

**THERMOREGULATION OF THE TESTICLE IN RESPONSE TO EXERCISE
AND SUBSEQUENT EFFECTS ON SEMINAL
CHARACTERISTICS IN STALLIONS**

A Thesis

by

JEANNETTE DIANE MAWYER

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

May 2011

Major Subject: Animal Science

Thermoregulation of the Testicle in Response to Exercise and Subsequent Effects on
Seminal Characteristics in Stallions

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Approved by:

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Major Subject: Animal Science

ABSTRACT

Thermoregulation of the Testicle in Response to Exercise and Subsequent Effects on
Seminal Characteristics in Stallions. (May 2011)

Jeannette Diane Mawyer, B.S.A., Murray State University

Chair of Advisory Committee: Dr. Clay A. Cavinder

Studies performed on stallions have characterized detrimental effects on semen quality resulting from thermal stress by testicular insulation, but few have investigated the effects of exercise-induced increases in core body temperature on stallion semen parameters. To our knowledge, this is the first study that correlates subcutaneous scrotal temperature and stallion spermatozoa quality using a subdermal scrotal thermal sensory device. Stallions were assigned to a non-exercised (non-ex; control; n=4) or exercised (ex; n=4) group. A motorized equine exerciser was used to work stallions 30 min/d for 4 d/wk during a 12-wk period from July through October. Temperatures (subcutaneous scrotal, subcutaneous neck, rectal, and ambient) were recorded before exercise, immediately after exercise, and 60 and 120 min post-exercise. Humidity data were obtained later to determine THI.

No deleterious effects were observed from implantation of thermal sensory devices. An interaction of treatment and time ($P < 0.0001$) was evident for rectal and neck temperatures. The relationship between scrotal and rectal temperatures was highest ($r_s = 0.761$), and other correlations existed between scrotal, neck, and ambient

temperatures, as well. Mean rectal temperature in the ex group increased 1.9°C ($P < 0.0001$), while there was a slight increase in scrotal temperature of 0.8°C ($P > 0.05$) from 0 min to 22 min. Although an increase in core body temperature was successfully induced by exercise protocol, scrotal temperatures were not significantly affected, and no treatment effects were found in any of the semen parameters measured ($P > 0.05$). Therefore, no significant changes in fresh or cooled semen parameters resulted from exercise or increases in core body temperature resulting from exercise protocol used in this study.

Results of this study indicate that thermal sensory devices are a safe and effective way to measure subcutaneous scrotal and neck temperatures. Although an increase in core body temperature was successfully induced by exercise protocol, scrotal temperatures were not significantly affected, indicating efficient thermoregulation of the testes by the scrotum. Since the testes experienced no significant thermal insult during the exercise protocol, no significant changes in fresh or cooled semen parameters were evident as a result of exercise or elevated core temperature.

DEDICATION

This thesis is dedicated to the two people I admire most, my parents. They forged the career path that I have chosen in life by providing me the opportunity to grow up with horses. Although this path has taken me far from home and family, I know that no matter the distances I travel, the unwavering love and support of my parents will always sustain me. Mom and Dad, I will forever be grateful for the sacrifices you have made to see me achieve my goals. Thank you; I love you; and I miss you every day.

To my beloved horse and friend of only 5 short years: you will always be the horse to which I will compare others. Your beauty, talent, and personality won the respect of everyone we encountered during and after our years in Murray, and I am quite certain that I will never find a more perfect horse. I miss you, Boomer.

*“When we think of those companions who traveled by our side down life’s road,
let us not say with sadness that they left us behind,
but rather say with gentle gratitude that they once were with us.”*

– Author Unknown

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Everyone working in the Theriogenology Laboratory deserves many thanks, especially Sheila Teague for being extremely helpful with semen analysis. I thoroughly enjoyed my time there. Thanks also go to my undergraduate student workers; I could not have completed this project without them. Another group that was absolutely essential to the completion of this study consisted of Krissy Schroeder, Carrie Mueller, Stephanie Standridge, Lindsey Huth, Kelly Winsco, and Jennifer Rosenberg. Many thanks go to them for not only assisting with semen collection and analysis, but also for being great friends. Finally, thanks to my parents for their continued love, support, and encouragement.

NOMENCLATURE

AA	Abnormal acrosome; spermatozoal morphological abnormality
AH	Abnormal head; spermatozoal morphological abnormality
AI	Artificial insemination
AM	Abnormal midpiece; spermatozoal morphological abnormality
AMBT	Ambient temperature
ART	Artificial reproductive technologies
AV	Artificial vagina
BCS	Body condition score
BM	Bent midpiece; spermatozoal morphological abnormality
bpm	Heartbeats per minute
BT	Bent tail; spermatozoal morphological abnormality
BW	Body weight
CONC	Concentration of spermatozoa
DD	Distal droplet; spermatozoal morphological abnormality
DH	Detached head; spermatozoal morphological abnormality
DSO	Daily spermatozoa output
EF	Extractable fat
EP	Exercise period
ex	Exercised (treatment) group of stallions
HR	Heart rate

HUM	Relative humidity
ITT	Intratesticular temperature
non-ex	Non-exercised (control) group of stallions
NORMCELL	Morphologically normal spermatozoa
PD	Proximal droplet; spermatozoal morphological abnormality
PGC	Premature germ cell; spermatozoal morphological abnormality
PMOT	Progressive motility of spermatozoa
post-EP	Post-exercise period; after the cessation of the exercise period
pre-EP	Pre-exercise period; prior to the onset of the exercise period
RCT	Rectal temperature
rf	Rump fat
RR	Respiration rate
SCSA	Sperm Chromatin Structure Assay
COMP _{at}	Sperm chromatin structure assay – cells outside main population
SET	Standard exercise test
SQNT	Subcutaneous neck temperature
SQST	Subcutaneous scrotal temperature
SST	Scrotal surface temperature
T0	Time 0; fresh semen sample
T24	Time 24; 24-h cooled semen
T48	Time 48; 48-h cooled semen
TD	Testicular degeneration

THI	Temperature-heat index
TMOT	Total motility of spermatozoa
TV	Testicular volume
VAP	Average path velocity of spermatozoa
VIAB	Viability of spermatozoa
VOL	Semen volume

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1. INTRODUCTION

Results of a population estimate conducted during the early 1990s indicated that 25% of horses in Texas were being used for reproductive purposes (Jones et al., 1993). A 2004 survey determined that Texas is home to more horses than any other state, accounting for approximately 10% of the U.S. horse population (Deloitte, 2005). Although the more recent study does not report the number of horses used for breeding purposes specifically in Texas, it does acknowledge the importance of breeding on the U.S. equine industry. It specifically reviews the impact of breeding on the racing industry: of the 9.2 billion horses in the U.S., 844,500 are used for racing, and more than half of the horses used for racing are involved in breeding (Deloitte, 2005).

Unlike food animals that are valued for their reproductive abilities, a stallion's most attractive assets are his performance records and his ability to produce offspring with similar performance capabilities. While many stallions are retired to breeding-only activity, others are required to maintain a performance career as well, requiring stallion managers to simultaneously maintain these stallions at a high level of fitness and training while maintaining quality semen. A present concern regards stallions subjected to high levels of activity that may not permit preservation of testicular health and production of maximal sperm quality. A reduction in sperm quality would reduce fertility, especially in processed semen, which would reduce the efficiency of widely-used artificial reproductive technologies (**ART**). For example, artificial insemination (**AI**) has proven

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beneficial to the success of the U.S. equine breeding industry; however, it has placed even more of a strain on stallion managers. High quality ejaculates are crucial to maximize the efficiency of cooled/shipped semen to facilitate the use of AI, as the process of extending, cooling or freezing, and shipping semen can be quite taxing on spermatozoa. A stallion with low quality semen may not be a candidate for shipment if his spermatozoa are unlikely to survive. Therefore, stallion managers must carefully control the many factors known to detrimentally affect stallion semen quality in order to optimize reproductive efficiency.

According to McKinnon and Voss (1993), thermal damage is often due to an increase in normal core body temperature of a stallion resulting from various factors such as systemic infection, prolonged increase in ambient temperature, or scrotal insulation from edema, dermatitis, or hemorrhage. Thermal damage is the most common cause of testicular degeneration that can result in subsequent decreased semen characteristics among stallions (McKinnon and Voss, 1993). Although equine breeders are aware that factors such as heat and exercise stress may hinder spermatozoal production, many are forced to subject their stallions to potential damages from these sources during the breeding season in an effort to maintain a level of training required for maximum athletic performance.

Hot and humid environmental temperatures add an additional challenge to stallion managers in south-central Texas, as ambient temperatures can become extremely high (24°C to 36°C) during peak breeding/show season (NOAA, 2010). These temperatures, coupled with high relative humidity, can be taxing on the efficiency of

sweating and other mechanisms responsible for cooling the body. Maintenance of a lower testicular temperature relative to core body temperature is achieved by means of the thermoregulatory tissues of the testes; namely, the pampiniform plexus, tunica dartos, cremaster muscle, and scrotum. Efficient thermoregulation is necessary for normal spermatogenesis; however, it is unclear whether a threshold exists due to high heat indices coupled with exercise and minimal airflow above which the scrotum and other thermoregulatory tissues are unable to thermoregulate. Many stallions endure rigorous training during the breeding season, which may cause a prolonged increase in body temperature resulting in thermal damage to the testes, leading to a decrease the quality of spermatozoa and potentially lower fertility.

Increased core body temperature achieved during excessive exercise (Webb et al., 1987; Scot, 1992; Kohn et al., 1999) may be a factor contributing to male infertility. Thermometers affixed to the skin of the scrotum have been used in past research to measure the scrotal surface temperatures (**SST**) of stallions during exercise (Staempfli et al., 2006) or testicular insulation (Love and Kenney, 1999; Blanchard et al., 2000); however, to our knowledge, no literature regarding stallions has documented subcutaneous scrotal temperatures resulting from increases in core body temperature due to exercise. Therefore, the objectives of the current study were to:

1. Determine whether subdermal thermal sensors could be applied to the measurement of rectal, subcutaneous neck, and subcutaneous scrotal temperatures,

2. Determine the effect of exercise on rectal, subcutaneous neck, and subcutaneous scrotal temperatures of stallions in hot and humid environments,
3. Determine the relationship between changes in rectal, subcutaneous neck, and subcutaneous scrotal temperatures and sperm quality.

2. LITERATURE REVIEW

2.1 Common Causes of Testicular Degeneration

Testicular degeneration (**TD**) can be defined as the process which causes deterioration in the structure of the testis with a consequent loss of testicular function (Oristalگو Turner, 2007). In stallions, TD is a common cause of acquired subfertility and infertility, which results in substantial economic losses due to loss of breeding fees, increased management costs, and loss of valuable male genetics (McKinnon and Voss, 1993). Stallions may experience TD subsequent to certain nutritional deficiencies, administration of exogenous androgens, spermatozoal outflow obstructions, neoplasia, infection, autoimmune disease, testicular trauma, exposure of the testes to heat or cold, radiation, toxins, or ischemia (Oristalگو Turner, 2007). However, the focus of literary review for the current study examined TD and subsequent effects on reproductive parameters in a variety of species relative to high ambient temperatures (**AMBT**), rectal temperatures (**RCT**), subcutaneous scrotal temperatures (**SQST**), SST, or exercise.

2.2 Relationship between Scrotal Surface and Subcutaneous Scrotal Temperatures and Intratesticular Temperatures

Studies in bulls and rams have used infrared thermography to obtain SST and needle thermistors to obtain SQST and intratesticular temperature (**ITT**) in order to determine whether correlations existed among the temperatures recorded from different areas of the testes (bulls: Kastelic et al., 1995; rams: Coulter et al., 1988). Relationships

between SST and SQST were moderate to high (mean $r = 0.73$), with a mean difference of 3.4°C ; those between SQST and ITT were low to moderate (mean $r = 0.45$), with a mean difference of 1.3°C ; and those between SST and ITT were low (mean $r = 0.29$), with a mean difference of 4.7°C (Kastelic et al., 1995). These correlations suggest that inferences should not be made about ITT based on other scrotal temperature measurements (Kastelic et al., 1995).

2.3 Influence of Increased Subcutaneous Scrotal Temperature on Reproductive Parameters

Needle thermistors have been used to measure SQST in rams as opposed to the aforementioned studies that used SST (Mieusset et al., 1992). Rams experienced a 1.4 to 2.2°C increase in SQST via scrotal insulation for 21 d which resulted in a significant increase in embryonic mortality rate (Mieusset et al., 1992). Similar to studies in bulls (Ross and Entwistle, 1979; Vogler et al., 1993) that used SST as the endpoint to measure thermal insult, deleterious effects were apparent as early as d 4 of insulation, suggesting that spermatozoa stored in the epididymis was initially affected, with significant decreases in viability, motility, and total number of spermatozoa subsequently observed (Mieusset et al., 1992).

