± 4.38	3.5 ± 2.07	5.83 ± 3.25	4.13 ± 2.80	3.17 ± 2.79	4.25 ± 0.71	10.00 ± 4.34
Bent Midpiece	1.63 ± 1.19	5.67 ± 3.20	2.13 ± 1.73	1.83 ± 1.72	2.25 ± 1.58	2.17 ± 3.92	1.75 ± 0.71	3.17 ± 2.40
Bent Tail	1.38 ± 1.69	0.50 ± 0.84	1.75 ± 2.05	3.17 ± 3.97	1.25 ± 1.58	2.17 ± 3.92	1.75 ± 1.49	1.83 ± 3.23
Coiled Tail	1.00 ± 1.07	1.50 ± 0.84	0.875 ± 0.64	1.17 ± 1.17	0.75 ± 1.16	0.50 ± 0.84	0.25 ± 0.46	0.33 ± 0.52
Premordial Germ Cell	0.13 ± 0.35	0.17 ± 0.41	0.38 ± 0.52	0.50 ± 0.54	0.125 ± 0.35	0.00 ± 0.00	0.00 ± 0.00	0.67 ± 0.82

*****No significance was found on normal cells, statistical analysis was not performed for abnormalities

5. DISCUSSION

High rate of heat production and a relatively small skin surface area in horses lead to inefficient heat dissipation (Hodgson et al., 1994; Geor et al., 1996; Lingdinger, 1999). Horses have 50% less skin surface area per unit of body mass than humans limiting the amount of heat loss from the skin to the environment via convection, radiation, and sweat evaporation (Geor et al., 1995, Lindinger et al., 1999). This is further increased when AMBT reaches 36°C because the negligible cutaneous heat loss by radiation and convection due to the small skin surface area to environment temperature gradient (Geor et al., 1996). In the current study even the lowest mean temperature during exercise, 38.08 ± 3.77, exceeded the thermoneutral zone. In horses, the thermoneutral zone is 5 to 25°C and is defined as the temperature in which the animal does not have to expend energy to maintain body temperature (Ott, 2005). High HUM adds to the inability to dissipate heat because the water pressure gradient between the skin and the environment is small and adds to THI, which raised AMBT up to 51.42 ± 6.49 in the current study. High environmental heat decreases the length of time a horse can exercise before reaching an elevated temperature causing concern for their health (Hodgson et al., 1994; Geor et al., 1995). Within this study a significant increase in RT, NT, and SQST were seen within the first 10 min of exercise concurring with previous findings (Hodgson et al., 1994; Geor et al., 1995). Furthermore, within a 30 min recovery period EX stallions were not able to return to pre-exercise temperatures and were bathed to help facilitate thermoregulation.

All temperature parameters (NT, RT, and SQST) of EX stallions were significantly increased during exercise. In horses, intense exercise has been shown to increase core body

temperature above 41°C (Staempfli et al, 2006). When core temperatures rise above 42°C multiple negative effects can occur in the horse including CNS damage, inability to exercise, reduced sweating rate, reduced peripheral perfusion and persistent tachycardia, with some of these conditions having long term effects (Hodgson et al., 1994; Geor et al., 1995; Lindinger, 1999; Staempfli et al., 2006). In the current study, stallions were hand-walked if RT exceeded above 40°C until temperatures decreased. Rectal temperatures are slower to increase than core temperatures; therefore, core temperatures could have been closer to critical values (Lindinger, 1999). The exercise protocol for this study included 5 min bouts of exercise followed by 5 min of standing to mimic what might be experienced by some performance horses (roping and cutting horses). Moments of high intensity exercise followed by rest can lead to a decrease in airflow resulting in an increase in core temperature (Kohn et al., 1999; Staempfli et al., 2006). Even though the EX stallions were walked to avoid RT elevating above 40°C, there was still a significant difference in RT, NT, and SQST in reference to temperatures of EX stallions and CN stallions throughout exercise for the duration of the study.

Human athletes that were introduced to hot and humid environments where their work capacity was reduced were able to adapt to the environment and improve cardiovascular capacity and sweat rate within a few d (Geor et al., 1995). Adaptation to exercise can be seen in horses, though not as adequately to environment. In cutting horses, the capacity for performance was enhanced by training specifically for the work over a 6 wk period (Webb et al., 1988). The current study compensated for these possible adaptations by simulating the effects of riding via increased weight loads added in 3 wk increments to the

EX stallions. With each increment of the study stallion temperatures continued to elevate as well as HR demonstrating a lack of adaptation.

The mean increase in SQST for EX vs. CN stallions was 1.5°C, over a 110 min period, which is lower than SST in some studies finding significant effects on semen quality (Freidman et al., 1991; Blanchard et al., 1996; Blanchard et al., 2000). In bulls and rams, it was shown that ITT is higher than SST and their thermoregulatory mechanisms are better at restoring SST than the interior structures of the scrotum, SQST and ITT (Coulter et al. 1988; Kastelic et al., 1996). Simply, the ITT may maintain higher temperatures for longer than the SST. The correlation between SQST and ITT was found to be moderate, but the correlation between SST and ITT was found to be very low (Kastelic et al., 1996). Scrotal temperature in the current study does not seem as pronounced as those in other studies (Freidman et al., 1991; Blanchard et al., 1996; Blanchard et al., 2000), but SST was not measured. It could be postulated, that since SQST is more correlated to ITT than SST and ITT has been found to be higher than SST, the SST of EX stallions may have been less than what is shown by SQST. In the current study, NT, RT, and SQST were found to be greatly correlated ($r_s \geq 0.891$) when stallions were exercised but only moderately correlated in CN stallions for RT and NT ($r_s = 0.543$), with SQST moderately correlating to RT ($r_s = 0.448$) but not to NT ($r_s = 0.222$). Thus, suggesting that at lower temperature the thermoregulatory mechanisms of the scrotum are better able to compensate for changes in the body than when body temperatures are increased in the stallion.

Blanchard et al. (1996) increased the SST temperature of the testis by 3 to 3.5°C, through insulation, and found multiple effects including a decrease in PMOT and NORMCELL. Further studies report SST ranging from 35.9 to 38.6°C in testes insulated

over a 24 or 48 h period with varying effects on sperm including a reduction in TMOT, PMOT, CONC, DSO, NORMCELL, and TOT (Freidman et al., 1991; Blanchard et al., 1996; Blanchard et al., 2000). Horses that are exercised to increase scrotal temperature have not seen as great of an effect on semen quality (Dinger et al., 1986; Lange et al. 1997; Janett et al., 2006; Mawyer et al., 2011). Stallions in competition during the breeding season were found to have significantly different sperm motility but it still resided in the normal range therefore not having a negative effect on fertility (Lange et al. 1997). The greatest effect of exercise on equine reproduction was in stallions exercised on a treadmill. These stallions showed significant reduction of normal acrosome in fresh semen and motility and VIAB in frozen-thawed semen (Janett et al., 2006). Two-year-old stallions that were exercised had a lower libido than non-exercised horses but this did not significantly effect semen parameters (Dinger et al., 1986). Stallions undertaking moderate (30 min) exercise resulting in an average increase of 0.8°C in subcutaneous scrotal temperature also demonstrated no significant effects on semen quality (Mawyer et al., 2011). The current study increased the time interval of elevated scrotal temperature (about 110 min, 1.5°C) by almost 3 times that of Mawyer et al. (2011) with no significant effects on semen quality. However, there was a large variation between stallions. A small sample size possibly led to a large standard deviation in TOT and NORMCELL which may have affected the statistical outcome. This is especially prevalent in the NORMCELL which did decline within the EX stallions from wk 0 to 12 (60.17 ± 7.19 vs. 50.00 ± 8.79, respectively). The time of thermal stress in which spermatogenesis is effected in stallions is between the parameters of an increase in SQST 1.5°C within a 110 min period, having no effect, and the findings of Blanchard et al. (1996), increasing the SST 3 to 3.5°C within a 24 h period, having multiple effects on

spermatogenesis. It is impractical that a stallion would be exercised for as long as Blanchard et al. (1996) insulated stallions, but it is within the means of a performance stallion to train for 120 min/d.

Two stallions were removed from the study due to circumstances conflicting with the limits of the study. One of the stallions, who was originally part of CN, developed an infection causing a fever and was moved to the Texas A&M Veterinary Hospital for several days during the study. The increase in core temperature due to the fever may have caused an effect on semen parameters leading to inconsistent results in the study. The second stallion, who was originally part of EX, was excluded due to the development of anhidrosis and an inability to maintain exercise protocol. The mechanisms of anhidrosis are not known but it has been related to performance horses in hot, humid climates (Hubert et al., 2002). Horses with this condition typically have a higher HR and body temperature than their counterparts with little to no sweat development during exercise. Respiratory distress is also seen in these horses in the form of panting (Hubert et al., 2002). Evaporation of sweat can account for up to 65% of heat loss in horses, thus, a horse that losses the ability to sweat is unable to dissipate heat efficiently and will become overheated more quickly than other horses (Hubert et al., 2002). Though this stallion did not become completely anhidroic until the last 4 wk of the study, signs of the pending condition were prevalent within the first couple of wk. From the beginning, this stallion was in noticeably worse condition than his peers due to an elevated HR that exceeded other EX stallions and body temperatures reaching elevated temperatures at a faster rate. As the study progressed he became prone to excessive panting and reduced sweating. During the first semen collection after the start of exercise (wk 4) there was a noticeable decrease in CONC (143.80 to 102.19 million sperm/ejaculate,

respectively), TMOT (73.00 to 50.50 percent, respectively), PMOT (63.00 to 38.50 percent, respectively) and NORMCELL (32.00 to 15.50 percent, respectively). These values remained at lower levels through the remainder of the study. The mean SQST for this stallion during exercise was 35.90°C ± 0.66, which was 1°C higher than what was seen in the rest of EX stallions (34.90°C ± 0.48) and 2.5°C higher than CN stallions (33.40°C ± 0.42). Speculation could arise that higher temperatures such as a 2.5°C increase in SQST either caused by extensive exercise and high ambient temperatures or an extraneous factor such as anhidrosis could cause a decrease in semen parameters. Further research needs to be conducted to determine if temperatures higher than produced in this study would affect sperm quality and if the decrease in semen parameters seen in this 1 stallion where a result of his anhidrotic condition or of the temperature effects cause by it. Although this study did not provide an exact plausible point where damage to a stallion's reproductive success might occur, it does shed some further light in determining what that range may be and that stallions can be exercised in excessive heat without being a detrimental to sperm quality.

6. CONCLUSION

Stallions may have to endure strenuous exercise in hot, humid climates to be successful in their equine discipline. It is with accomplishments in competition that owners are able to demand high breeding fees to their stallion. The objectives of this study were to compare sperm quality parameters of EX and CN stallions in an adverse environment, and to determine the relationship between RT, NT, and SQST and semen quality. The results of this study indicate the stallion has a great ability to overcome hyperthermia which may develop in the scrotum due to exercise stress. Even though a significant increase in RT, NT, and SQST occurred, sperm parameters were not adversely affected. Though some studies have seen minimal effects on reproductive quality due exercise the results of this study indicate, trainers who are exercising horses for prolonged periods in the hot summer days may not need to be concerned about the semen quality of their stallions. An exception may be made for horses that develop a dysfunction in their thermoregulatory system, such as anhidrosis, but further research is necessary in both areas.

LITERATURE CITED

Barth, A. D., and P. A. Bowman. 1994. The sequential appearance of sperm abnormalities after scrotal insulation or dexamethasone treatment in bulls. Can. Vet. J.-Rev. 35: 93-102.

Blanchard, T. L., J. B. Jorgensen, D. D. Varner, D. W. Forrest, and J. W. Evans. 1996. Clinical observations on changes in concentrations of hormones in plasma of two stallions with thermally-induced testicular degeneration. J.Equine Vet. Sci 16: 195-201.

Blanchard, T. L., D. D. Varner, L. Johnson, J. Roser, J. Hill, C. Miller. 2000. Testicular and hormonal changes in stallions with thermally induced testicular degeneration. J. Reprod. and Fertil. Suppl. 56: 51-59.

Bonde, J. P. 1992. Semen quality in welders exposed to radiant heat. Br. J Ind. Med. 49: 5-10.

Casady, R. B., R. M. Myers, and J. E. Legates. 1953. The effect of exposure to high ambient temperature on spermatogenesis in the dairy bull. J. Dairy Sci. 36: 14-23.

Coetzee, K., T. Kruge, and C. Lombard. 1998. Predictive value of normal sperm morphology: a structured literature review. Human Reprod. Update 4: 73-82.

Coulter, G. H., P. L. Senger, and D. R. C. Bailey. 1988. Relationship of scrotal surface temperature measured by infrared thermography to subcutaneous and deep testicular temperature in the ram. J. Reprod. Fertil. 84: 417-423.

Desouza, M. J., J. C. Arce, L. S. Pescatello, H. S. Scherzer, and A. A. Luciano. 1994. Gonadal-hormones and semen quality in male runners - a volume threshold effect of endurance training. Int. J. Sports Med. 15: 383-391.

Dinger, J. E., E. E. Noiles, and T. A. Hoagland. 1986. Effect of controlled exercise on semen characteristics in two-year-old stallions. Theriogenology 25: 525-535.

Freidman, R., M. Scott, S.E. Heath, J.P. Hughes, P.F. Daels, T.Q. Tran. 1991. The effects of increase testicular temperature on spermatogenesis in the stallion. J. Reprod. Fertil. Suppl. 44: 127-134.

Gebauer, M. R., B. W. Pickett, J. L. Voss, and E. E. Swietstra. 1974. Reproductive physiology of the stallion: daily sperm output and testicular measurements. J. Am. Vet. Med. Assoc. 165: 711-713.

Gebreegziabher, Y., E. Marcos, W. McKinon, and G. Rogers. 2004. Sperm characteristics of endurance trained cyclists. Int. J. Sports Med. 25(4): 247-251.

Geor, R. J., J. L. McCutcheon, G. L. Ecker, and M. I. Lindinger. 1995. Thermal and cardiorespiratory responses of horses to submaximal excerise under hot and humid conditions. Equine Vet. J. 20: 125-132.

Geor, R. J., J. L. McCutcheon, and M. I. Lindinger. 1996. Thermoregulation in the Exercising Horse: Physiological Adaptations Associated with Heat Acclimation. The Equine Athlete 9: 14-15.

Henneke, D. R., G. D. Potter, J. L. Kreider, and B. F. Yeates. 1983. Relationship between condition score, physical measurements and body fat percentages in mares. Equine Vet. J. 15(4): 371-372.

Hodgson, D.R., R.E. Davis, F.F. McConaghy. 1994. Thermoregulation in the horse in response to excise. Brit. Vet. J. 150: 219-235.

Hubert, J.D., R.E. Beadle, G. Norwood. 2002. Equine anhidrosis. The Veterinary Clinics Equine Practice. 18: 355-369.

Janett, F. C. Burkhardt, D. Burger, I. Imboden, M. Hassig, R. Thun. 2006. Influence of repeated treadmill exercise on quality and freezability of stallion semen. Theriogenology 65: 1737-1749.

Jasko, D. J., T. V. Little, D. H. Lein, and R. H. Foote. 1992. Comparison of spermatozoal movement and semen characteristics with fertility in stallions: 64 cases (1987-1988). J. Am. Vet. Med. Assoc. 200: 979-985.

Kastelic, J. P., R. B. Cook, G. H. Coulter, and R. G. Saacke. 1996. Insultaing the scrotal neck affects semen quality and scrotal/testicular temperatures in the bull. Theriogenology 45: 935-942.

Kastelic, J. P., G. H. Coulter, and R. B. Cook. 1995. Scrotal surface, subcutaneous, intratesticular, and intraepididymal temperatures in bulls. Theriogenology 44: 147-152.

Kenney, R. M., J. P. Hurtgen, and R. Pierson. 1983. Theriogenology and the equine part II, the stallion. Theriogenology. 9: 88-90.

Kohn, C. W., K. W. Hinchcliff, and K. H. McKeever. 1999. Effect of ambient temperature and humidity on pulmonary artery temperature of exercising horses. Eq. Vet. J. 30: 404-411.

Kunavongkrit, A., A. Suriyasomboon, N. Lundeheim, T. W. Heard, and S. Einarsson. 2005. Management and sperm production of boars under differing environmental conditions. Theriogenology 63: 657-667.

Lange, J., S. Matheja, E. Klug, C. Aurich, and J. E. Aurich. 1997. Influence of training and competition on the endocrine regulation of testicular function and on semen parameters in stallions. Reprod. Dom. Anim. 32: 297-302.

Levine, R. J., R.M. Mathew, C.B. Chenault, M.H. Brown, M.E. Hurtt, K.S. Bently, K.L. Mohr, P.K. Working. 1990. Differences in the quality of semen in outdoor workers during summer and winter. N. Engl. J. Med. 323: 12-16.

Lindinger, M. I. 1999. Exercise in the heat: Thermoregulatory limitations to performance in humans and horses. Can. J. Appl. Physiol. 42(2): 152-163.

Love, C. C, M. C. Garcia, F. R. Riera, R. M. Kenney. 1991. Evaluation of measures taken by ultrasonography and caliper to estimate tesicular volume and predict daily sperm output in the stallion. J Reprod Fertil. Suppl. 44: 99-105.

Love, C. C., and R. M. Kenney. 1998. The relationship of increased susceptibility of sperm DNA to denaturation and fertility in the stallion. Theriogenology 52: 955-972.

Love, C. C., and R. M. Kenney. 1999. Scrotal heat stress induces altered sperm chromatin structure associated with a decrease in protamine disulfide bonding in the stallion. Biol. Reprod. 60: 615-620.

Lucía, A., J. L. Chicharro, M. Perez, L. Serratosa, F. Bandres, J. C. Legido. 1996. Reproductive function in male endurance athletes: sperm analysis and hormonal profile. J. Appl. Physiol. 81: 2627-2636.

Marai, I. F. R., A. A. M. Habeeb, and A. E. Gad. 2002. Reproductive traits of male rabbits as affected by climatic conditions, in the subtropical environment of Egypt. J. Anim. Sci. 75: 451-458.

Mawyer, J. D., R. K. Gordon, C. A. Cavinder, M. M. Vogelsang, D. H. Sigler, C. C. Love, S. P. Brinsko, T. L. Blanchard. S. Teague. 2011. Thermoregulation of the testicle in response to exercise and subsequent effects on seminal characteristics in stallions. J. Eq. Vet. Sci. 31: 317-317.

McNitt, J. I., and N. L. First. 1970. Effects of 72 hour heat stress on semen quality in boars. Int. J. Biometeor 14: 373-380.

Mieusset, R., and L. Bujan. 1994. The potential of mild testicular heating as a safe, effective and reversible contraceptive method among fertile men. Int. J. Androl. 17: 1884-1886.

Mieusset, R., P. I. Quintana Casares, L. G. Sanchez-Partida, S. F. Sowerbutts, J. L. Zupp, B. P. Setchell. 1992. Effects of heating the testes and epididymides of rams by scrotal insulation on fertility and embryonic mortality in ewes inseminated with frozen semen. J. Reprod. Fertil. 94.

Momen, M. N., F. B. Ananian, I. M. Fahmy, and T. Mostafa. 2010. Effect of high environmental temperature on semen parameters among fertile men. Fertil. Steril. 93: 1884-1886.

National Oceanic and Atmospheric Administration (NOAA). 2011. Hourly relative humidities recorded during the summer of 2011. Accessed April 30, 2012. http://www.noaa.com/pastweather.html.

Oristaglio Turner, R. M. 2007. Pathogenesis, diagnosis, and management of testicular degeneration in stallions. Clin. Tech. Equine Pract. 6: 278-284.

Ott, E.A. 2005. Influence of temperature stress on the energy and protein metabolism of the working horse. Livest. Prod. Sci. 92: 123-130.

Paccamonti, D. L., J. M. Buiten, J. M. Parleviet, and B. Colenbrander. 1999. Reproductive parameters of miniture stallions. Theriogenology 51: 1343-1349.

Pérez-Crespo, M., B. Pintado, and A. Gutiérrez-Adán. 2008. Scrotal heat stress effects on sperm viability, sperm DNA integrity, and the offspring sex ratio in mice. Mol. Reprod. Dev. 75: 40-47.

Rahman, M. B., L. Vandaele, T. Rigsselaere, D. Maes, M. Hoogewijs, A. Frijters, J. Noordman, A. Granados, E. Dernelle, M. Shamsuddin, J. Parrish, A. Van Soom. 2011. Scrotal insulation and its relationship to abnormal morphology, chromatin protamination and nuclear shape of spermatozoa in Holstein-Friesian and Belgian Blue bulls. Theriogenology 76: 1246-1257.

Rockett, J. C., F.L. Mapp, B.J. Garges, C.J. Luft, C. Mori, D.J. Dix. 2001. Effects of Hyperthermia on Spermatogenesis, Apoptosis, Gene Expression, and Fertility in Adult Male Mice1. Biol. Reprod. 65: 229-239.

Ross, A. D., and K. W. Entwistle. 1979. The effect of scrotal insulation on spermatozoal morphology and the rates of spermatogenesis and epididymal passage of spermatozoa in the bull. Theriogenology 11(2): 111-129.

Scott, B. D., G. D. Potter, L. W. Greene, P. S. Hargis, and J. G. Anderson. 1992. Efficacy of a fat-supplemented diet on muscle glycogen concentrations in exercising Thoroughbred horses maintained in varying body conditions. Equine Nutrition and Physiology Society 12_{th} Symposium 12: 109-113.

Senger, P. L. 2003. Pathways to Pregnancy and Parturition. Current Concepts, Inc., Pullman, WA.

Shilkina, L. A. 1976. Effect of hyperthermia on spermatogenesis in mice and role of heat training in adaptation of sex cells to high-temperatures. Bull. Exp. Biol. Med. 82: 1860-1863.

Shiraishi, K., H. Takihara, and H. Matsuyama. 2010. Elevated scrotal temperature, but not varicocele grade, reflects testicular oxidative stress-mediated apoptosis. World J. Urol. 28: 359-364.

Staempfli, S., F. Janett, D. Burger, H. Kundig, I. Imboden, M. Hassig, R. Thun. 2006. Effect of exercise and suspensory on scrotal surface temperature in the stallion. Theriogenology 66: 2120-2126.

Stone, B. A. 1982. Heat induced infertility of boars: the inter-relationship between depressed sperm output and fertility and an estimation of the critical air temperature above whic sperm output is impaired. Anim. Reprod. Sci. 4: 283-299.

Swietstra, E. E., B. W. Pickett, and M. R. Gecauer. 1975. Spermatogenesis and duration of transit of spermatozoa through the excurrent ducts of the stallion. J. Reprod. Fertil. 23: 53-57.

Thompson, J. A., C.C. Love, K.L. Stich, S.P. Brinsko, T.L. Blanchard, D.D. Varner. 2004. A Bayesian approach to prediction of stallion daily sperm output. Theriogenology 62: 1607-1617.

Vaamonde, D., M. E. Da Silva, M. S. Poblador, and J. L. Lancho. 2006. Reproductive profile of physically active men after exhaustive endurance exercise. Int. J. Sports Med. 27: 680-689.

Vogler, C. J., J. H. Bame, J. M. DeJarnette, M. L. McGilliard, and R. G. Saacke. 1993. Effects of elevated testicular temperature on morphology characteristics of ejaculated spermatozoa. Theriogenology 40: 1207-1219.

Wang, C., V. McDonald, A. Leung, L. Superlano, N. Berman, L. Hull, R.S. Swerdloff. 1997. Effect of increased scrotal temperature on spermatozoa production in normal men. Fertil. Steril. 68: 334-339.

Webb, S. P., G. D. Potter, J. W. Evans, C. A. Schwab, and B. D. Scott. 1987. Physiological responses of cutting horses to exercise testing and to training. Pages 351-356 in Proceedings of the 10th Equine Nutritional Physiology Symposium Ft. Colling, CO.