A study conducted on men working in a steel-casting plant and being exposed to 38.9°C for 5 h/d found no effects on semen parameters, as internal scrotal temperatures obtained through scrotal invagination thermography of these men did not differ from those of control subjects not exposed to high temperatures. Authors attributed this

phenomenon to acclimatization of treated subjects (Momen et al., 2010). However, a similar study reported that welders working in temperatures of 31 to 45°C for 5 h/d for 6 wk increased groin temperature by 1.4°C, and a subsequent decrease in percent normal spermatozoa was noted (Bonde, 1992).

2.4 Influence of Increased Scrotal Surface Temperature on Reproductive Parameters

Scrotal heating or insulation has been used to increase stallion SST by approximately 3°C for 24 to 48 h (Freidman et al., 1991), 3 to 3.5°C for 36 h (Blanchard et al., 2000), and 2 to 3°C for 48 h (Love and Kenney, 1999). Results of these studies showed a decrease in spermatozoal motility, concentration, number of spermatozoa per ejaculate, percentage of morphologically normal spermatozoa (Freidman et al., 1991; Blanchard et al., 2000), as well as decreased DNA quality (Love and Kenney, 1999). Similar studies in bulls and boars maintained SST at 35°C for 10 or 20 h (bulls; Ross and Entwistle, 1979), 33.3 to 36.4°C for 48 h (bulls; Vogler et al., 1993), and 39.5°C for 3 h (boars; McNitt and First, 1970). Results of these studies demonstrated a decrease in spermatozoal concentration and percent live and motile spermatozoa (Ross and Entwistle, 1979; McNitt and First, 1970), as well as an increase in percent abnormal spermatozoa (Ross and Entwistle, 1979; Vogler et al., 1993). Studies aimed at more closely examining the effects of elevated temperature on morphological characteristics in the bovine agree that a 1 to 1.5°C increase in SST for 2 or 4 d interfered with spermatogenic processes, as indicated by observed increases in primary morphologic abnormalities of spermatozoa (Vogler et al., 1993; Barth and Bowman, 1994). After 8 d

of insulation, a direct effect of heat on epididymal spermatozoa was apparent, shown by increases in dead spermatozoa and detached heads, which were found to be directly related to loss of cell membrane integrity (Barth and Bowman, 1994). Severity of the changes in seminal characteristics varied in these studies, and no assumptions can be made about the extent of these changes based on duration of thermal stress.

Similar studies conducted on men are consistent with the findings in domestic livestock species. Research investigating male contraception via genital heat stress introduced an apparatus for the immersion of the scrotum into a 45°C water bath (Watanabe, 1959). Hot water bath treatment for 30 min/d for 12 d dramatically compromised semen quality for 5 to 12 wk, after which time spermatozoal parameters returned to normal (Watanabe, 1959). Men who wore close-fitting underwear that held the testes close to the inguinal canal for 2 to 6 mo experienced a decrease in concentration of motile spermatozoa to fewer than 5% of pre-treatment values (Mieusset and Bujan, 1994). Although significant increases in scrotal temperature appear to adversely affect male fertility, slight increases, such as 0.8 to 1.0°C, appear to have no affect on spermatogenesis or spermatozoa function in the human male (Wang et al., 1997).

2.5 Influence of Increased Ambient Temperature on Reproductive Parameters

Numerous reports exist on the influence of increased ambient temperature on lab animal reproductive parameters. When exposed to a single episode of 43°C for 10 min, mice experienced a decrease in testes weight 7 d post-exposure (Shilkina, 1976). Other

studies have shown that DNA quality was reduced when mice were exposed to temperatures as low as 38°C (Sailer et al., 1997) and as high as 42°C for a period of 30 min (Perez-Crespo et al., 2008). Mice mated at predetermined intervals after heat treatment displayed subfertility consistent with damage to spermatocytes (Rockett et al., 2001), which is consistent with reports that damage to spermatocytes occurred within the testes at the time of heat stress (Perez-Crespo et al., 2008). Rabbits, a species extremely sensitive to heat stress, were exposed to an estimated temperature-humidity index (**THI**) value of 30.1 (very severe heat stress) for 3 mo, after which they presented significantly lower ejaculate volume, concentration of spermatozoa, and total sperm output; however, quality of spermatozoa remained unaffected (Marai et al., 2002). When compared to control group boars that were maintained at a temperature of 20°C, boars exposed to ambient and radiant temperatures of 33°C for 72 h showed significant decreases in spermatozoal concentration, total motility of spermatozoa, and total spermatozoa per ejaculate, as well as an increase in primary and secondary abnormalities (McNitt and First, 1970). These results suggest that exposure to relatively low temperatures for an extended period of time may induce heat stress leading to fertility problems that can persist for 2 mo after initial exposure. Similarly, decreases in spermatozoal motility and percent normal spermatozoa was observed after boars were exposed to either 34.5°C for 8 h/d or 31°C for 16 h/d for 90 d (Wettemann et al., 1976). Pregnancy rates in gilts bred with semen from the heat stressed boars were significantly lower, as was embryonic survival at 30 d (Wettemann et al., 1976). Boars initially housed at a temperature of 20°C were subjected to an increase in air temperature of 1°C/d for 20 d, which elicited

SST as high as 39°C (Stone, 1982). Seminal parameters were affected due to thermal insult to the testes, with a decrease in motility of spermatozoa observed when air temperature reached 30°C and a decrease in percent normal spermatozoa noted during the last week of heat treatment (Stone, 1982). These studies suggest that boars are more sensitive to lower ambient temperatures than other species, and heat stress is an extremely important factor to consider in their management (Konavongkrit et al., 2005). This information has prompted swine breeders to develop temperature management protocols to optimize the fertility of boars (Konavongkrit et al., 2005). Increased sensitivity to thermal insult may be due to the location of the testes close to the body wall, which differs from the more pendulous conformations of the scrota of stallions and bulls. The particularly pendulous structure of the bull scrotum allows the testes to remain farther from the body and to dissipate heat more efficiently. Nevertheless, when bulls were exposed to ambient temperatures higher than 29°C, motility and concentration of spermatozoa decreased, as did total numbers of spermatozoa, for as long as 2 mo following heat stress (Casady et al., 1953).

2.6 Influence of Exercise on Reproductive Parameters of Other Species

Previous studies have been performed examining the reproductive parameters of men who exercise strenuously on a regular basis (Jung and Schuppe, 2007). An increase in spermatozoa morphological abnormalities was observed in endurance-trained cyclists (Gebreegziabher et al., 2004), while a decrease in spermatozoal concentration, ejaculate

volume, and total number of spermatozoa was apparent in recreational male athletes subjected to more strenuous workouts (Vaamonde et al., 2006).

2.7 Influence of Exercise on Stallion Reproductive Parameters

A limited number of studies have explored the effects of thermal insult on stallion reproductive parameters, especially regarding thermal insult as a result of exercise, and results of these studies are conflicting. The first study of its kind, showed that daily minimal exercise by lunging at the walk and trot during a 16-wk period had no influence on semen quality (Dinger et al., 1986). However, the results were likely confounded by immaturity of the stallions as well as seasonal effects on spermatozoa production (Dinger et al., 1986). In contrast, Lange et al. (1997) recorded significantly better progressive spermatozoa motility in breeding stallions being trained and used in dressage and jumping competitions than in those used for breeding alone, although stallions were not blocked for age or other factors, and the extent of training was not defined. Results similar to those of Lange were obtained from a survey that found significantly higher fertility in intensively-trained Icelandic stallions when compared to moderately trained or untrained stallions (Davies Morel and Gunnarsson, 2000); however, again, intense versus moderate training was not clearly characterized. A more recent study with a better-defined exercise regimen consisting of repeated strenuous exercise (until exhaustion) on a high-speed treadmill 2 d/wk for 4 wk was found to cause a distinct stress reaction in stallions that compromised the quality of fresh and frozen-thawed semen during and up to 1 mo after exercise (Janett et al., 2006). Increases in the

percentage of major spermatozoa defects immediately following the exercise period were observed, as well as increases in acrosome defects and nuclear vacuoles during the exercise period (Janett et al., 2006). Additionally, a decrease in motility of spermatozoa was observed 16 wk after the exercise period (Janett et al., 2006). Related research conducted by Staempfli et al. (2006) sought to determine how SST would be affected by treadmill or ridden exercise in stallions wearing a net-like testicular sling (suspensory) designed to hold the testicles close to the body during jumping, eventing, or fox hunting. The study showed that treadmill and ridden exercise significantly influenced SST, with levels being higher with suspensory than without, which was likely due to the impairment of airflow with suspensory (Staempfli et al., 2006). Also, SST of stallions subjected to treadmill exercise were significantly higher than those that were ridden, and scrotal cooling seemed to be directly related to gait in ridden horses, with a decrease in initial SST of approximately 3°C during cantering and an increase of more than 3°C from initial SST measurements during the walking cool-down (Staempfli et al., 2006). Semen characteristics were not evaluated during this study.

2.8 Exercise and Related Increases in Core Body Temperatures in the Equine

Numerous equine exercise physiology studies have documented the increase in body temperature that occurs when a horse is exercised. Several reports have recorded rectal temperatures in the range of 39.4 to 41.1°C for exercised horses, as well as persistent elevated temperatures during 30 min recovery periods (Webb et al., 1987; Scott, 1992; Kohn et al., 1999).

2.9 Inferences

Research examining the effects of exercise on male reproductive processes is questionable; however, increased thermal stress due to extreme ambient temperature or scrotal insulation clearly adversely affects reproductive processes in males of all species. Although literature has not conclusively elucidated the ambient temperatures at which spermatogenesis might be adversely affected, we can infer that animals dwelling in a hot and humid environment like south-central Texas may be more likely to suffer thermal testicular insult resulting in decreased reproductive parameters. Stallions being rigorously exercised during extreme temperatures may be at increased risk. If exercise can maintain a substantial increase in body temperature long enough to cause thermal insult to the testes, then many stallions could potentially suffer severe reproductive losses. High quality ejaculates are absolutely essential for success in artificial reproductive technologies such as AI, which is becoming increasingly popular in the equine industry. If stallions do suffer reproductive losses preventing the use of AI, then substantial economic losses will ensue due to an increase in management costs, as well as loss of breeding fees and valuable male genetics. Research in other domestic livestock species has led to changes in management in order to minimize thermal stress. Further research is required to determine correlations between ambient, scrotal, and core body temperatures that are capable of causing impaired reproductive capacity so that stallion management practices can be improved in an effort to preserve the fertility of performance stallions, and thus their value as athletes and sires.

3. MATERIALS AND METHODS

3.1 Stallions, Housing, and Diets

Eight mature Miniature Horse stallions (4 to 16 y, 72.1 to 110.7 kg) were used in a 12 wk study from early July – early October (Table A.1). The stallions were brought to the Texas A&M University Horse Center 90 d prior to the onset of the study and remained there throughout. Stallions were confined to individual stalls (1.8 m x 1.8 m) that allowed visual access to other horses and featured attached small dry paddock runs (7.3 m x 1.8 m) to allow each stallion free exercise every other day. Project approval was granted by the Texas A&M University Institutional Agricultural Animal Care and Use Committee using guidelines set forth by the Federation of Animal Science Societies (1999).

Body weight (**BW**), rump fat (**rf**), and body condition scores (**BCS**) of each stallion were measured weekly to aid in diet determination, and percent extractable fat (**EF**) was determined as previously described (Westervelt et al., 1976). Each stallion was fed 12.5% CP coastal hay (Table B.1) in amounts equal to 1.5% of his BW daily, and a 16.1% CP concentrate (Table B.2) twice daily in amounts consistent with each stallion's body condition (Henneke et al., 1983; Westervelt et al., 1976). Each stallion was allowed free access to fresh water. Table 1 lists mean \pm SD values for each measurement of body condition at the onset (Wk 0) and the end (Wk 12) of the project.

TABLE 1. Mean (\pm SD) body condition endpoints of non-exercised (non-ex; control) and exercised (ex) stallions at the onset (Week 0) and at the end (Week 12) of the study (n = 4/group).