APPENDIX A

PROJECT ORGANIZATION

Table A.1 Timeline of Study Protocol

Dates (2011)	Wk	Sun	Mon	Tues	Wed	Thurs	Fri	Sat
4/22-5/22				Initial rest and training period				
5/22-5/28	0		SCE	SCE	SCE	SCE	SCE	
5/29-6/4	0a		WT			Implant		
6/5-6/11	1		Ex w/ TR	Ex		EX w. TR	Ex	
6/12-6/18	2		Ex w/ TR	Ex		EX w. TR	Ex	
6/19-6/25	3		Ex w/ TR	Ex		EX w. TR	Ex, BCS	
6/26-7/2	4		SCE	SCE	SCE	SCE	SCE	
7/3-7/9	5		Ex w/ TR	Ex		EX w. TR	Ex, WT, BCS	
7/10-7/16	6		Ex w/ TR	Ex		EX w. TR	Ex	
7/17-7/23	7		Ex w/ TR	Ex		EX w. TR	Ex, WT, BCS	
7/24-7/30	8		SCE	SCE	SCE	SCE	SCE	
7/31-8/6	9		Ex w/ TR	Ex		EX w. TR	Ex, WT, BCS	
8/7-8/13	10		Ex w/ TR	Ex		EX w. TR	Ex	
8/14-8/20	11		Ex w/ TR	Ex		EX w. TR	Ex, WT, BCS	
8/21-8/27	12		SCE	SCE	SCE	SCE	SCE	

SCE = Semen collection and evaluation
WT = Weight measurement
BCS = Body condition score measurements
EX w/ TR = Exercise with temperature recordings

APPENDIX B

DIET

TABLE B.1 Chemical composition of forage diet.

	AMOUNT	
INGREDIENT	%	ppm
Crude protein	12.5	
Acid degergent fiber	27.5	
Calcium	0.5	
Phosphorus	0.25	
Potassium	1.54	
Magnesium	0.18	
Sodium		702
Zinc		39
Iron		34
Copper		13
Manganese		96

TABLE B.2 Chemical composition of concentrate diet.

	AMOUNT	
INGREDIENT	%	ppm
Crude protein	16.1	
Acid degergent fiber	12.1	
Calcium	0.93	
Phosphorus	0.6	
Potassium	1.06	
Magnesium	0.27	
Sodium		3418
Zinc		98.5
Iron		29.5
Copper		36
Manganese		193

APPENDIX C

HEART RATE VARIATION AMONG EXERCISED STALLIONS DURING EXERCISE

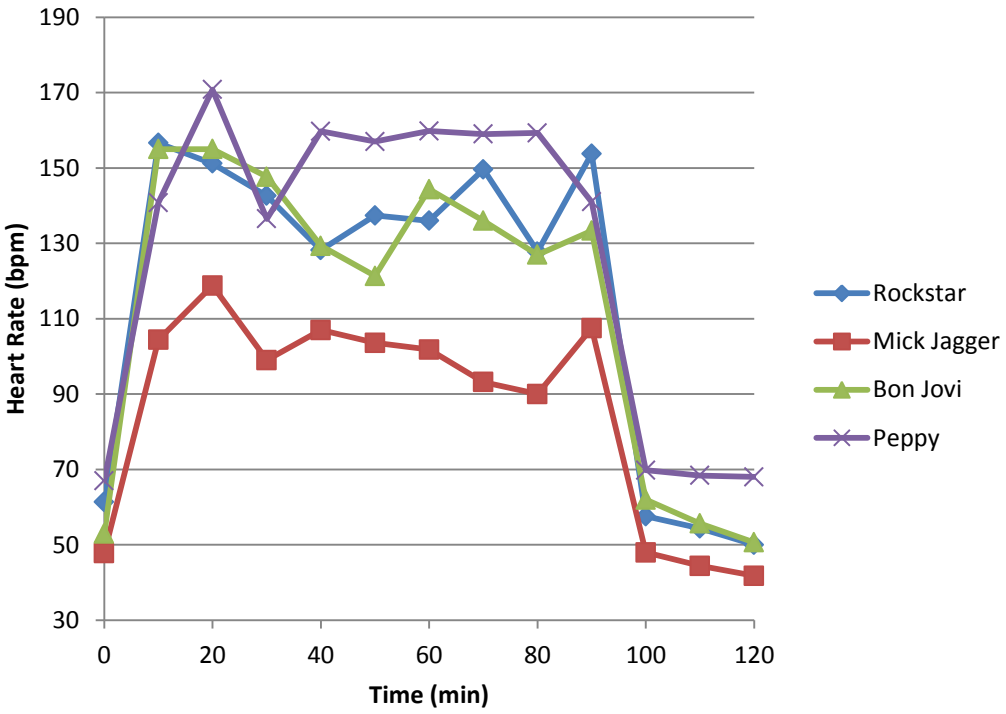
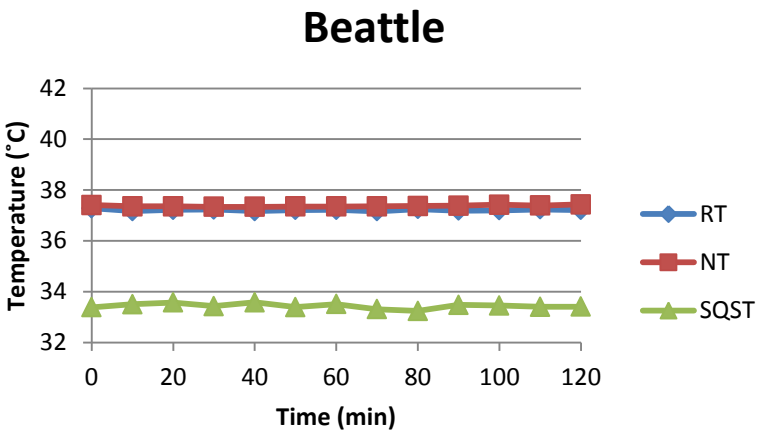
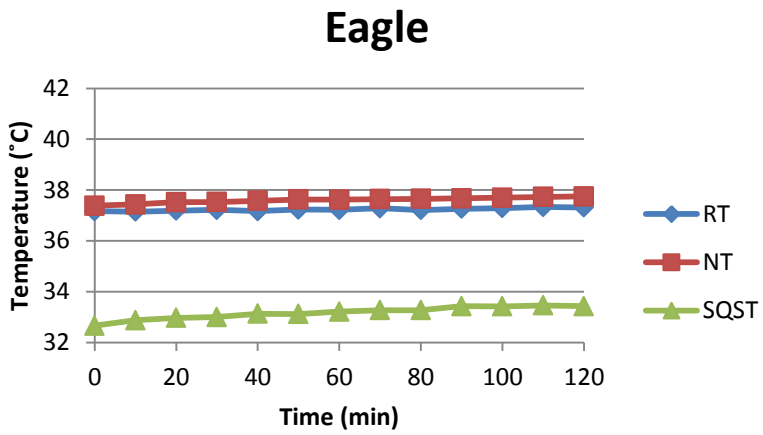


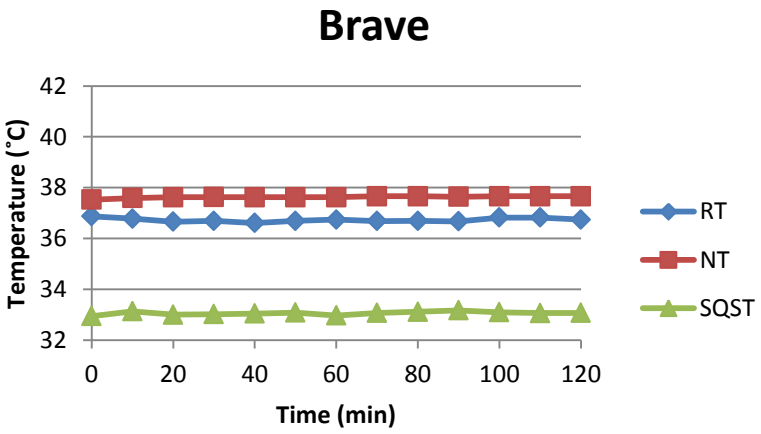
FIGURE C.1 Mean heart rate variation among exercised stallions during exercise

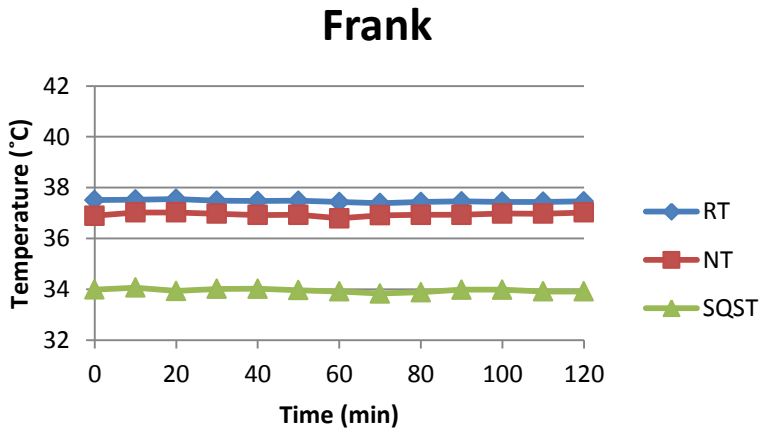
APPENDIX D

TEMPERATURE VARIATION OF STALLION WITHIN GROUP









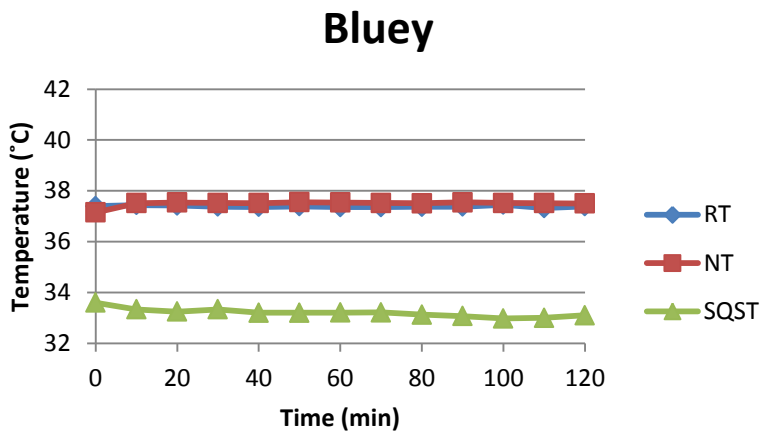


FIGURE D.1 Mean temperatures (rectal, subcutaneous neck, and subcutaneous scrotal) of individual stallions in non-exercising group (CN)

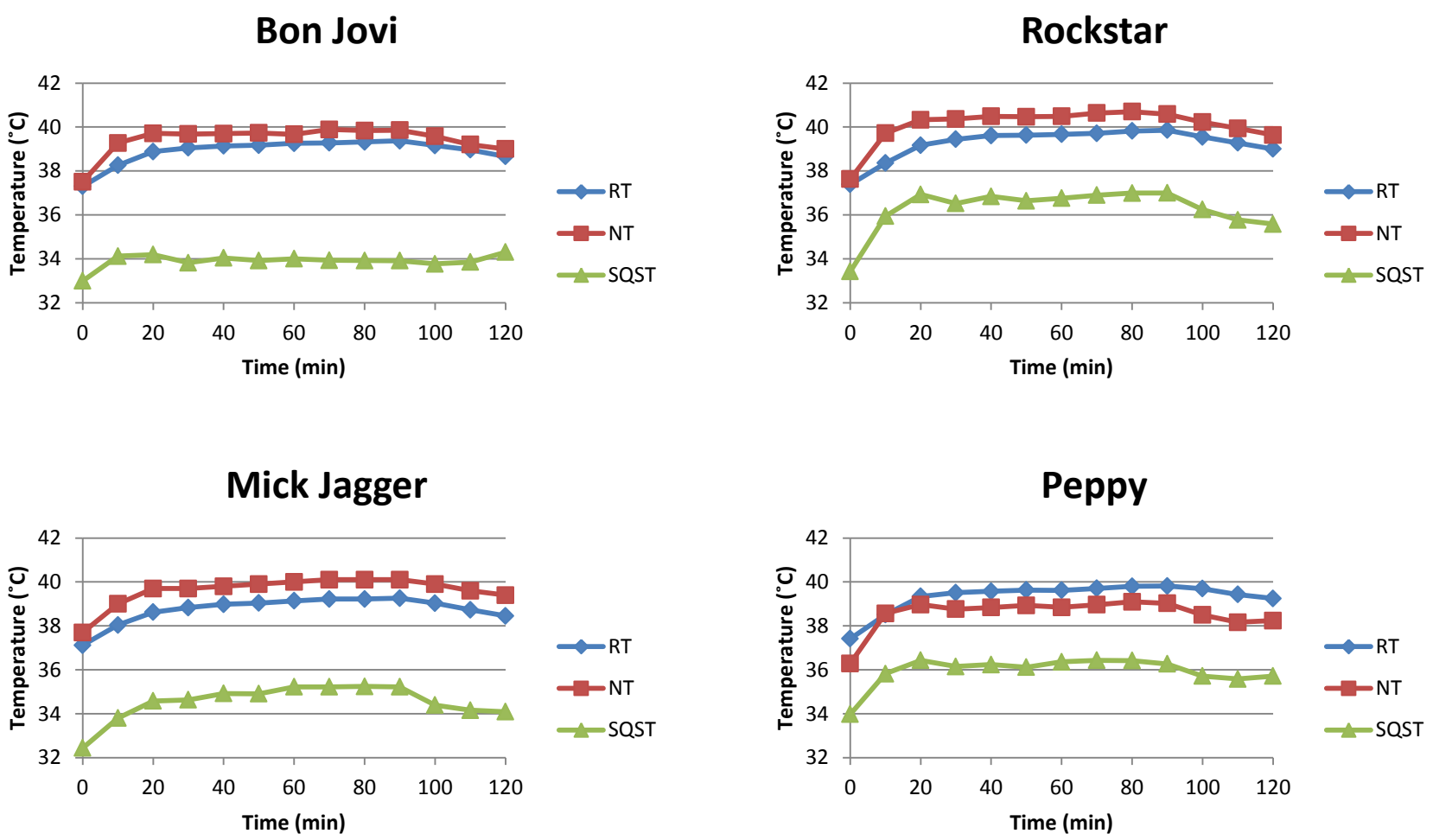


FIGURE D.2 Mean temperatures (rectal, subcutaneous neck, and subcutaneous scrotal) of individual stallions in exercising group (EX)

APPENDIX E

MEAN STALLION SPERM PARAMETERS BY GROUP

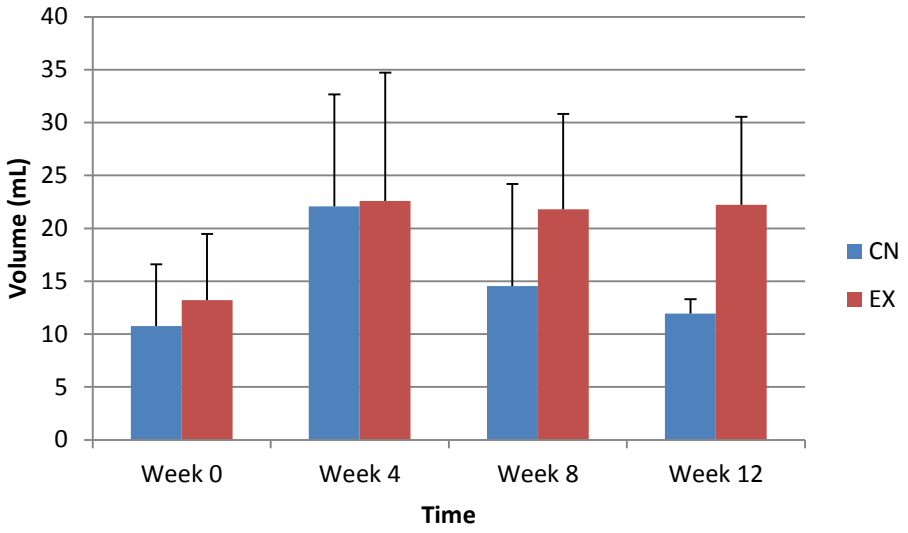


FIGURE E.1 Mean (± SD) semen volume within group, exercising (EX) and non-exercising (CN)

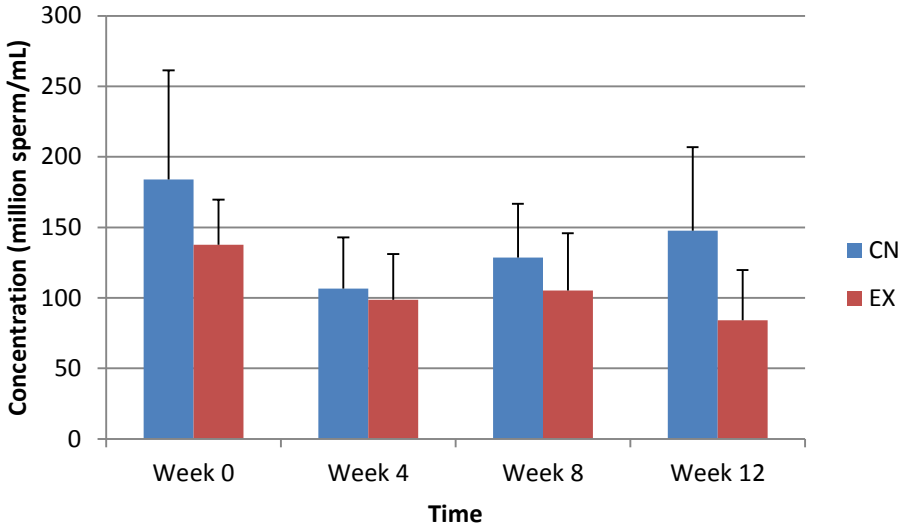


FIGURE E.2 Mean (± SD) sperm concentration per ejaculate within group, exercising (EX) and non-exercising (CN)

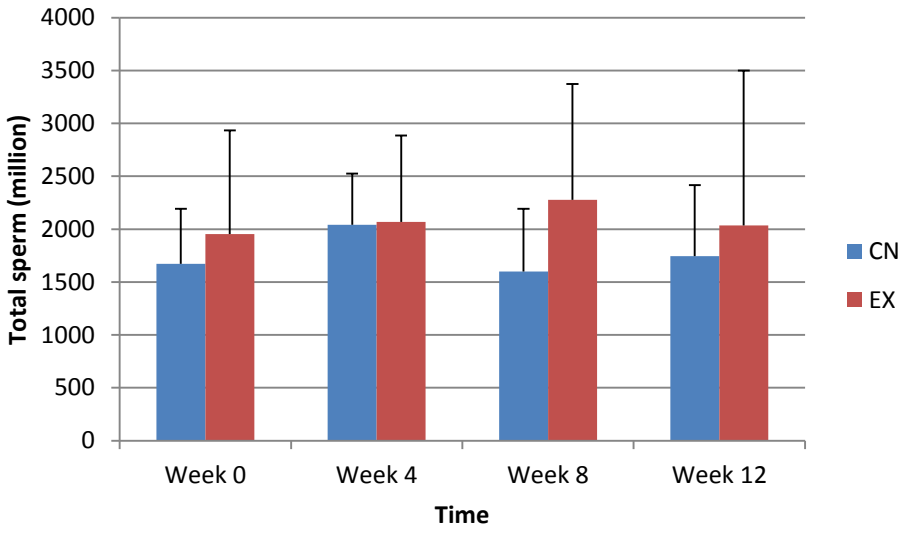


FIGURE E.3 Mean (± SD) total sperm per ejaculate within group, exercising (EX) and non-exercising (CN)

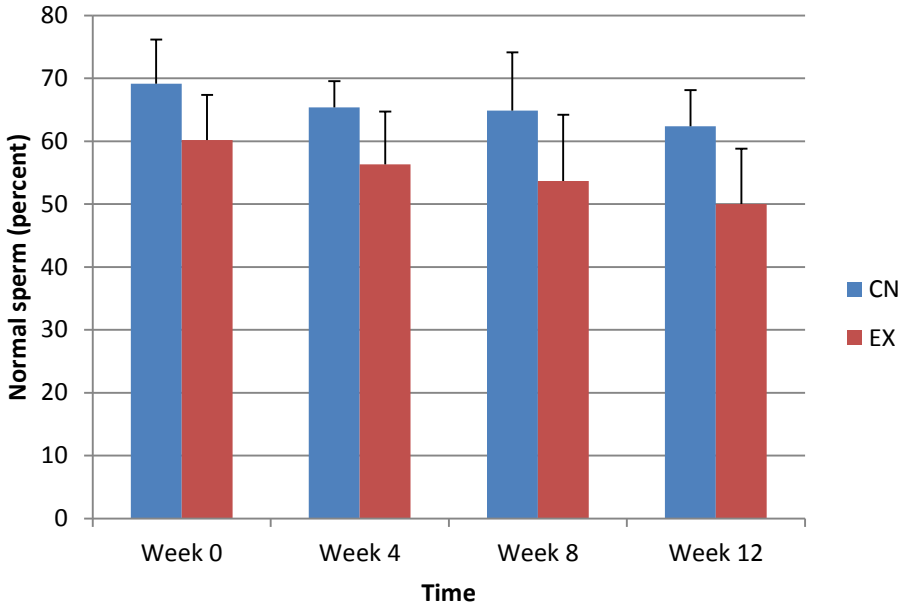


FIGURE E.4 Mean (± SD) percent of morphologically normal sperm within group, exercising (EX) and non-exercising (CN)

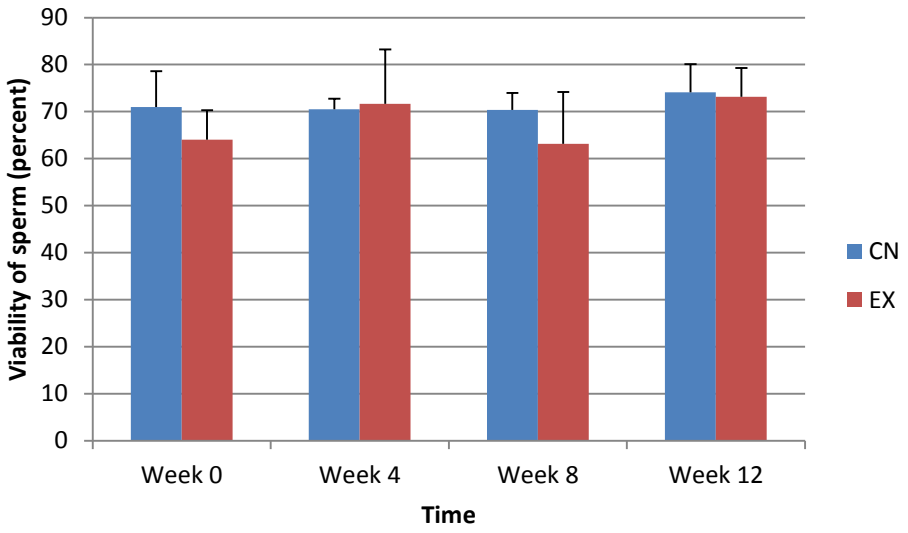


FIGURE E.5 Mean (± SD) percent of viable sperm within group, exercising (EX) and non-exercising (CN)

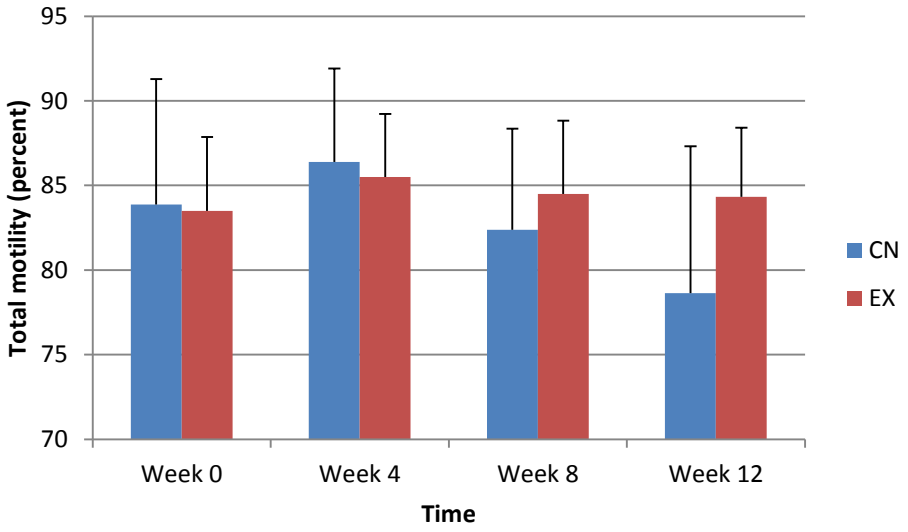


FIGURE E.6 Mean (± SD) percent of motile sperm within group, exercising (EX) and non-exercising (CN)