	WEEK 0		WEEK 12	
	NON-EX	EX	NON-EX	EX
Body Weight (kg)	87.09 \pm 10.27	95.82 \pm 15.93	93.67 \pm 8.16	98.32 \pm 0.01
Body Condition Score	4.75 \pm 0.87	5.20 \pm 0.24	5.13 \pm 0.63	5.38 \pm 0.29
Rump Fat Thickness (cm)	0.35 \pm 0.17	0.58 \pm 0.38	0.34 \pm 0.13	0.54 \pm 0.29
Extractable Fat (%)	5.78 \pm 0.95	7.07 \pm 2.15	5.71 \pm 0.73	6.82 \pm 0.29

3.2 Initial Rest/Acclimation Period

The stallions were allowed an initial 90-d rest period, during which they were stalled and only allowed free exercise every other day. This time period was also used to acclimatize the stallions to the breeding barn and the procedures involved with semen collection. Each stallion was trained to mount a breeding phantom and ejaculate into an artificial vagina (AV).

3.3 Thermal Sensor Implants

Two subdermal thermal sensory devices (Digital Angel Corp., St. Paul, MN) were surgically implanted into each stallion by a veterinary surgeon. Prior to surgery, an intramuscular (IM) tetanus toxoid (1 mL) was administered in addition to intravenous (IV) flunixin meglumine (1mg/kg) to control inflammation and analgesia. Xylazine (1 mg/kg, IV) was administered as a sedative prior to induction with ketamine (2 mg/kg, IV), and stallions were placed in dorsal recumbency. A 10 x 10 cm area on the left of the neck over the trapezius muscle was clipped, and the neck area and scrotum were

aseptically prepared for surgery with a betadine and alcohol scrub. A sterile, pre-packaged telemetric probe was preloaded into a syringe with a 12-g needle. The most ventral portion of the scrotum was grasped and the vaginal tunic identified within the scrotum. By tenting the skin of the scrotum, the needle was inserted past the skin into the vaginal tunic, and the plunger was deployed. The skin surrounding the needle was tightly grasped as the needle was withdrawn in order to avoid removing the transponder with the needle. Transponder placement in the scrotum was verified by manual palpation. A second transponder was placed beneath the skin and subcutaneous tissue into the trapezius muscle on the left side of the neck using a similar technique. No closure of skin was needed at either site. Scanners (Pocket Reader EX™, Digital Angel Corp., St. Paul, MN) were used to read internal temperatures measured by the implanted microchips during the study.

3.4 Predicted Daily Spermatozoa Output

Testicular volume was computed from ultrasonographic measurements of width, height, and length of the testes. Predicted daily spermatozoa output (**DSO**) was calculated using a regression formula, as previously reported (Love et al., 1991) (Table 2).

TABLE 2. Predicted daily spermatozoa output (DSO; in billions) for all stallions.

STALLION	DSO
Bon Jovi	0.88
Rock	0.88
Rockstar	0.57
Scooter	0.87
Beatle	0.55
Brave	1.13
Eagle	1.07
Mick Jagger	1.63

3.5 Blocking and Treatment Groups

Ejaculates from each stallion were collected each day for 5 consecutive days, which is sufficient for miniature stallions, although standard protocol for full-sized stallions requires 10 consecutive collections. The mean value for sperm output during the final 2 d of semen collection was used to represent actual DSO, as the numbers of spermatozoa no longer varied significantly (Gebauer et al., 1974; Thompson et al., 2004). No semen was collected for at least 7 d prior to each DSO determination.

Baseline DSO parameters for each stallion were determined at 0 wk. Subsequently, stallions were blocked by age, testicular volume (**TV**), total number of spermatozoa/ejaculate, and percent normal spermatozoa before being randomly assigned to either a non-exercised (**non-ex**; control) or exercised (**ex**; treatment) group (Table 3).

TABLE 3. Mean (\pm SD) values of blocking criteria used to assign stallions to non-ex (control) or ex (treatment) groups (n=4/group).

	NON-EX	EX
Age (yr)	8.25 \pm 4.35	9 \pm 5.10
TV (mL)	67.5 \pm 9.81	74.25 \pm 18.84
Total # Cells ($\times 10^9$)	1.217 \pm 0.34	1.684 \pm 1.057
Normal Cells (%)	63.75 \pm 17.50	70 \pm 8.98

3.6 Fitness Evaluation – Standard Exercise Tests

A high speed, motorized equine treadmill was used to complete a standard exercise test (**SET**) on each stallion before (**pre-EP**) and after (**post-EP**) the exercise period (**EP**) in order to determine whether exercised stallions achieved a higher level of fitness as a result of the exercise protocol prescribed during the study. Prior to the SET, each stallion was fitted with a Polar Equine WearLink™ heart rate monitor (Polar Electro Oy, HQ, Finland). Resting heart rate (**HR**), respiration rate (**RR**), subcutaneous neck temperature (**SQNT**), RCT, SQST, AMBT, and relative ambient humidity (**HUM**) were recorded. The 28-min SET protocol is listed in Table 4. Heart rate was recorded at 5, 10, 15, 20, and 25 min during exercise. Upon completion of the SET at 28 min, HR, RR, and all temperatures (SQNT, RCT, and SQST) were recorded. The HR monitor remained on each stallion during a 10 min hand-walking cool-down to record the HR, RR, and temperatures (SQNT, RCT, and SQST) at 10 min post-SET. The monitor was then removed, and the stallion was tied in a stall for 20 min. All temperatures (SQNT, RCT, and SQST) were recorded at 30 min post-SET. Additionally, blood was collected from each stallion immediately before and after SET via jugular venipuncture with

lithium heparinized vacutainer tubes (BD Vacutainer[®], Franklin Lakes, NJ). Tubes of blood were immediately placed in a 16°C cooler (Koolatron PC3, Koolatron[™], Brantford, Ontario) until delivery to the Clinical Pathology Laboratory at the Texas A&M University Veterinary Medical Teaching Hospital within 4 h of collection, where blood lactate concentrations were analyzed.

TABLE 4. Standard exercise test (SET) protocol for all stallions; used before (pre-EP) and after (post-EP) the exercise period (EP).

TIME (min)	VELOCITY (m/s)	GRADE (%)
0	1.8	0
5	2.4	0
10	2.4	2.1
15	4	2.1
20	4.5	2.8
25	5.5	2.8
28		

3.7 Exercise

During a 12-wk period (between DSO determinations at wk 0, 4, 8, and 12), ex stallions (n=4) were exercised on a high-speed motorized equine exerciser. Exercise protocol was divided into 3 phases: Phase I (8 min walking warm-up), Phase II (15 min long trot/canter), and Phase III (8 min walking cool-down) (Table 5). Exercise began at 1200 h on each day of exercise. A target heart rate of 150 to 160 beats per minute (**bpm**) was achieved and maintained during Phase II. Temperatures (SQST, SQNT, and RCT) were recorded before exercise, immediately after Phase II, immediately after

Phase III, and at 60 and 120 min post-exercise. Ambient temperatures were recorded hourly via iButton® temperature loggers (Maxim DS1923 iButton®, Maxim Integrated Products, Inc., Sunnyvale, CA) located in the shaded stall area (where pre- and post-exercise temperatures were measured) and in the sun near the motorized equine exerciser (where Phase II and III temperatures were measured). Hourly HUM percentages were obtained (NOAA, 2010) and used to calculate the hourly THI experienced during exercise periods. Non-exercised stallions were tied outside while the ex stallions were being exercised, which subjected both groups to consistent ambient temperatures.

TABLE 5. Exercise protocol (4 d/wk) used for ex (treatment) stallions during a 12-wk exercise period (EP) with temperatures recorded at each time point.

TIME POINTS	DESCRIPTION	DURATION	INTENSITY	LOCATION
0	Resting			Shade
	Phase I	8 min	Walk	Sun
22	Phase II	15 min	Long trot/Canter	Sun
30	Phase III	7 min	Walk	Sun
60 min-post	Recovery	60 min		Shade
120 min-post	Recovery	60 min		Shade

^aExercise began at 1200 h on each day of exercise

3.8 Semen Collection

Throughout the study, 4 DSO determinations were completed for each stallion at 0, 4, 8, and 12 wk, with 0 wk representing baseline values. Ejaculates were collected at the Texas A&M University Horse Center Breeding Farm facilities using a 33 cm Missouri-model AV (Nasco, Ft. Atkinson, WI). Each AV was lubricated thoroughly

with Priority Care Lube® (Nasco, Ft. Atkinson, WI) and then filled with 48.8 to 54.4°C water, depending on stallion preference. All collection materials were kept in an incubator at 37°C. A sterilized liner (Playtex® Drop-Ins®, Neenah, WI) was placed inside of a collection container with a micromesh semen filter stretched across the opening to filter gel and debris from the semen sample. Gel was discarded upon semen collection, and the sample was taken immediately to the laboratory and placed into a 37°C incubator.

3.9 Semen Processing

Equipment used for semen analysis during this study was maintained at the Texas A&M School of Veterinary Medicine in the Theriogenology Laboratory, which was located approximately 2 km (5 min) from the Texas A&M University Horse Center. Therefore, semen was initially processed at the Horse Center and had to be transported to the Theriogenology Laboratory for analysis. For short-term storage, semen may be cooled to room temperature (20°C) in an effort to reduce the metabolic rate of spermatozoa, thus preventing a decline in viability and motility (Davies Morel, 2008). Additionally, spermatozoa are susceptible to ultraviolet light (Davies Morel, 2008). Therefore, during the present study, semen samples were placed in a placed in a 16°C cooler (Koolatron™ PC3, Koolatron™, Brantford, Ontario), which allowed for maintenance of the samples at a temperature of approximately 20°C in an environment free from ultraviolet light.

Gel-free volume (**VOL**) of each ejaculate was measured in the Horse Center Laboratory using a 10-mL graduated cylinder. Two 0.5-mL aliquots of raw semen were prepared; one aliquot was placed in a 16°C cooler until analysis for concentration and viability, and the second aliquot was frozen in liquid nitrogen and maintained at -80°C for later assessment of DNA quality using the Sperm Chromatin Structure Assay (**SCSA**). Raw semen was also diluted in buffered formol saline for pending morphological analysis. Three additional 1.5-mL aliquots were prepared using the remaining fresh semen diluted with a commercial semen extender (INRA-96, Breeder's Choice, Aubrey, TX) at a 1:4 dilution. One aliquot was placed in the 16°C cooler for later analysis of motion characteristics, and the other two aliquots were stored in separate cooling devices (Equitainer-11, Hamilton-Thorne Biosciences, Beverly, MA), with one sample analyzed after 24 h and the other after 48 h of storage. All the materials used in processing ejaculates were maintained at 37°C in an incubator until time of use. The 16°C cooler, containing a 0.5-mL aliquot of raw semen and a 1.5-mL aliquot of extended semen, was transported to the Theriogenology Laboratory for analysis within 30 min of collection.

3.10 Semen Analysis

Concentration of Spermatozoa. Upon arrival at the Theriogenology Laboratory, fresh (**T0**) samples were removed from the 16°C cooler. Raw semen was analyzed for fluorescence-based spermatozoa concentration analysis (**CONC**) and membrane integrity (**VIAB**) (NucleoCounter SP-100; ChemoMetec, Allerød, Denmark).

Spermatozoal Motility. Extended semen was analyzed for percentage of total motile (**TMOT**) and progressively motile (**PMOT**) spermatozoa via computer-assisted spermatozoa motion analysis (CASMA, IVOS version 12.2 L, Hamilton-Thorne Biosciences, Beverly, MA). Prior to analysis, extended semen samples were further diluted with INRA-96® extender to achieve a concentration of 25 to 30 x 10⁶ spermatozoa/mL. Samples were then placed in a 37°C incubator for 15 min prior to motility analysis (Jasko et al., 1992). 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) were loaded with a 6-µL volume of extended semen and inserted into the CASMA instrument for evaluation. A total of 10 microscopic fields and a minimum of 500 spermatozoa were examined per sample. Settings for the IVOS system included the following: 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-0.60 to 2.00; static head intensity,-0.20 to 2.01; static elongation-40 to 85; LED illumination intensity-2200.

Spermatozoal Morphology. Fresh semen samples preserved in 10% buffered formol saline were evaluated using a wet mount preparation and differential interference contrast microscopy (Olympus BX60, Olympus America, Inc., Melville, NY, 1250X magnification). Specifically, 1 to 2-µl drop was placed under a coverslip, and a total of 100 cells were counted per sample. The percent of morphologically normal sperm

(**NORMCELL**) were identified, as well as specific morphologic abnormalities including abnormal heads (**AH**), acrosomes (**AA**), and midpieces (**AM**), detached heads (**DH**), bent tails (**BT**), coiled tails (**CT**), and premature germ cells (**PGC**) (Kenney et al., 1983). Percentages of each abnormality were determined by counting all abnormalities present on a sperm.