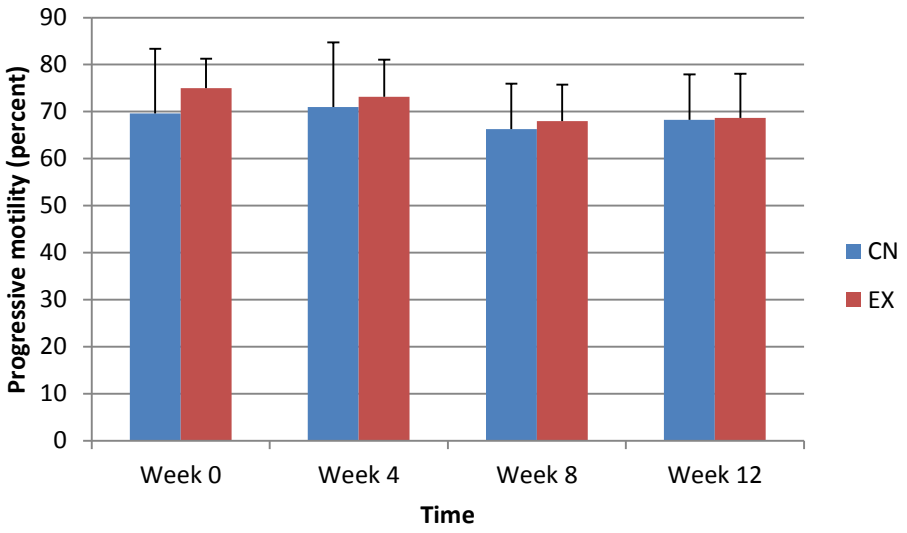


FIGURE E.7 Mean (± SD) percent of progressively motile sperm within group, exercising (EX) and non-exercising (CN)

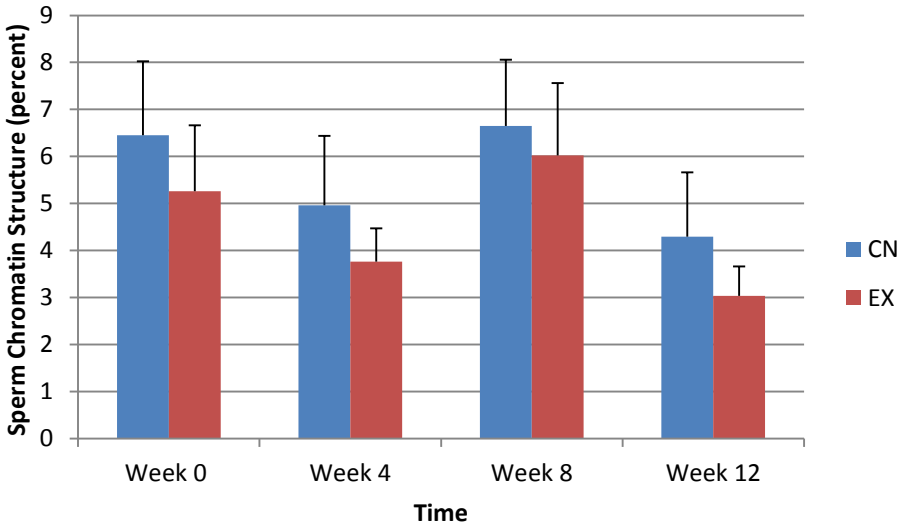
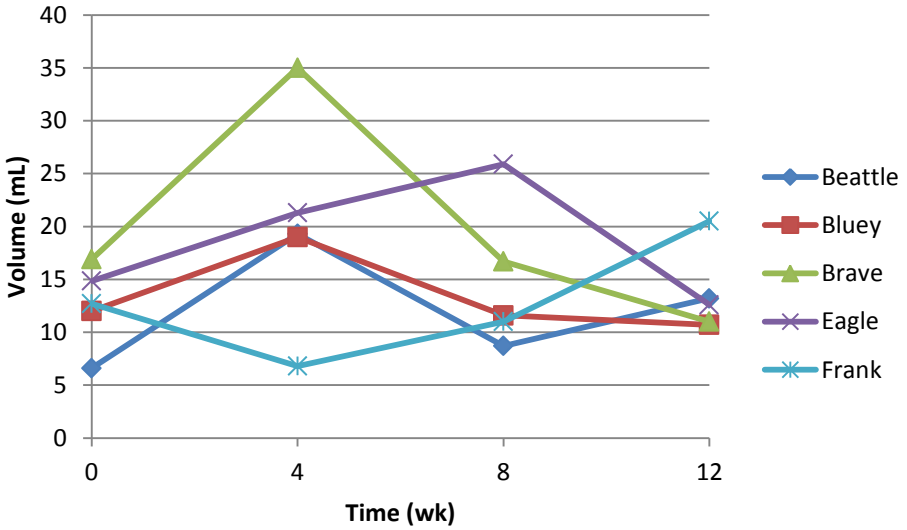


FIGURE E.8 Mean (± SD) $COMP_{\alpha t}$ sperm chromatin structure analysis within group, exercising (EX) and non-exercising (CN)

APPENDIX F

INDIVIDUAL STALLION SEMEN PARAMETERS WITHIN GROUP

a)



b)

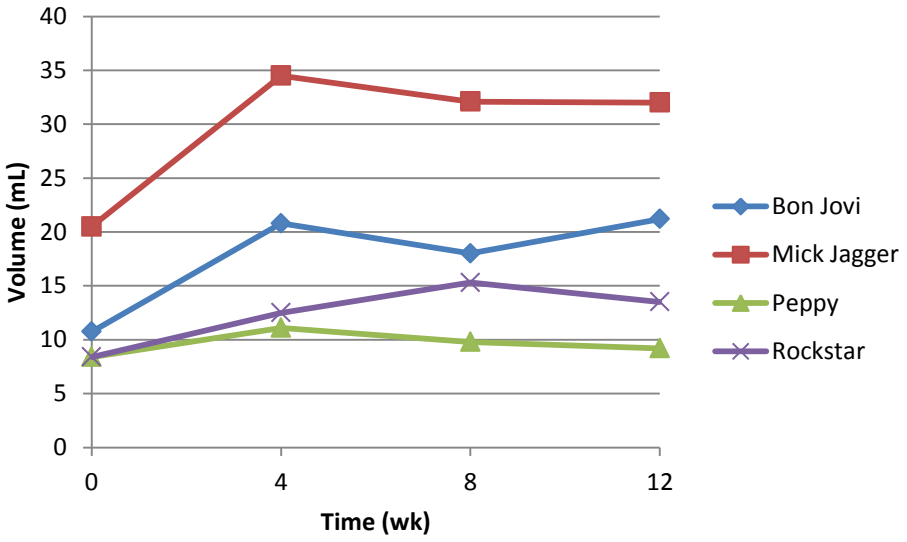
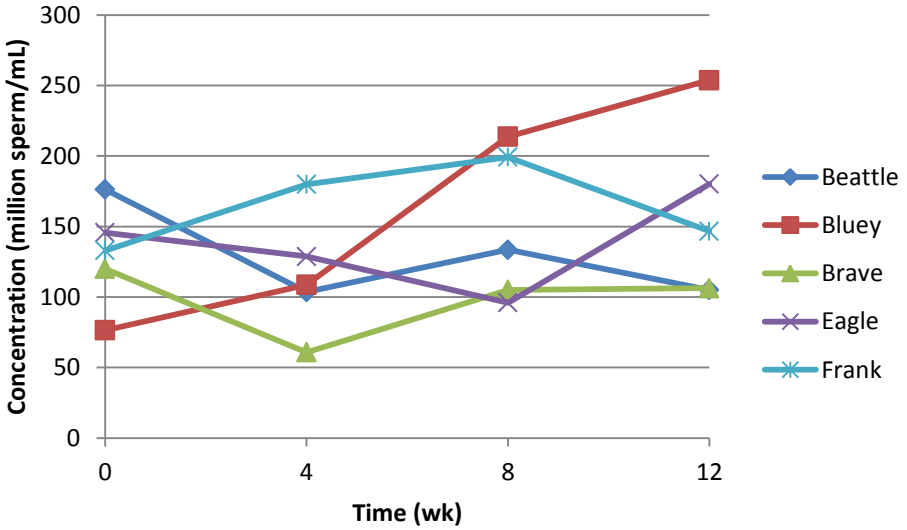


FIGURE F.1 Mean semen volume of individual stallions within group: (a) non-exercising and (b) exercising

a)



b)

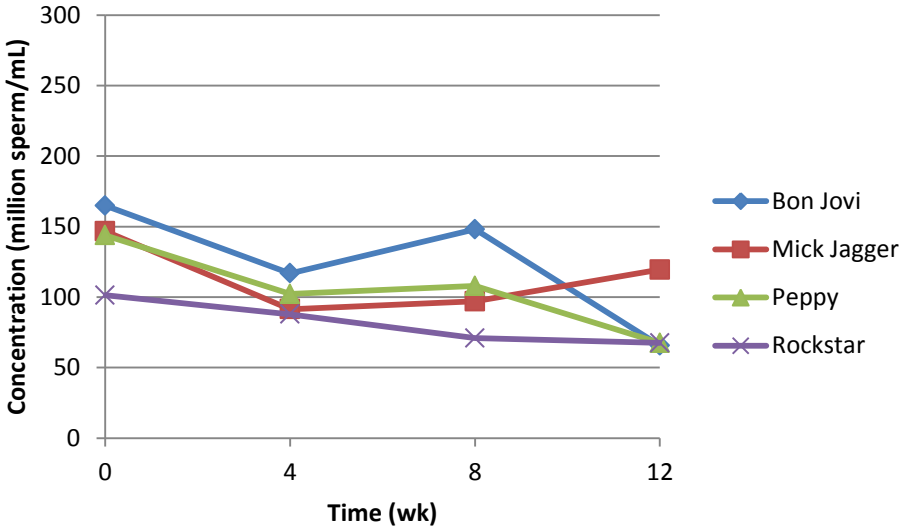
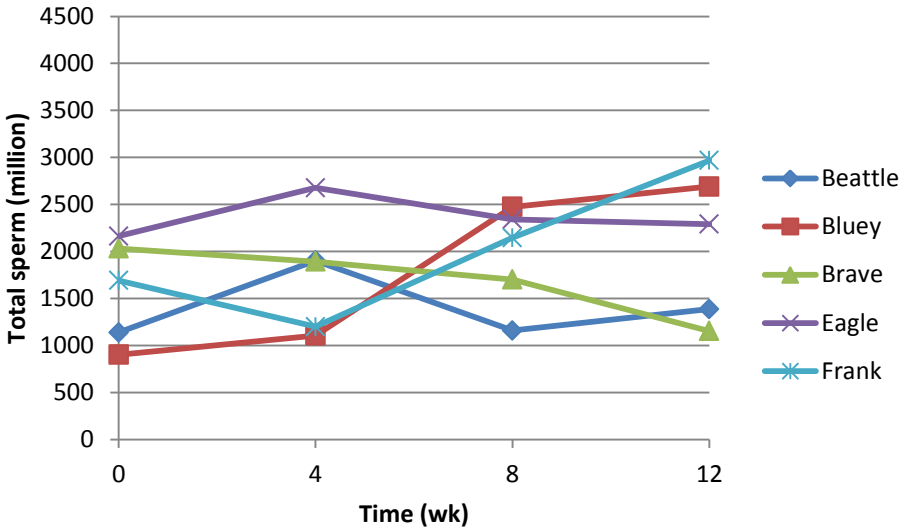


FIGURE F.2 Mean sperm concentration per ejaculate of individual stallions within group: (a) non-exercising and (b) exercising

a)



b)