Sperm Chromatin Quality. Quality of DNA was assessed using the SCSA as previously described (Love and Kenney, 1998). Samples were thawed in a 37°C water bath. Aliquots (approximately 5 µL) of thawed semen 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 a 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 detergent) 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 it was 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 as the ratio of red/ (red + green fluorescence). The alpha-t (αt) designation is used to describe the relationship between the amounts of green (native, double-stranded DNA) and red (single-stranded DNA) fluorescence. The endpoint evaluated was the percent of cells with abnormal DNA, or the cells outside the main population (**COMP αt**). This was determined by selecting those sperm cells to the right of the main population and represents the number of sperm cells outside the main population as a percentage of the total number of sperm cells evaluated.

Cooled Samples. Samples stored for 24 h (**T24**) and 48 h (**T48**) were evaluated for concentration and viability of spermatozoa, TMOT, PMOT, and DNA quality.

3.11 Statistical Analysis

Spermatozoa and SET 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 group (non-ex 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 that made no assumptions based on distribution of the data, as the data were not normally distributed. A Spearman's Rank Correlation test was performed to determine relationships between SQST, SQNT, RCT, AMBT, HUM, and THI during exercise.

4. RESULTS

4.1 Implantation of Subcutaneous Scrotal Devices

No fever, edema, or other deleterious effects were noted from the subdermal implantation of thermal sensory devices into the necks or scrotums of the stallions in this study.

4.2 Ambient Temperature, Humidity, and Temperature-Heat Index

Hourly AMBT measurements from iButton® temperature loggers fixed in the stall area (shade) and exercise area (sun) did not differ among weeks of the study; however, a difference among time points ($P < 0.0001$) was observed (Fig. 1). The increase in AMBT at 22 and 30 min were recorded during Phases I-III of exercise in the heat of the sun, while 0 min, 60 min-post, and 120 min-post were recorded in the shaded stall area. A steady decrease in HUM was observed during exercise time points but was not significant. Ambient temperature and HUM were used to calculate THI, which did not differ among weeks, but did increase at 22 and 30 min of exercise ($P < 0.0001$) (Fig. 1). Mean AMBT, HUM, and THI for each time point during exercise are listed in Table 6.

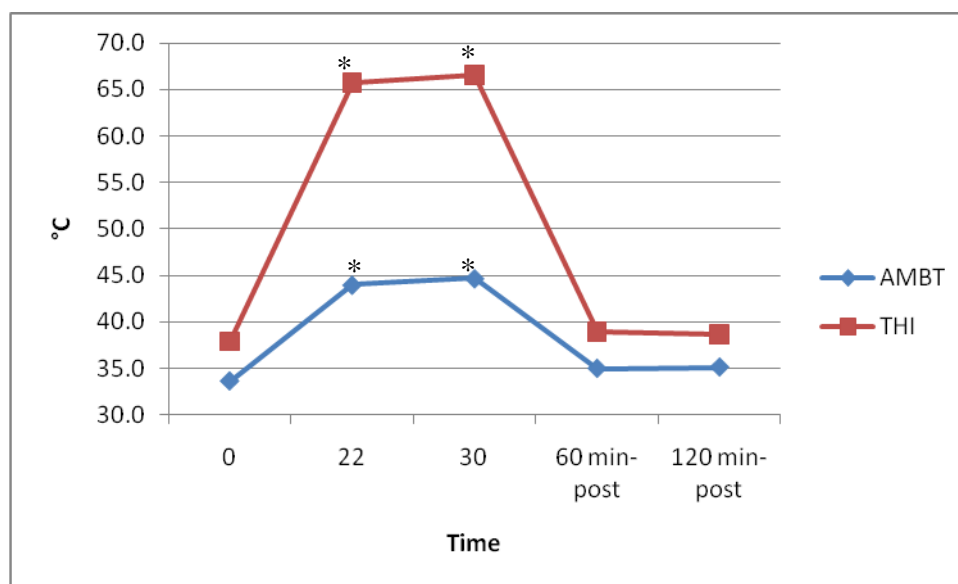


FIGURE 1. Mean ambient (AMBT) and temperature-heat index (THI) to which all stallions were subjected at each time point during exercise protocol.

* Indicates significant increase from other time points along the same line ($P < 0.0001$)

TABLE 6. Mean ambient (AMBT), humidity (HUM), and temperature-heat index (THI) at each time point during exercise.

	0	22	30	60 min-post	120 min-post
AMBT (°C)	33.6	44.0*	44.7*	35.0	35.1
HUM (%)	51.3	47.6	46.7	45.9	44.0
THI (°C)	37.9	65.7*	66.5*	38.9	38.7

* Indicates significant increase from other time points ($P < 0.0001$)

4.3 Changes in Stallion Temperatures Due to Exercise

During exercise protocol, ex stallions achieved a mean average HR of 149 bpm with a mean peak value of 164 bpm. Temperatures (RCT, SQNT, and SQST) during exercise were significantly higher ($P < 0.0002$) in both ex and non-ex stallions at time

points 22 and 30 min compared to other times (0 min, 60 min-post, and 120 min-post; Tables 7 to 9). Rectal and SQNT of ex stallions increased ($P < 0.0001$) by 1.9°C and 2.4°C, respectively, at 22 min of exercise (Fig. 2). Although not significant ($P > 0.05$), a mean increase in SQST of 0.8°C from was achieved from 0 to 22 min of exercise (Fig. 3). Changes in temperature of individual stallions within groups can be viewed in Tables C.1 and C.2.

TABLE 7. Mean (\pm SD) rectal temperatures (RCT) of non-ex (control) and ex (treatment) stallions during exercise protocol (n=4/group).

TIME	RCT	
	NON-EX	EX
0	37.60 \pm 0.18 ^{ac}	37.30 \pm 0.15 ^{ac}
22	37.78 \pm 0.24 ^{ac}	39.22 \pm 0.43 ^{bd}
30	37.82 \pm 0.20 ^{ac}	39.21 \pm 0.47 ^{bd}
60 min-post	37.56 \pm 0.17 ^{ac}	37.44 \pm 0.14 ^{ac}
120 min-post	37.41 \pm 0.17 ^{ac}	37.23 \pm 0.19 ^{ac}

^{ab}Means not sharing the same superscript within a column are different ($P < 0.03$)

^{cd}Means not sharing the same superscript within a row are different ($P < 0.03$)

TABLE 8. Mean (\pm SD) subcutaneous neck temperatures (SQNT) of non-ex (control) and ex (treatment) stallions during exercise protocol (n=4/group).

TIME	SQNT	
	NON-EX	EX
0	37.72 \pm 0.23 ^{ac}	37.46 \pm 0.23 ^{ac}
22	38.03 \pm 0.32 ^{ac}	39.94 \pm 0.98 ^{bd}
30	38.01 \pm 0.36 ^{ac}	39.71 \pm 0.90 ^{bd}
60 min-post	37.66 \pm 0.31 ^{ac}	37.55 \pm 0.23 ^{ac}
120 min-post	37.54 \pm 0.25 ^{ac}	37.46 \pm 0.06 ^{ac}

^{ab}Means not sharing the same superscript within a column are different ($P < 0.0001$)

^{cd}Means not sharing the same superscript within a row are different ($P < 0.0001$)

TABLE 9. Mean (\pm SD) subcutaneous scrotal temperatures (SQST) of non-ex (control) and ex (treatment) stallions during exercise protocol (n=4/group).

TIME	SQST	
	NON-EX	EX
0	33.80 \pm 0.23 ^a	33.91 \pm 1.02 ^a
22	34.23 \pm 0.50 ^a	34.68 \pm 0.92 ^b
30	34.31 \pm 0.44 ^a	34.66 \pm 0.99 ^b
60 min-post	33.83 \pm 0.24 ^a	33.69 \pm 0.24 ^a
120 min-post	33.97 \pm 0.26 ^a	33.59 \pm 0.11 ^a

^{ab}Means not sharing the same superscript within a column are different ($P < 0.03$)

Means across rows are not different ($P > 0.05$)

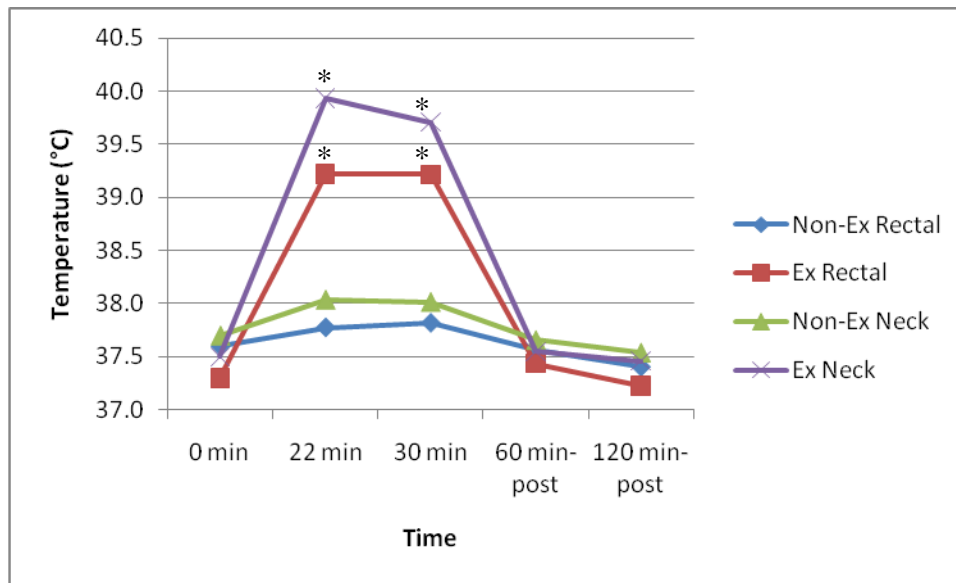


FIGURE 2. Mean rectal and neck temperatures for non-ex (control) and ex (treatment) stallions during the exercise period (n=4/group).
*Indicates significant increase from like time points along the same line ($P < 0.0001$)

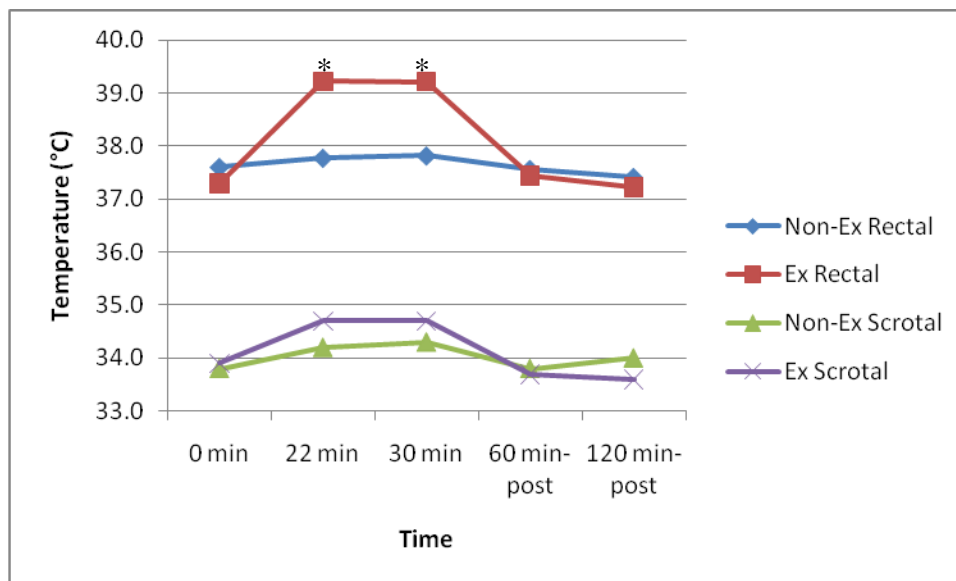


FIGURE 3. Mean rectal and scrotal temperatures for non-ex (control) and ex (treatment) stallions during the exercise period (n=4/group).
*Signifies significant increase from like time points along the same line ($P < 0.0001$)

4.4 Correlation Analysis between Different Temperature Recordings

Significant, positive correlations existed between SQST, SQNT, RCT, AMBT, and THI (Table 10). Humidity showed no significant relationships with any of the measured temperatures in this study.

4.5 Fresh Semen

Semen parameter measurements from the final 2 d of each DSO determination were averaged to determine mean values of fresh semen for wk 0, 4, 8, and 12 (Table 11). No effects of treatment by time were observed for any of the endpoints measured.

TABLE 10. Mean correlations (r_s) of subcutaneous scrotal temperature (SQST), subcutaneous neck temperature (SQNT), rectal temperature (RCT), ambient temperature (AMBT), humidity (HUM), and temperature-heat index (THI).

	SQST	SQNT	RCT
SQNT	0.476*		
RCT	0.761*	0.453*	
AMBT	0.625*	0.513*	0.558*
HUM	-0.074*	-0.263*	-0.025
THI	0.629*	0.453*	0.573*

* indicates $P < 0.0001$

TABLE 11. Mean (\pm SD) semen characteristics for all samples (T0, T24, and T48) of non-ex (control) and ex (treatment) stallions (n=4/group).

	WEEK 0		WEEK 4		WEEK 8		WEEK 12	
	NON-EX	EX	NON-EX	EX	NON-EX	EX	NON-EX	EX
VOLUME (mL)								
T0	9.1 \pm 4.1	9.1 \pm 4.3	12.0 \pm 7.7	7.7 \pm 4.8	11.8 \pm 5.2	11.9 \pm 2.6	7.9 \pm 5.5	10.1 \pm 8.2
CONCENTRATION (million/mL)								
T0	139.9 \pm 35.5	193.5 \pm 39.0	172.1 \pm 29.6	203.8 \pm 51.3	100.5 \pm 24.1	169.8 \pm 35.1	102.6 \pm 61.0	218.1 \pm 127.7
TOTAL CELLS (million spermatozoa)								
T0	1217 \pm 339.7	1683.9 \pm 1057.2	1676.6 \pm 1215.5	1466.3 \pm 808.0	1100.0 \pm 628.1	1908.0 \pm 899.5	667.6 \pm 422.4	1573.4 \pm 592.9
NORMAL CELLS (%)								
T0	61.0 \pm 17.5	72.5 \pm 5.8	58.8 \pm 16.0	74.5 \pm 3.7	51.8 \pm 7.3	73.3 \pm 6.1	57.8 \pm 4.6	71.3 \pm 6.5
VIABILITY (%)								
T0	72.6 \pm 2.4	72.9 \pm 5.5	72.1 \pm 1.9	75.8 \pm 4.1	62.1 \pm 11.3	72.6 \pm 5.7	67.0 \pm 12.8	76.1 \pm 7.2
T24	66.1 \pm 9.0	69.0 \pm 3.1	64.6 \pm 6.2	70.6 \pm 5.5	66.4 \pm 2.9	65.1 \pm 6.8	68.5 \pm 6.9	77.9 \pm 5.4
T48	58.8 \pm 13.8	66.3 \pm 2.4	64.4 \pm 11.5	70.8 \pm 5.1	59.4 \pm 5.0	68.6 \pm 7.4	74.0 \pm 5.7	79.8 \pm 2.4
TOTAL MOTILITY (%)								
T0	71.8 \pm 7.1	75.8 \pm 6.1	71.5 \pm 5.8	77.5 \pm 4.7	63.3 \pm 5.9	70.1 \pm 3.8	58.3 \pm 15.4	76.0 \pm 6.8
T24	68.6 \pm 12.6	73.4 \pm 9.9	67.4 \pm 12.7	74.9 \pm 9.9	55.5 \pm 8.2	65.9 \pm 4.6	49.6 \pm 12.7	64.1 \pm 9.2
T48	49.9 \pm 13.4	56.3 \pm 11.0	44.1 \pm 20.7	56.6 \pm 15.0	35.3 \pm 8.1	47.6 \pm 14.7	40.1 \pm 14.3	56.3 \pm 13.9
PROGRESSIVE MOTILITY (%)								
T0	48.4 \pm 10.7	52.9 \pm 8.8	46.1 \pm 3.6	53.5 \pm 8.7	36.8 \pm 7.2	46.3 \pm 9.1	35.6 \pm 10.9	55.1 \pm 8.5
T24	37.8 \pm 14.2	50.3 \pm 11.8	39.4 \pm 15.3	50.4 \pm 13.4	26.6 \pm 8.4	42.9 \pm 6.4	23.1 \pm 10.7	34.6 \pm 13.6
T48	24.4 \pm 14.7	31.5 \pm 10.7	23.5 \pm 15.3	34.4 \pm 15.4	11.6 \pm 6.1	27.5 \pm 12.7	17.5 \pm 7.1	32.6 \pm 13.0
% COMP-at								
T0	12.7 \pm 8.3	10.8 \pm 6.7	13.3 \pm 6.3	8.3 \pm 0.8	12.8 \pm 3.3	11.2 \pm 6.4	12.1 \pm 4.5	8.8 \pm 2.9
T24	20.9 \pm 11.2	16.0 \pm 5.1	20.4 \pm 8.3	13.9 \pm 3.7	21.9 \pm 3.7	16.9 \pm 5.2	19.6 \pm 3.9	15.1 \pm 4.0
T48	22.7 \pm 13.5	18.0 \pm 3.2	22.9 \pm 7.0	13.8 \pm 1.4	25.2 \pm 4.4	18.0 \pm 4.4	22.3 \pm 3.4	16.9 \pm 4.3

*No significant differences were observed ($P > 0.05$)

No significant difference was observed in gel-free semen volume or spermatozoal concentration between groups (Figures D.1 and D.2, respectively). Total spermatozoal number was calculated by multiplying semen concentration by gel-free semen volume, and no significant difference was observed (Figure D.3). Analysis of spermatozoal viability showed no significant differences between treatment groups (Figure D.4). No effects of treatment by time were observed on TMOT, PMOT, or DNA quality (Figures D.5, D.6, and D.7, respectively). Variations in fresh semen parameters of individual stallions within groups can be viewed in Figures E.1 to E.7.

Mean percentages of normal spermatozoa and morphological abnormalities are presented in Table 12. Percent normal spermatozoa differed consistently between groups for the duration of the study, with ex stallions having a greater percentage of normal spermatozoa than non-ex ($P < 0.02$) (Figure D.8). No effect of treatment by week was observed. Comparisons of respective morphological abnormalities between groups can be viewed in Figures F.1 to F.10.

4.6 Cooled Semen

Cooled semen samples (T24 and T48) were analyzed for VIAB, TMOT, PMOT, and COMPat. No significant differences were reported for any of the endpoints measured (Table 11).

TABLE 12. Mean (\pm SD) percentages of normal spermatozoa and morphologically abnormal spermatozoa observed in non-ex (control) and ex (treatment) stallions at 4 daily spermatozoa output determinations (Week 0, 4, 8, and 12) (n=4/group).

	WEEK 0		WEEK 4		WEEK 4		WEEK 8	
	NON-EX	EX	NON-EX	EX	NON-EX	EX	NON-EX	EX
NORMAL CELL	60.88 \pm 17.43	7.25 \pm 5.92	58.63 \pm 15.99	74.25 \pm 3.97	51.75 \pm 7.27	73.25 \pm 6.13	5.75 \pm 4.65	71.25 \pm 6.50
ABNORMAL HEAD	14.63 \pm 9.69	7.63 \pm 4.13	11.75 \pm 0.65	8.25 \pm 0.96	12.25 \pm 2.63	7.25 \pm 4.27	13.75 \pm 8.42	10.50 \pm 6.40
ABNORMAL ACROSOME	1.50 \pm 1.29	0.25 \pm 0.50	3.88 \pm 3.33	0.88 \pm 0.63	4.50 \pm 4.65	1.25 \pm 1.26	4.75 \pm 2.06	1.75 \pm 1.71
DETACHED HEAD	2.25 \pm 1.71	3.00 \pm 3.67	3.00 \pm 3.46	1.38 \pm 1.25	5.25 \pm 3.30	2.25 \pm 1.89	4.50 \pm 2.65	3.25 \pm 2.50
PROXIMAL DROPLET	12.50 \pm 9.60	10.75 \pm 2.75	11.38 \pm 7.02	7.88 \pm 4.13	10.75 \pm 2.87	9.00 \pm 5.60	9.75 \pm 1.50	6.25 \pm 1.71
DISTAL DROPLET	6.358 \pm 3.28	4.88 \pm 4.25	4.00 \pm 2.80	4.88 \pm 3.40	5.25 \pm 3.30	5.75 \pm 5.91	6.00 \pm 7.35	3.25 \pm 1.26
ABNORMAL MIDPIECE	6.13 \pm 3.68	3.25 \pm 1.32	7.38 \pm 4.99	4.88 \pm 1.93	16.00 \pm 4.32	4.50 \pm 2.08	11.75 \pm 3.77	7.75 \pm 6.24
BENT MIDPIECE	1.25 \pm 0.29	0.63 \pm 0.75	4.00 \pm 3.83	1.63 \pm 1.31	3.50 \pm 1.91	1.25 \pm 0.50	3.50 \pm 2.38	1.50 \pm 0.58
BENT TAIL	1.00 \pm 0.71	0.75 \pm 0.29	2.00 \pm 1.58	1.38 \pm 0.48	2.50 \pm 1.29	0.75 \pm 1.50	2.25 \pm 0.96	2.25 \pm 1.26
COILED TAIL	1.88 \pm 1.44	0.88 \pm 0.48	3.38 \pm 5.76	0.75 \pm 0.50	3.25 \pm 0.96	1.00 \pm 0.00	2.50 \pm 1.00	0.75 \pm 0.50
PREMATURE GERM CELL	0.13 \pm 0.25	0.38 \pm 0.48	0.13 \pm 0.25	0.13 \pm 0.25	0.25 \pm 0.50	0.00 \pm 0.00	0.25 \pm 0.50	0.00 \pm 0.00

*No significant differences in normal cells were observed ($P > 0.05$); statistical analyses were not performed on abnormalities

4.7 Evaluation of Fitness Level

Mean (\pm SD) HR, RR, and blood lactate concentrations were analyzed to determine whether ex stallions achieved a higher level of fitness as a result of exercise protocol (Table 13). Although decreases in all endpoints (HR, RR, and lactate) at 28 min of SET were observed in both groups from pre-EP to post-EP, none were significant (Figures G.1 to G.3). However, differences in RR were reported at the 28 min time point of SET from pre-EP to Post EP ($P < 0.0002$).

TABLE 13. Mean (\pm SD) of heart rate (HR) and respiration rate (RR) of non-ex (control) and ex (treatment) stallions, recorded at 28 min of standard exercise tests conducted before exercise period (pre-EP) and upon completion of the exercise period (post-EP) (n=4/group).

		HR (beats/min)		RR (breaths/min)	
NON-EX					
	PRE-EP	165.75	\pm 19.36 ^a	129.50	\pm 24.30 ^a
	POST-EP	176.75	\pm 22.11 ^a	92.50	\pm 20.02 ^b
EX					
	PRE-EP	189.50	\pm 17.52 ^a	116.00	\pm 25.51 ^{ab}
	POST-EP	172.00	\pm 22.02 ^a	88.00	\pm 19.87 ^{bc}

^{abc}Means not sharing the same superscript within a column are different ($P < 0.0002$)

5. DISCUSSION

To our knowledge, no research has attempted to implant a thermal sensory device into the scrota of stallions. Therefore, the first and foremost objective of the current study sought to determine whether subdermal thermal sensors could be applied to the measurement of neck and scrotal temperatures. No complications arose during scrotal implantation surgery, and one day after the surgery, no swelling, edema, increase in temperature, or trauma to the testicle was apparent. Similarly, no adverse reactions were recorded for the implantation of devices into the subdermis of the neck. One stallion did lose his scrotal implant within 1 wk of surgery; however, it was replaced with no complications. Subdermal thermal sensors can be applied and maintained to the measurement of neck and scrotal temperatures for 3 to 4 mo.

The second objective of the current study was to determine the effect of exercise on RCT, SQST, and SQNT of stallions in hot and humid conditions. Horses exercising in such conditions are not extremely effective at thermoregulation, as they use twice the muscle mass as do humans for locomotion, yet they only have about 50% the skin surface area per unit body mass for evaporative heat loss (Lindinger, 1999). Additionally, since humid conditions cause a decrease in the water vapor gradient for evaporation of sweat, the rate of evaporative heat loss is reduced (Lindinger, 1999). Peak mean AMBT and corresponding HUM during exercise protocol of the current study ranged between 44.0 to 44.7°C and 46.7 to 47.6%, respectively. Ambient temperature and HUM were used to calculate THI, which can be defined as the apparent

temperature in the shade (NOAA, 2010). Peak mean THI during exercise protocol of this project was calculated using AMBT measurements in the heat of the sun, resulting in an extremely high range of 65.7 to 66.5°C, considered extremely dangerous to humans (NOAA, 2010). If humans have a higher capacity for evaporative cooling than do horses, then extreme THI to which stallions were subjected during this study could be potentially more dangerous for horses than for humans. Ambient temperature, HUM, and THI to which stallions were subjected during exercise protocol of the current study were extremely hot and humid, as is characteristic for south-central Texas. Stallions being intensely exercised in such climates may experience difficulty dissipating body heat, resulting in inefficient thermoregulation of the body and, subsequently, the scrotum.