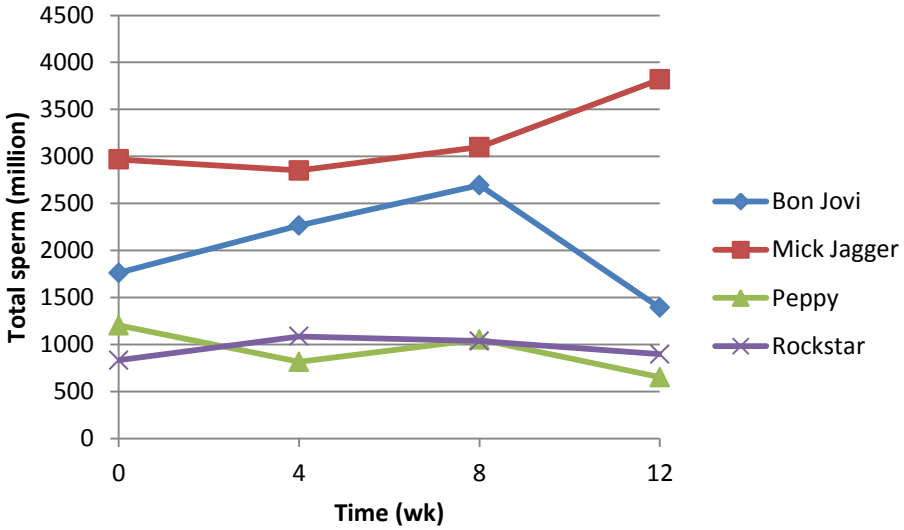
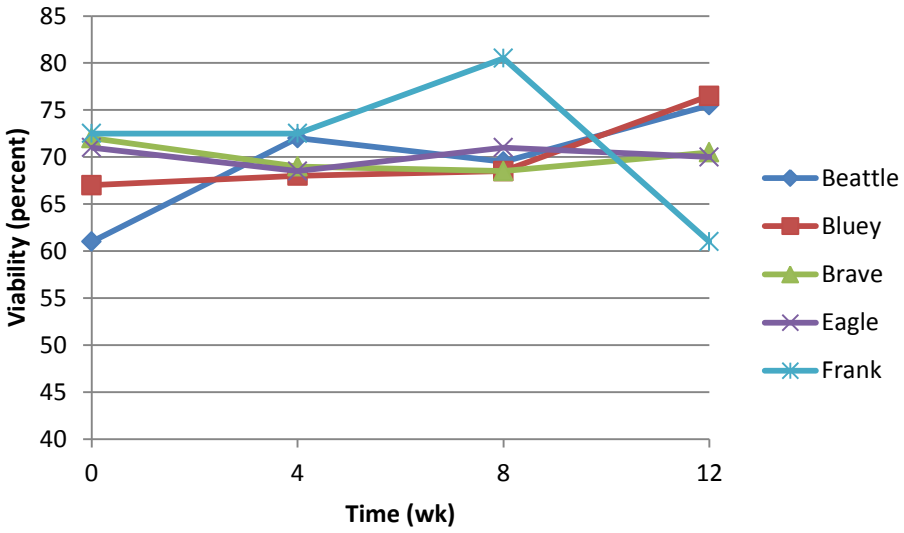


FIGURE F.3 Mean total sperm per ejaculate of individual stallions within group: (a) non-exercising and (b) exercising

a)



b)

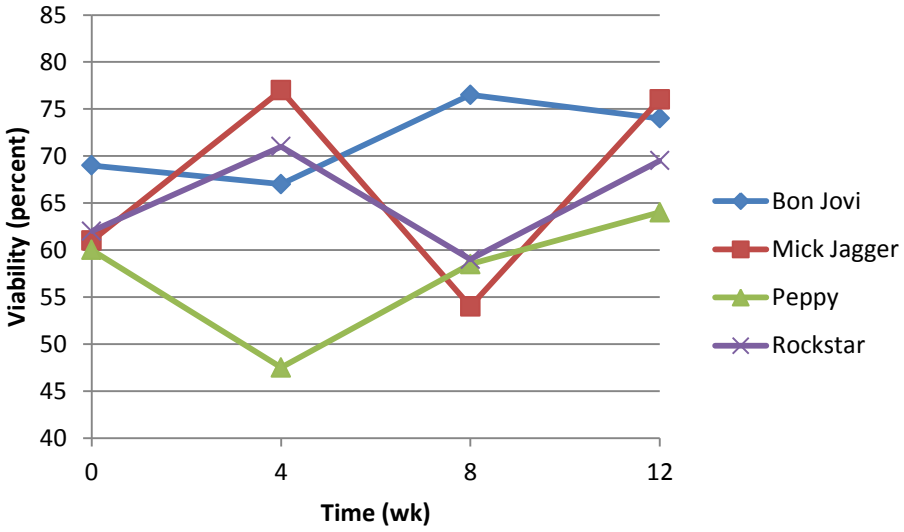
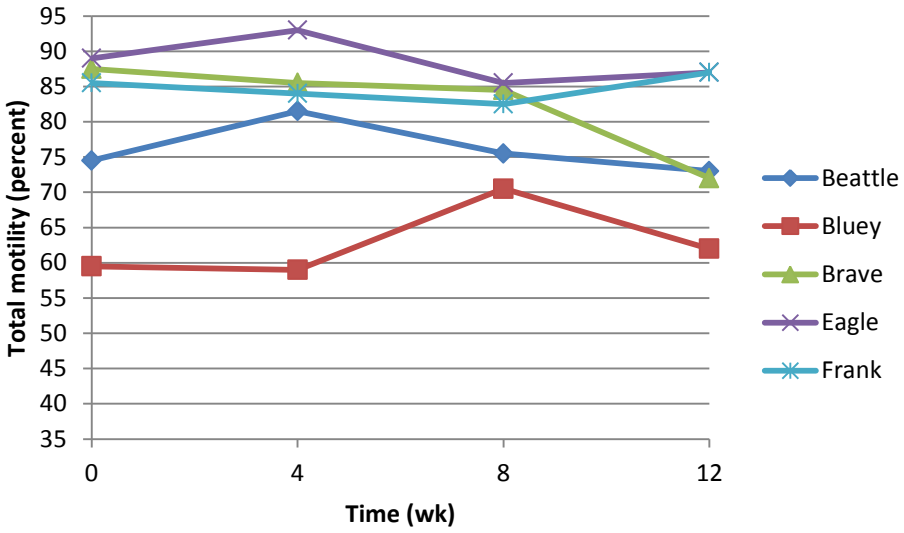


FIGURE F.4 Mean percent of viable sperm of individual stallions within group: (a) non-exercising and (b) exercising

a)



b)

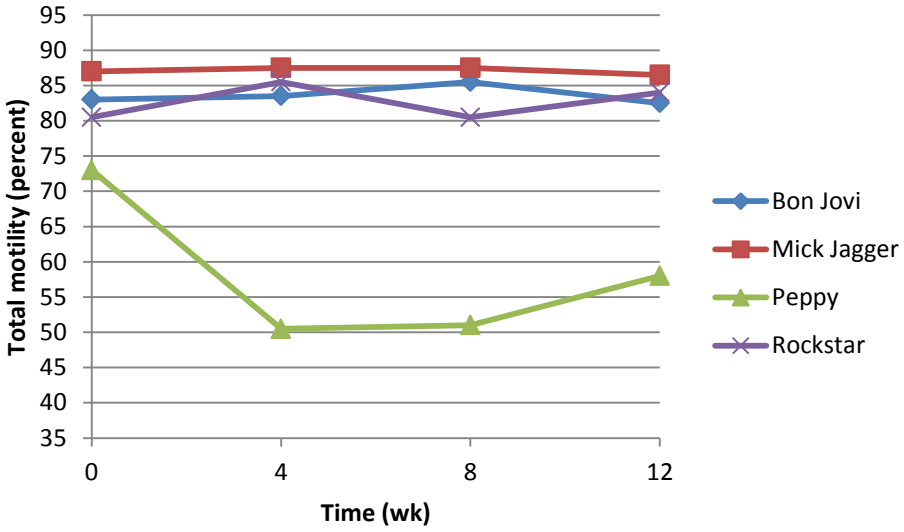
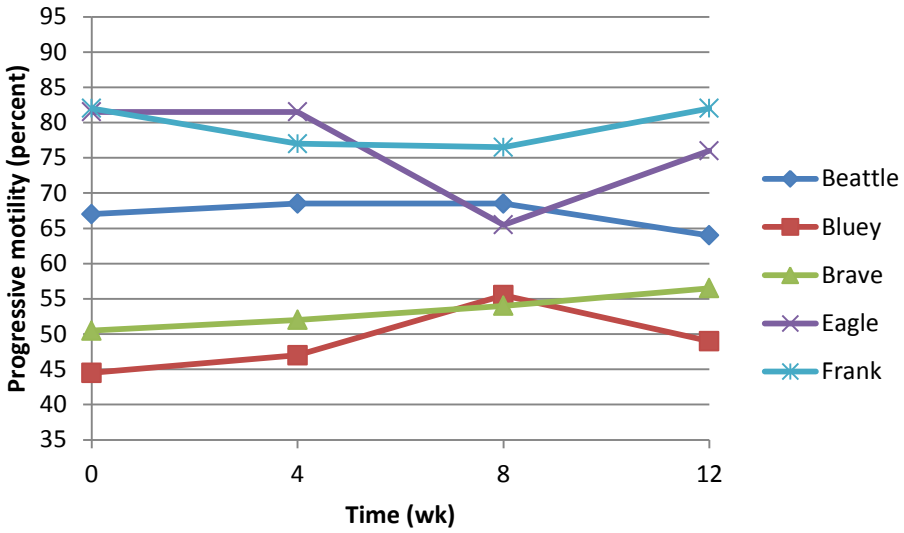


FIGURE F.5 Mean percent of motility sperm of individual stallion within group: (a) non-exercising and (b) exercising

a)



b)

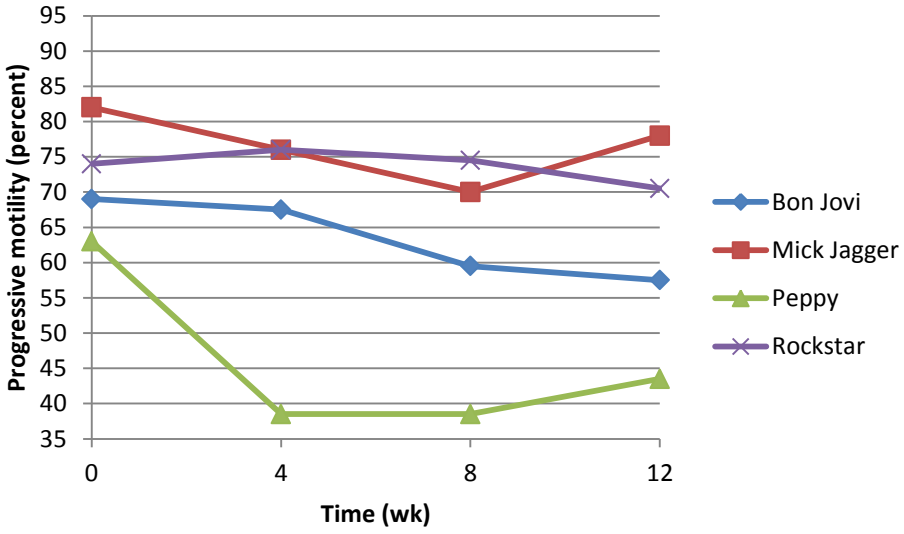
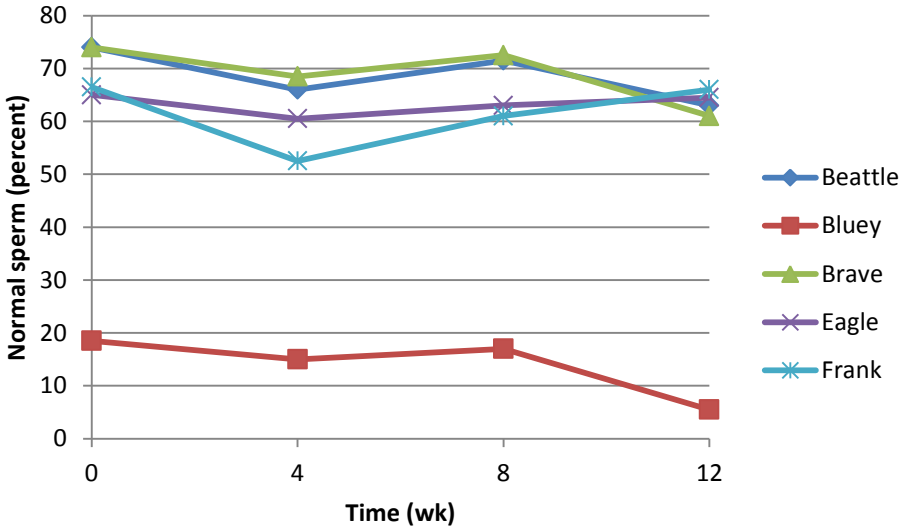


FIGURE F.6 Mean percent of progressively motile sperm of individual stallions within group: (a) non-exercising and (b) exercising

a)



b)

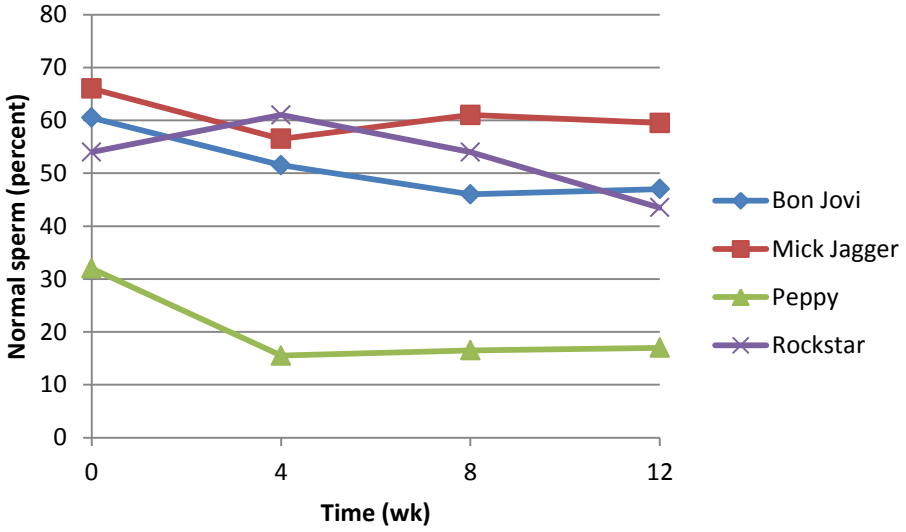
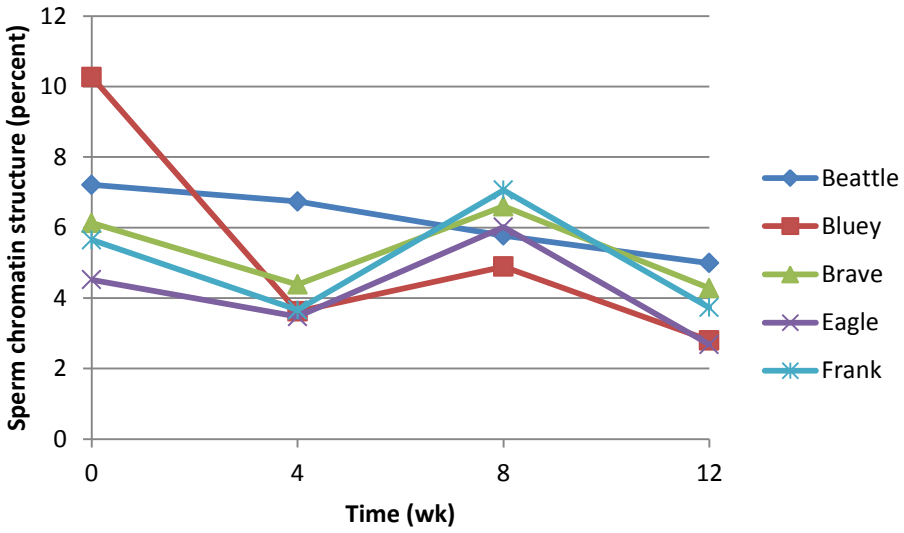


FIGURE F.7 Mean percent of morphologically normal sperm of individual stallions within group: (a) non-exercising and (b) exercising

a)



b)

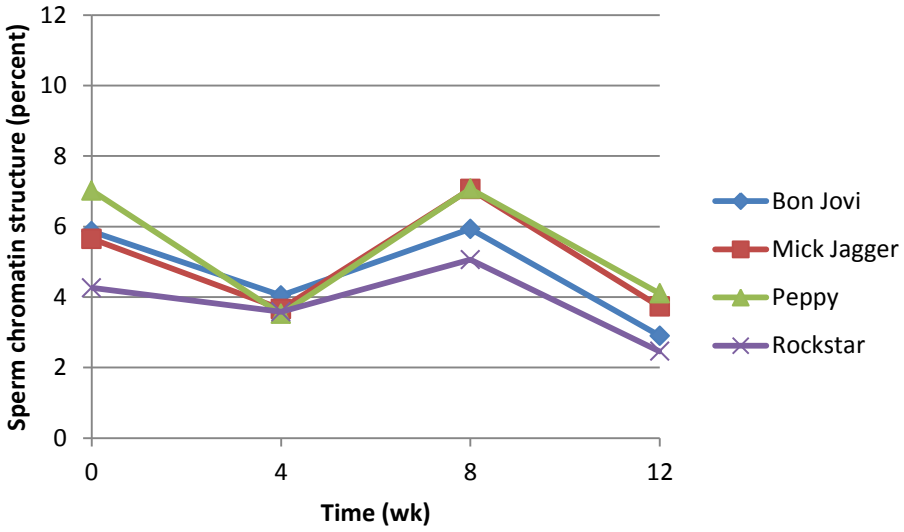


FIGURE F.8 Mean percent of $COMP_{\alpha t}$ sperm chromatin structure analysis of individual stallions: (a) non-exercising and (b) exercising

APPENDIX G

MORPHOLOGICAL PARAMETERS OF SPERMATOZOA BETWEEN GROUPS

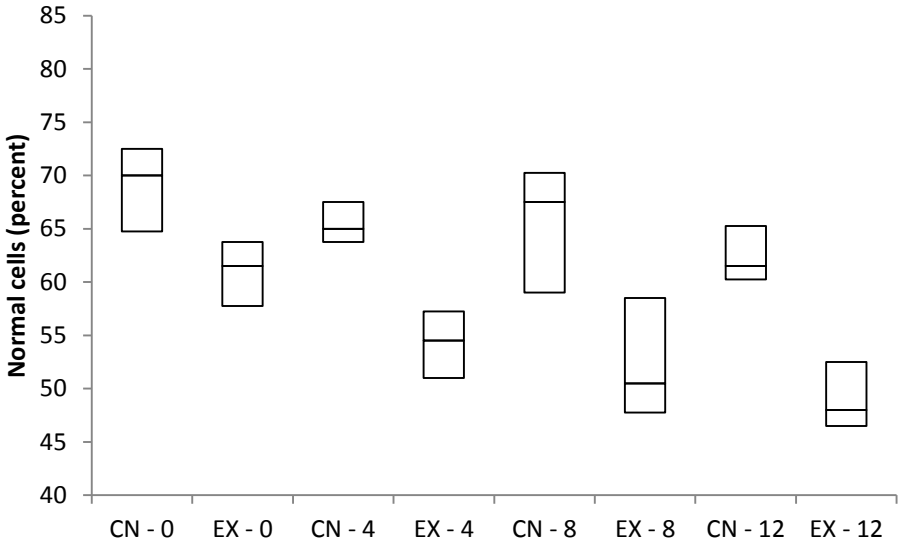


FIGURE G.1 Box plots representing mean percentages of normal spermatozoa within non-exercising and exercising groups at wk 0, 4, 8, 12

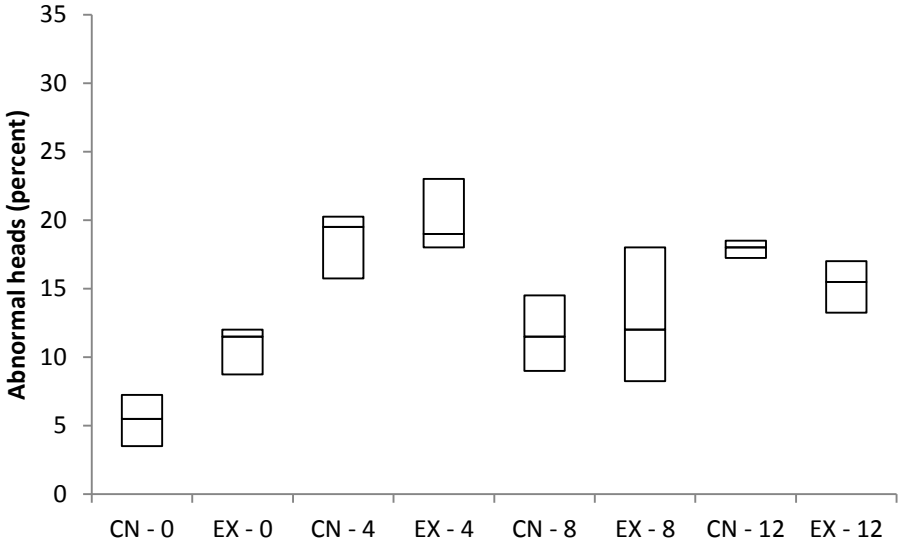


FIGURE G.2 Box plots representing mean percentages of abnormal heads within non-exercising and exercising groups at wk 0, 4, 8, 12

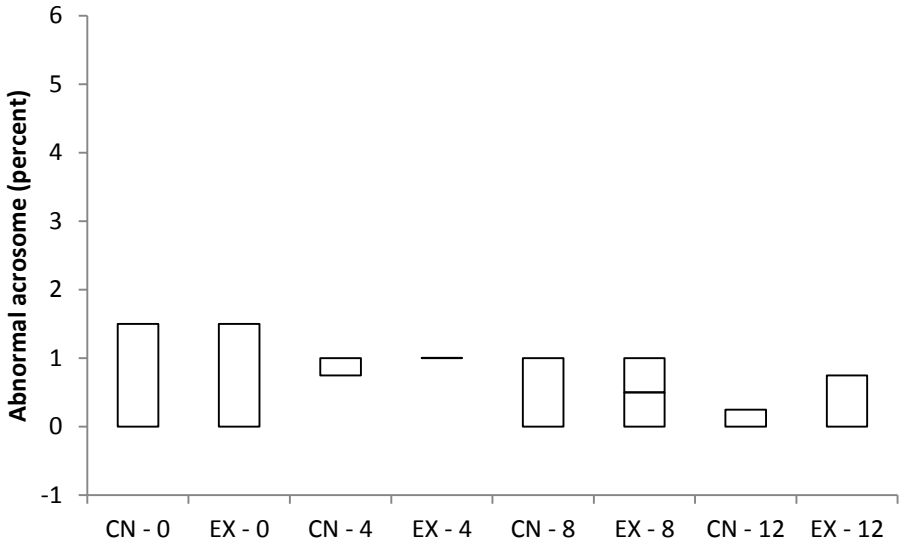


FIGURE G.3 Box plots representing mean percentages of abnormal acrosomes within non-exercising and exercising groups at wk 0, 4, 8, 12

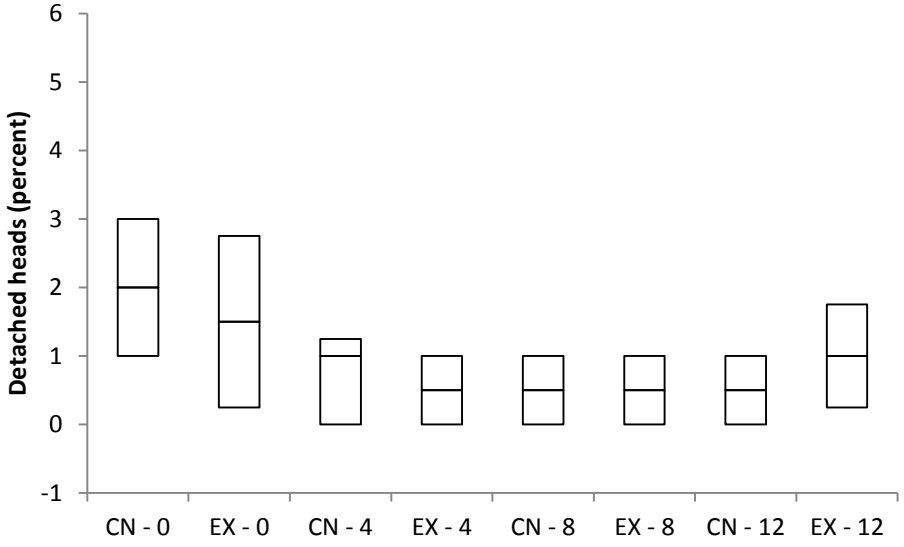


FIGURE G.4 Box plots representing mean percentages of detached heads within control and exercised groups at wk 0, 4, 8, 12