However, stallions used in the current study did not appear to have difficulty dissipating heat generated from the exercise protocol. Increases in RCT of stallions in the present study were not as high as increases in core temperature previously documented in exercised horses (Webb et al., 1987; Scott, 1992; Kohn et al., 1999). Quarter horses conditioned aerobically and trained for cutting work in 45-min bouts experienced a mean RCT of approximately 38.9°C during exercise, with a higher mean temperature of 40.5°C recorded at 30 min recovery (Webb et al., 1987). Daily aerobic conditioning by galloping around an oval track for approximately 50 d prior to a final anaerobic exercise test consisting of four 600-m sprints resulted in maximal core body temperatures averaging 41°C at 15 min recovery (Scott, 1992). Thoroughbred horses were also subjected to 46-min standard exercise tests in varying AMBT and HUM on a

high speed treadmill, with RCT reaching 41.5°C in hot and humid conditions (Kohn et al., 1999). The core temperatures achieved during the exercise protocols employed in these projects were approaching 42°C, which is considered the critical body temperature in the equine that may elicit voluntary exhaustion (Lindinger, 1999). Although mean peak RCT of 39.2°C and SQNT of 39.9°C were achieved, these increases in temperature were not extreme. Therefore, exercise protocol used on the current study cannot be considered as an intense regimen capable of producing maximal core body temperatures. Also, the current study cannot define the period of recovery, as the earliest recovery temperatures were recorded at 60 min-post exercise when temperatures had returned to resting values. By recording stallion temperatures every 10-min for a period of 60-min post exercise, a more defined recovery period would have been apparent.

All stallions experienced an increase in RCT, SQNT, and SQST at 22 and 30 min of exercise protocol, which was likely due to the extreme THI and related AMBT and HUM during those time points. All stallions were removed from their shaded stalls at 0 min and were either tied (non-ex) or exercised (ex) in the heat of the sun; this explains the temperature increases seen in the control (non-ex) group. All stallions were returned to their shaded stalls immediately following the 30 min time point, which elicited an apparent decrease in RCT, SQNT, and SQST at 60 min-post and 120 min-post exercise in all stallions.

The third and final objective of the present study sought to determine the relationship between changes in RCT, SQST, and SQNT and sperm quality. Abundant literature describes changes in concentration, number of spermatozoa per ejaculate,

motility, morphology, and integrity of spermatozoal chromatin structure resulting from SST increases of 1 to 3.5°C via scrotal insulation for prolonged periods of time (Freidman et al., 1991; Blanchard et al., 2000; Love and Kenney, 1999; Ross and Entwistle, 1979; Vogler et al., 1993; McNitt and First, 1970; Barth and Bowman, 1994). In the present study, a mean increase in SQST of only 0.8°C was achieved in ex stallions from 0 to 22 min of exercise. No adverse effects on semen parameters were observed, which is similar to results from studies in men that reported no changes in semen quality due to SQST increases of 0.8 to 1.0°C (Wang et al., 1997). Rams subjected to a 1.4 to 2.2°C increase in SQST for 21 d experienced a significant decrease in fertility (Mieusset et al., 1992). Clearly, prolonged increases in scrotal temperature due to insulation have detrimental effects on reproductive parameters, as the scrotum cannot regulate and dissipate heat. Stallions in the current study did experience elevated RCT and SQNT during exercise; however, the increase only lasted a maximum of 30 min/d for 4 d/wk, and SQST never increased significantly. The implemented exercise protocol more appropriately mirrored current industry standard exercise regimens for performance horses, and only intermittent increases in temperature due to exercise were noted. Since Miniature Horse stallions were used, effects of breed differences may exist. Nevertheless, results of the current study suggest that daily short-term exercise in hot and humid climates has little effect on RCT and SQST and no adverse effects on semen quality.

Most studies have used SST as the endpoint to determine thermoregulatory efficiency of the testes. However, studies utilizing intratesticular thermistors measured

the temperature of the testicular parenchyma and found a low correlation between SST and ITT (Kastelic et al., 1995). Subcutaneous scrotal temperature displayed a low to moderate correlation with ITT (Kastelic et al., 1995), which suggests that SQST may be a slightly more accurate measurement than SST for predicting ITT. Subcutaneous scrotal temperature in the current study showed a correlation with RCT ($r_s = 0.761$), AMBT ($r_s = 0.625$) and THI ($r_s = 0.629$), which implies that further studies might be successful in formulating an equation to determine SQST based on RCT.

Standard exercise tests to which the stallions were subjected during the current study displayed no significant evidence that a higher level of fitness was achieved by the exercise protocol employed. Although respiration rates of ex stallions were significantly lower during the post-EP SET, heart rates did not differ from the pre- to post-EP SET. Blood lactate levels may have indicated a higher level of fitness post-EP; however, some samples were hemolyzed, which compromised the integrity of the assay. Therefore, the exercise protocol can only be considered as minimally intense, as it did not achieve greater fitness in the stallions subjected to it.

Results of the current study are consistent with reports by Dinger et al. (1986) that showed no change in semen parameters of stallions being subjected to minimal or short term daily exercise. A conflicting report claimed that stallions trained for intermediate and advanced levels of dressage or show jumping for 1 to 2 h/d had significantly higher spermatozoal motility than stallions used for breeding alone (Lange et al., 1997). However, these stallions were not blocked into groups according to age or reproductive parameters, and no specific exercise protocol was defined. Further

contradictory literature reported a decrease in the percent normal cells and motility of fresh and frozen-thawed semen during and up to 1 month after intense exercise until exhaustion for 2 d/wk (Janett et al., 2006). Neither rectal nor scrotal temperatures were measured during this study; therefore, it cannot be determined whether the detriment to spermatozoal parameters was due to increased temperatures or stress levels.

Future research on this topic may benefit from a longer exercise protocol. In the current study, the stallion scrotum appeared to efficiently dissipate heat during and after exercise; however, prolonged exercise protocol would likely result in prolonged elevated body temperatures, which might have more of an effect on reproductive parameters. Staempfli et al. (2006) recorded peak SST values when horses were at rest following exercise; the faster the horse moved, the more efficient the scrotal cooling. Roping and cutting stallions are subjected to intense but interrupted exercise regimens, stopping between performances. These horses likely experience elevated temperatures during exercise, followed by a period of standing at rest, followed by exercise. During rest periods, the scrotum may not receive optimal air flow; therefore, efficient scrotal cooling could be hindered. A future study could examine the effects of interrupted exercise on stallion reproductive efficiency.

6. CONCLUSION

The objectives of the current study were to: determine whether subdermal thermal sensors could be applied to the measurement of SQNT, RCT, and SQST; determine the effect of exercise on RCT, SQST, and SQNT of stallions in hot and humid environments; determine the relationship between changes in RCT, SQST, and SQNT and sperm quality. The results indicate that SQST was not affected by exercise protocol, and thus semen parameters remained unaffected.

Although an increase in core body temperature was successfully induced by exercise protocol, scrotal temperatures were not significantly affected, indicating efficient thermoregulation of the testes by the scrotum. Since the testes experienced no significant thermal insult during the exercise protocol, no significant changes in fresh or cooled semen parameters were evident as a result of exercise or elevated core temperature.

No fever, edema, or other deleterious effects were noted from the subdermal implantation of thermal sensory devices into the necks or scrotums of the stallions in this study. Therefore, the implantation of thermal sensory devices into the subdermis of the scrotum can be considered as a safe and effective method to monitor testicular thermal stress.

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APPENDIX A
PROJECT ORGANIZATION

TABLE A.1 Timeline of study protocol.

Dates (2010)	Week	Sun	Mon	Tues	Wed	Thurs	Fri	Sat
4/4 - 7/3				INITIAL REST PERIOD				
5/27 & 6/3				IMPLANT SURGERIES				
7/4 - 7/10	0	BCS	DSO 1	DSO 1	DSO1	DSO 1	DSO 1	EX
7/11 - 7/17	1	BCS	pre-EP SET	pre-EP SET			EX	EX
7/18 - 7/24	2	BCS	EX	EX			EX	EX
7/25 - 7/31	3	BCS	EX	EX			EX	EX
8/1 - 8/7	4	BCS	DSO 2	DSO 2	DSO 2	DSO 2	DSO 2	EX
8/8 - 8/14	5	BCS	EX	EX			EX	EX
8/15 - 8/21	6	BCS	EX	EX			EX	EX
8/22 - 8/28	7	BCS	EX	EX			EX	EX
8/29 - 9/4	8	BCS	DSO 3	DSO 3	DSO 3	DSO 3	DSO 3	EX
9/5 - 9/11	9	BCS	EX	EX			EX	EX
9/12 - 9/18	10	BCS	EX	EX			EX	EX
9/19 - 9/25	11	BCS	EX	EX			EX	EX
9/26 - 10/2	12	BCS	DSO 4	DSO 4	DSO 4	DSO 4	DSO 4	EX
10/2 - 10/5	13	BCS	post-EP SET	post-EP SET				

^aBCS=measurement of weight, body condition, and rump fat

^bDSO1-DSO4=daily spermatozoa output determinations

^cEX=exercise

^dpre-EP SET=standard exercise test performed prior to the exercise period

^epost-EP SET=standard exercise test performed at the cessation of the exercise period

APPENDIX B

DIET

TABLE B.1 Chemical composition of forage diet.

INGREDIENT	AMOUNT	
	%	ppm
Crude protein	12.5	
Acid detergent 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.

INGREDIENT	AMOUNT	
	%	ppm
Crude protein	16.1	
Acid detergent 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

TEMPERATURE CHANGES OF INDIVIDUAL STALLIONS WITHIN GROUPS

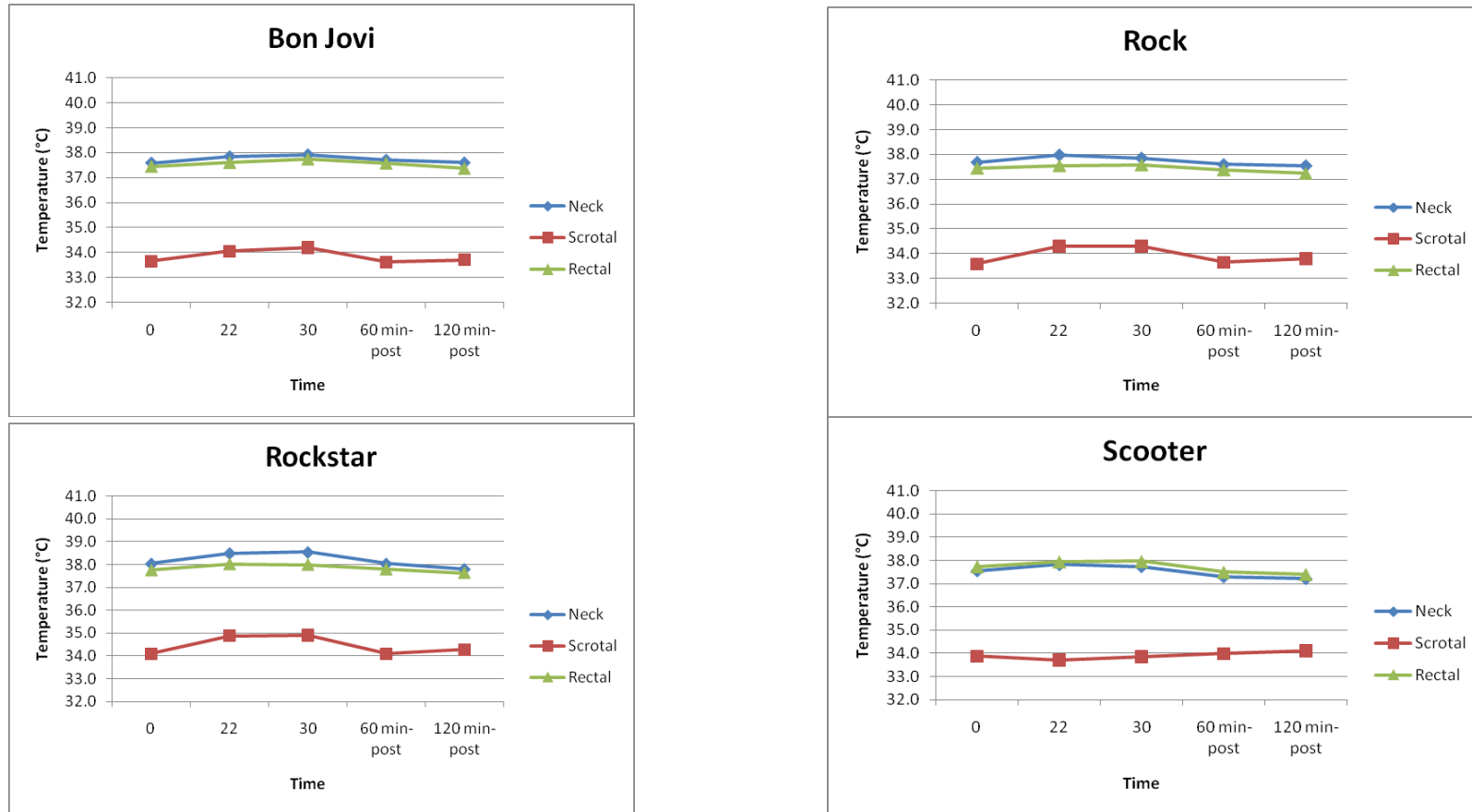


FIGURE C.1 Variations in mean temperature (neck, scrotal, and rectal) of individual stallions within the non-exercised (control) group (n=4).

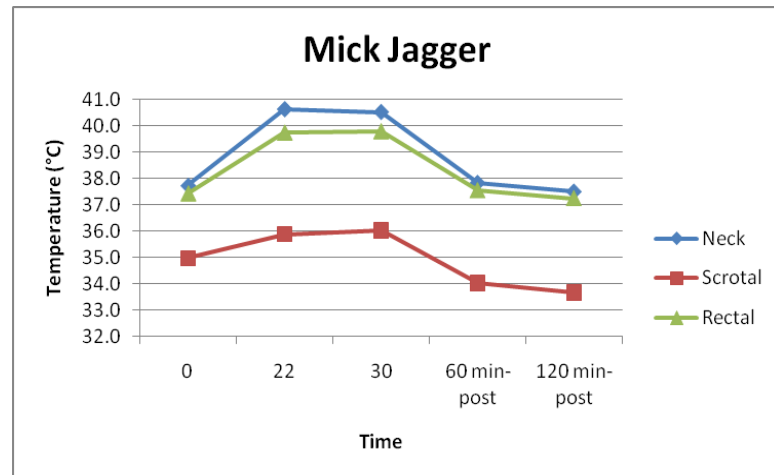
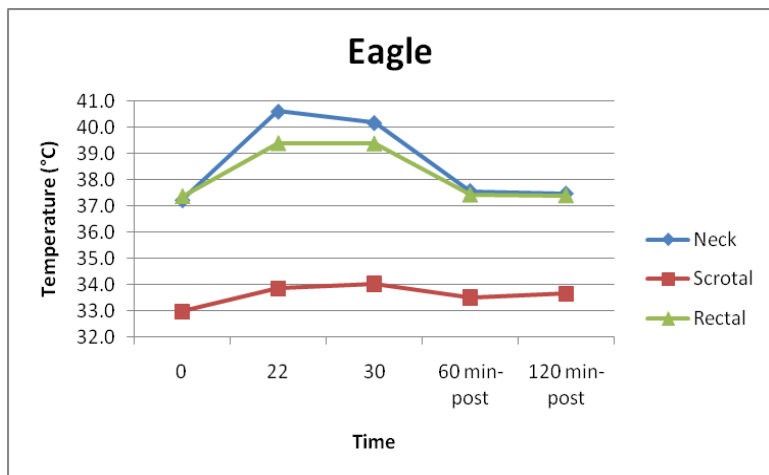
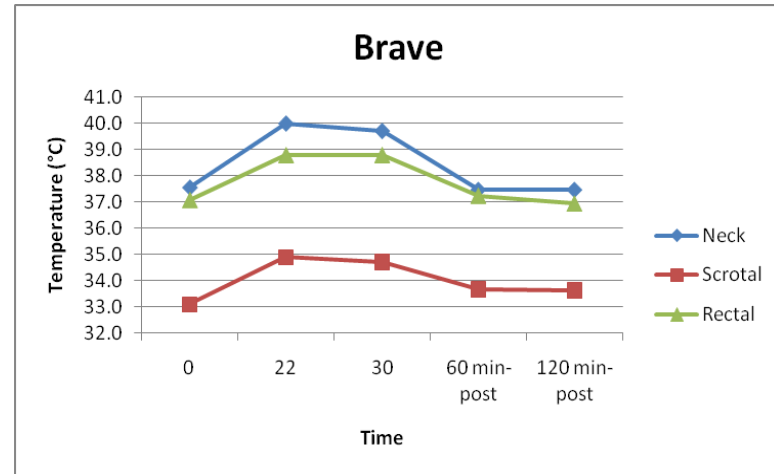
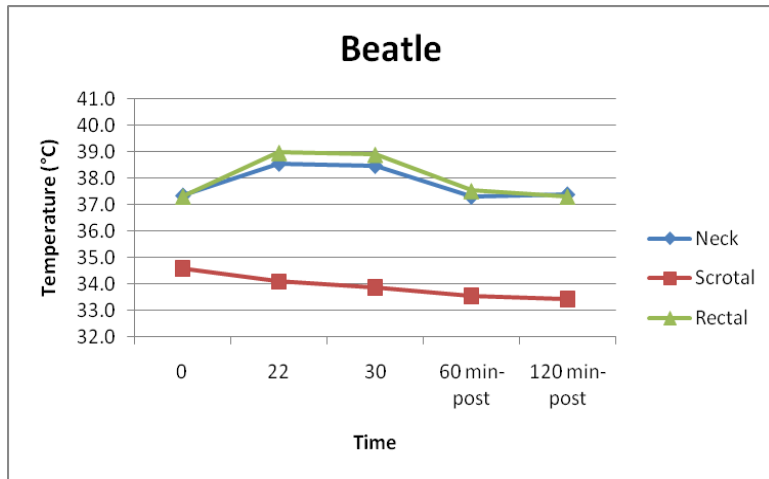


FIGURE C.2 Variations in mean temperature (neck, scrotal, and rectal) of individual stallions within the exercised (treatment) group (n=4).

APPENDIX D

FIGURES DISPLAYING MEAN SEMEN PARAMETERS WITHIN GROUPS

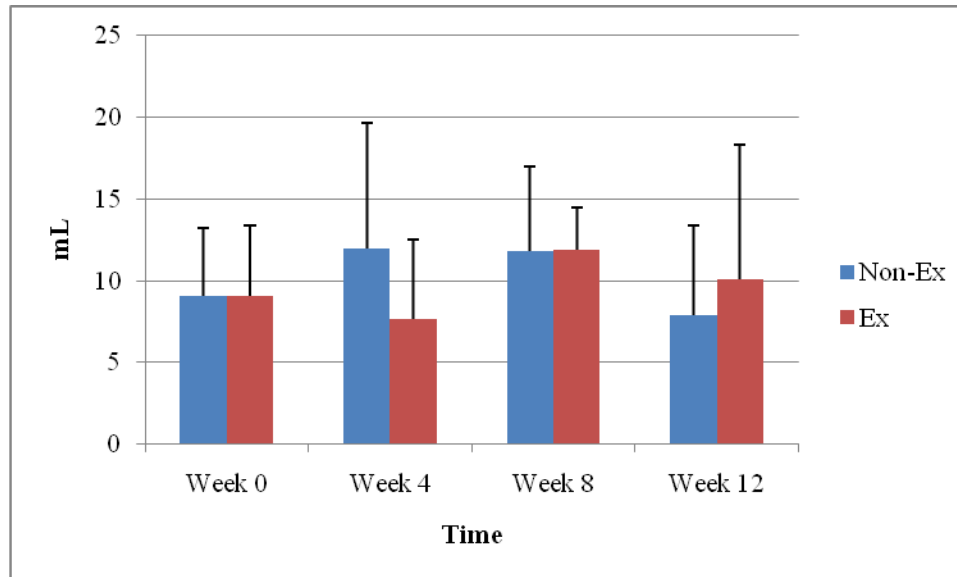


FIGURE D.1 Mean (\pm SD) gel-free semen volume among non-ex (control) and ex (treatment) groups (n=4/group).

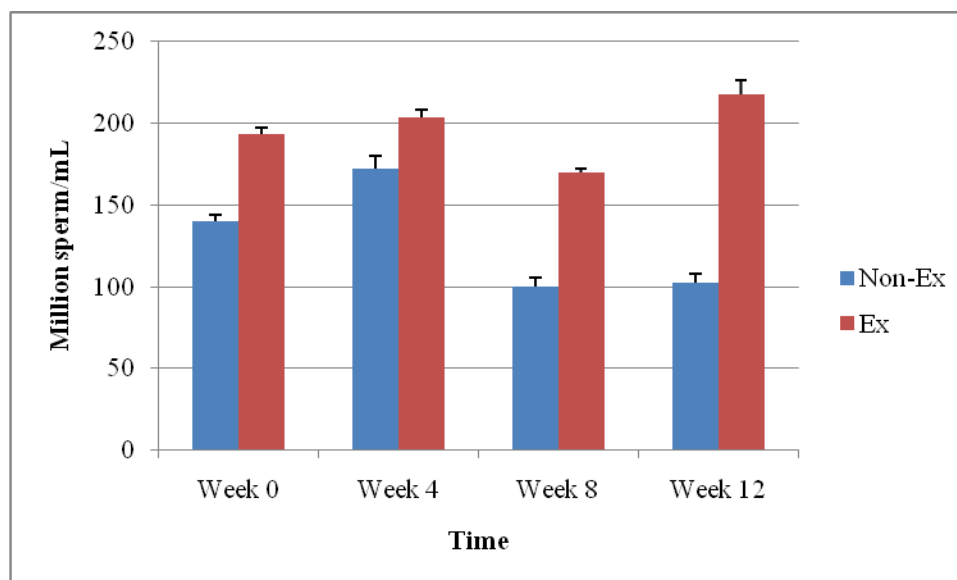


FIGURE D.2 Mean (\pm SD) spermatozoal concentration among non-ex (control) and ex (treatment) groups (n=4/group).

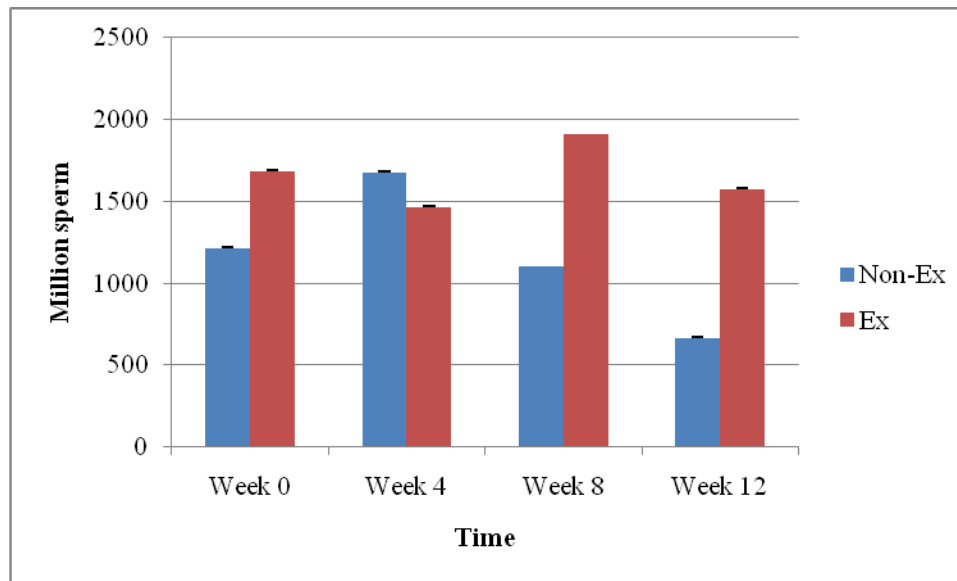


FIGURE D.3 Mean (\pm SD) total number of cells among non-ex (control) and ex (treatment) groups (n=4/group).