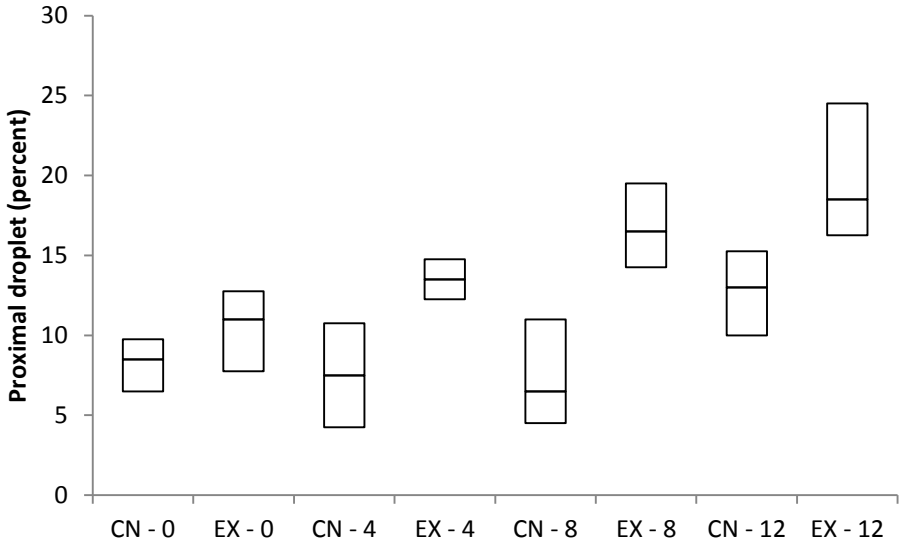


FIGURE G.5 Box plots representing mean percentages of proximal droplets within non-exercising and exercising groups at wk 0, 4, 8, 12

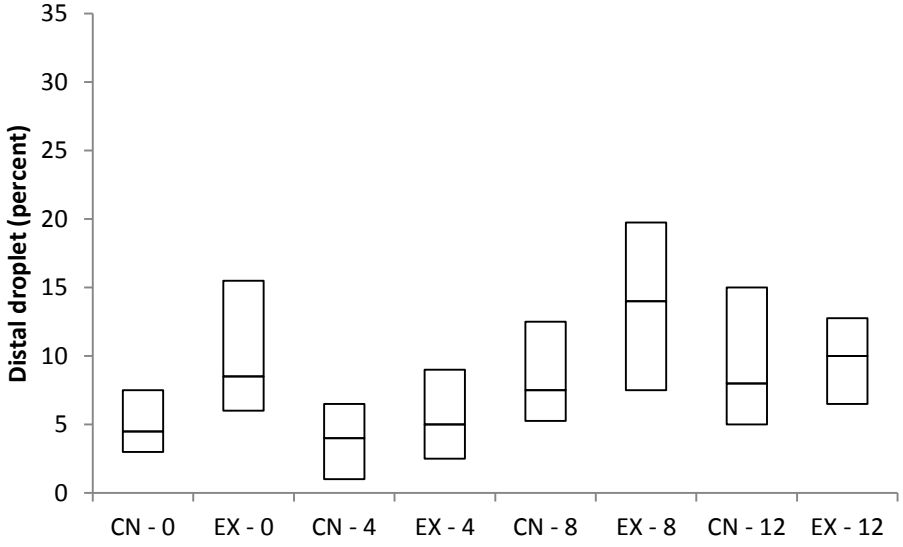


FIGURE G.6 Box plots representing mean percentages of distal droplets within non-exercising and exercising groups at wk 0, 4, 8, 12

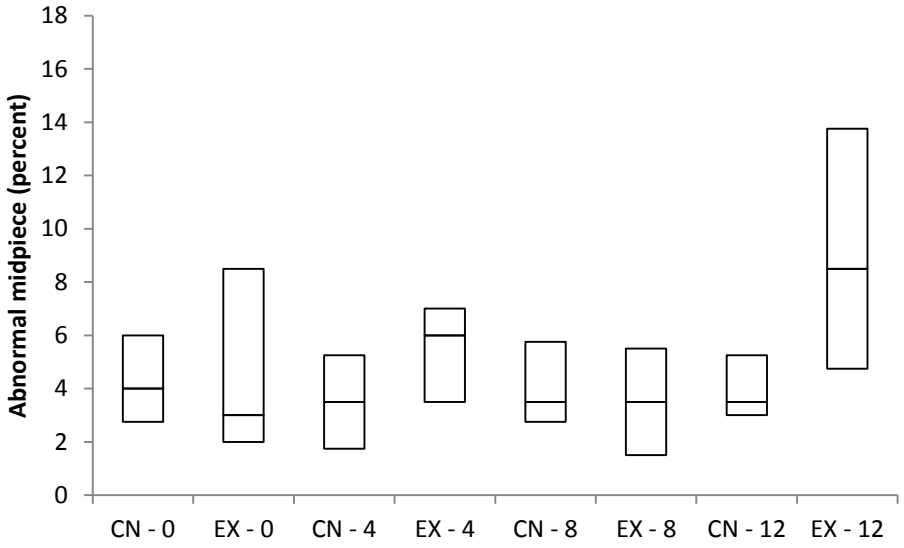


FIGURE G.7 Box plots representing mean percentages of abnormal midpieces within non-exercising and exercising groups at wk 0, 4, 8, 12

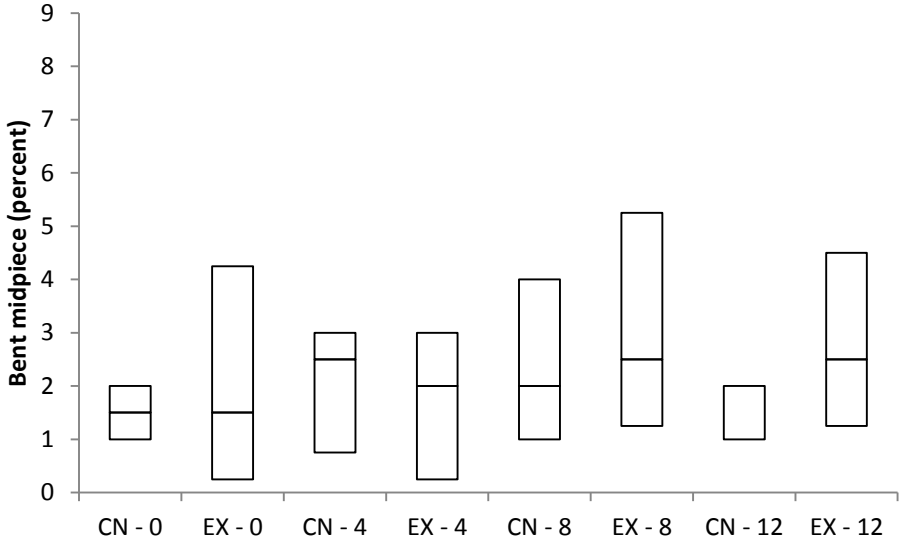


FIGURE G.8 Box plots representing mean percentages of bent midpieces within non-exercising and exercising groups at wk 0, 4, 8, 12

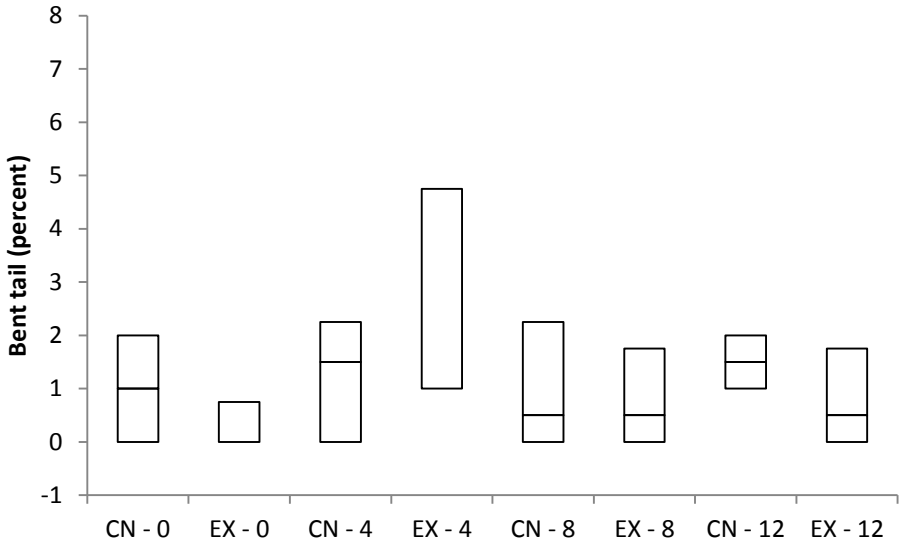


FIGURE G.9 Box plots representing mean percentages of bent tails within non-exercising and exercising groups at wk 0, 4, 8, 12

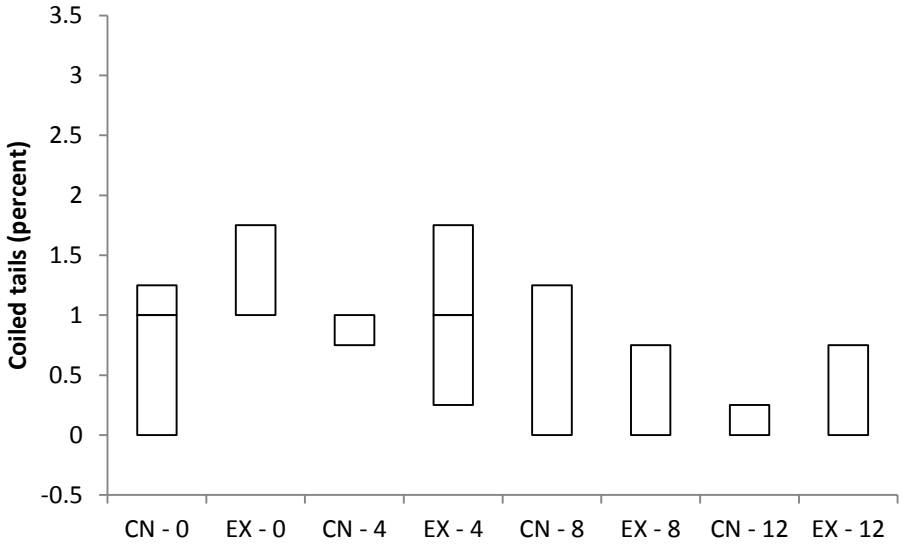


FIGURE G.10 Box plots representing mean percentages of coiled tails within non-exercising and exercising groups at wk 0, 4, 8, 12

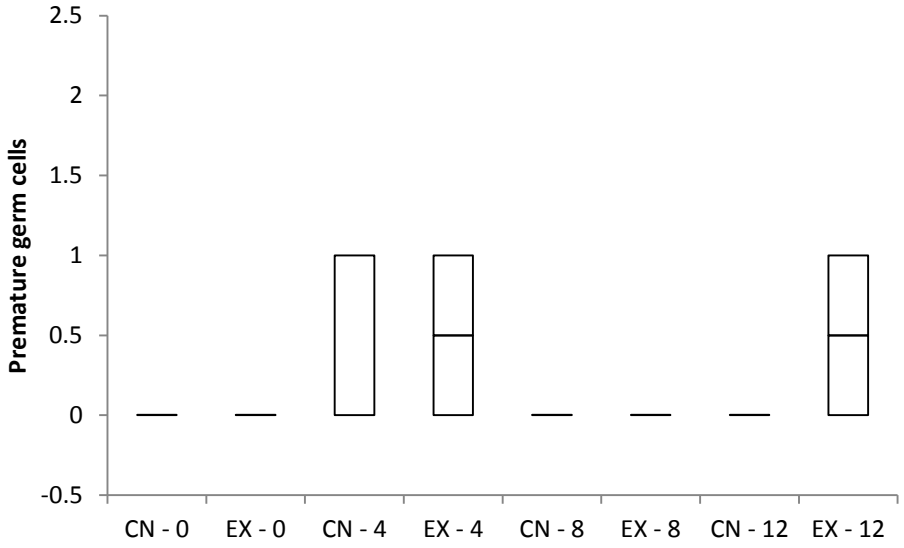


FIGURE G.11 Box plots representing mean percentages of premature germ cells within non-exercising and exercising groups at wk 0, 4, 8, 12

VITA

Name:	Jennifer L. Rosenberg
Address:	133 Kleberg Bldg. 2471 TAMU Texas A&M University College Station, TX 77843
Email Address:	jlrosenberg12@gmail.com
Education:	B.S.A., Animal Science, California Polytechnic State University, San Luis Obispo, 2010 M.S., Animal Science, Texas A&M University, College Station, 2012