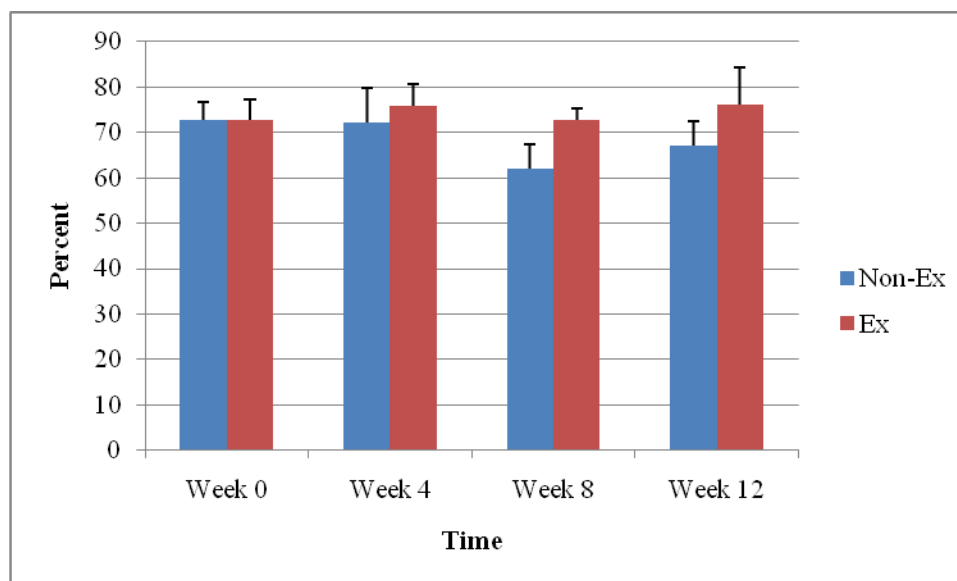


FIGURE D.4 Mean (\pm SD) viability of spermatozoa among non-ex (control) and ex (treatment) groups (n=4/group).

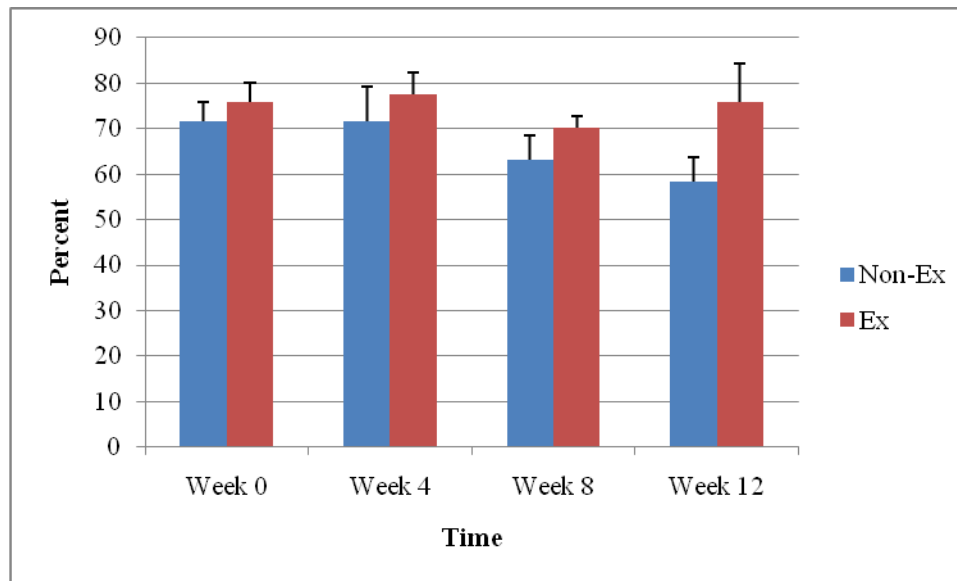


FIGURE D.5 Mean (\pm SD) total motility of spermatozoa among non-ex (control) and ex (treatment) groups (n=4/group).

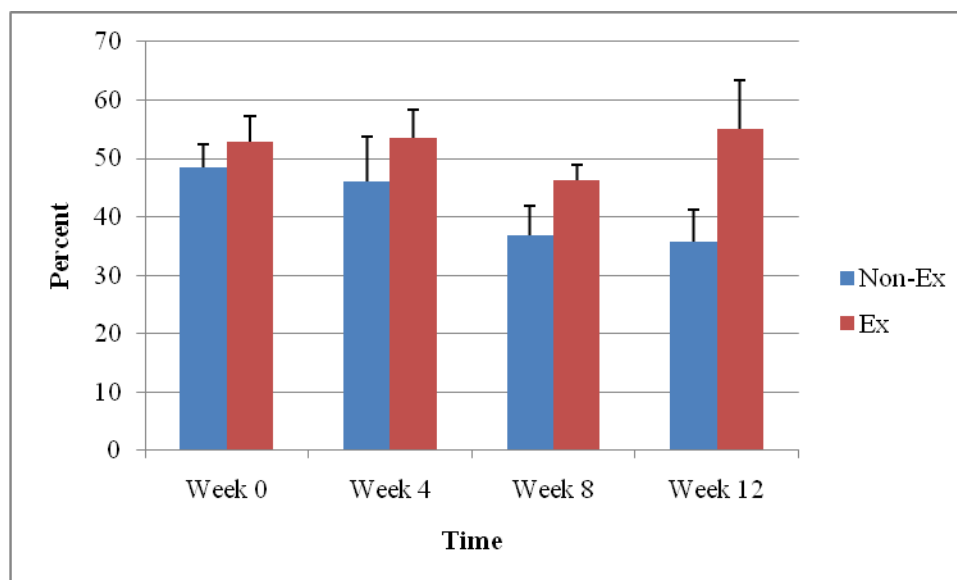


FIGURE D.6 Mean (\pm SD) progressive motility of spermatozoa among non-ex (control) and ex (treatment) groups (n=4/group).

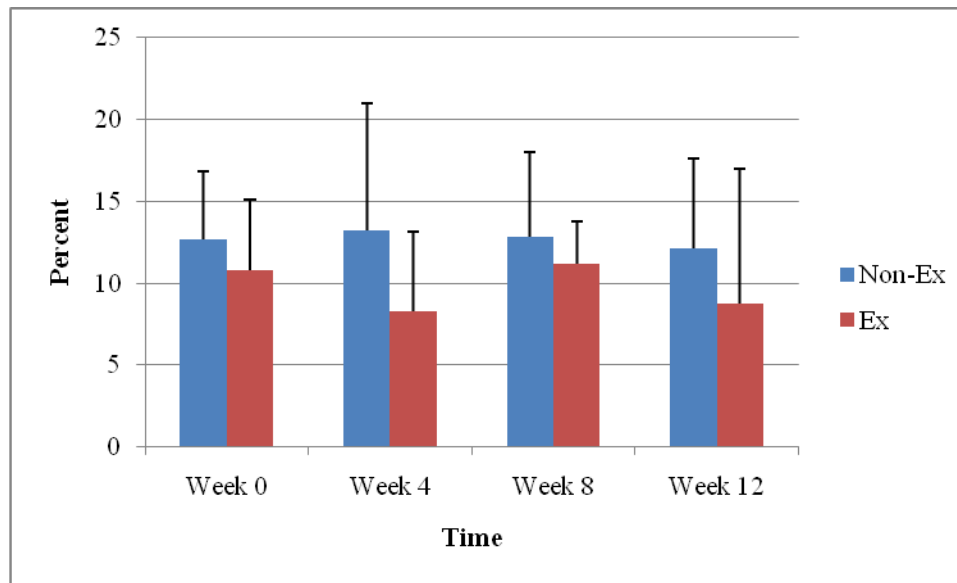


FIGURE D.7 Mean (\pm SD) COMPat of Sperm Chromatin Structure Assay among non-ex (control) and ex (treatment) groups (n=4/group).

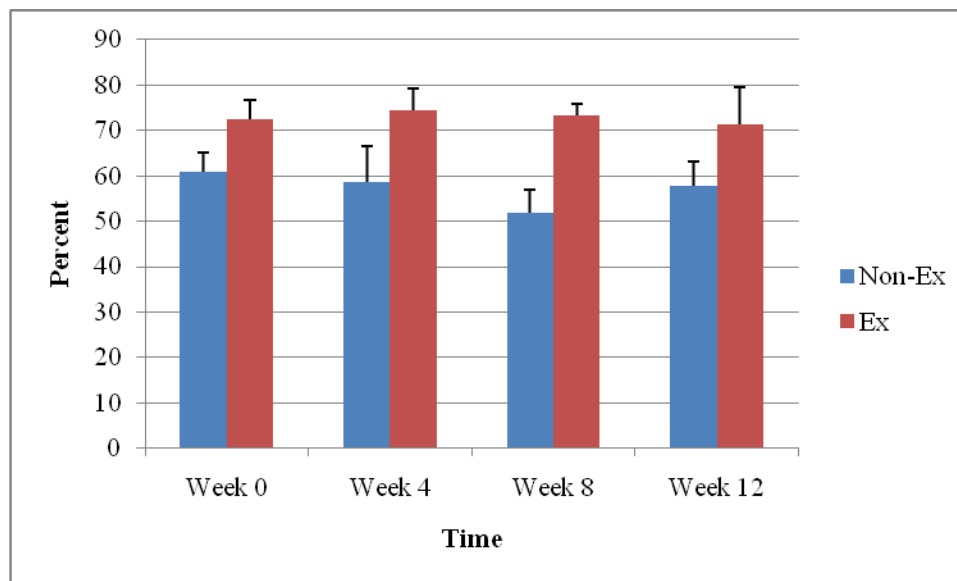
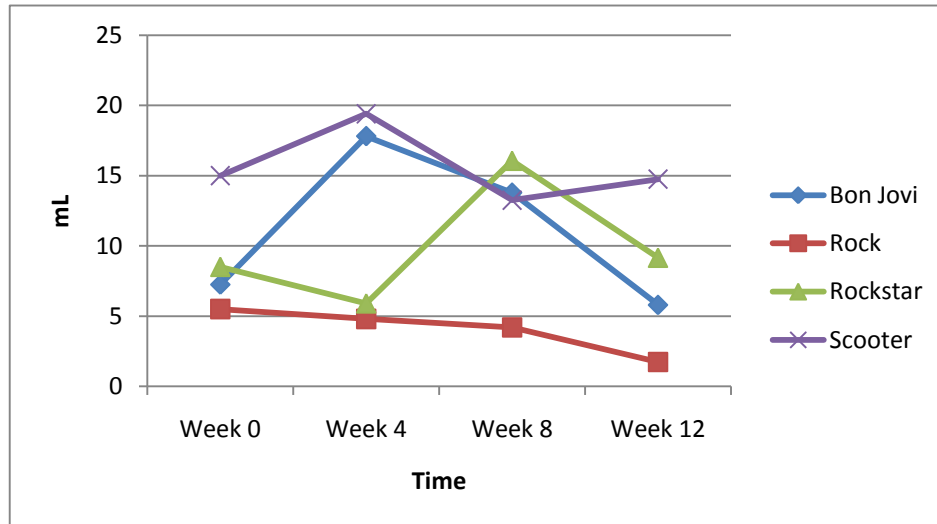


FIGURE D.8 Mean (\pm SD) percentages of normal spermatozoa among non-ex (control) and ex (treatment) groups (n=4/group).

APPENDIX E

INDIVIDUAL STALLION SEMEN PARAMETER FIGURES

a)



b)

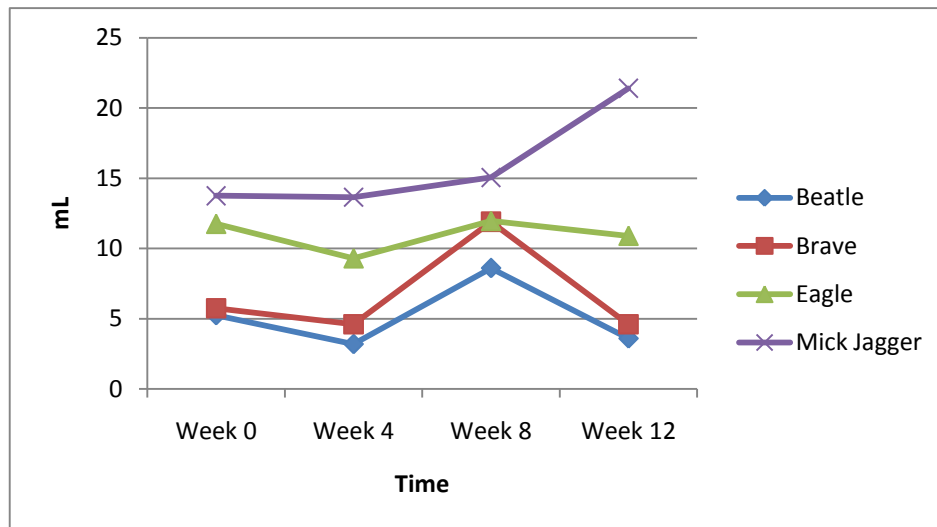
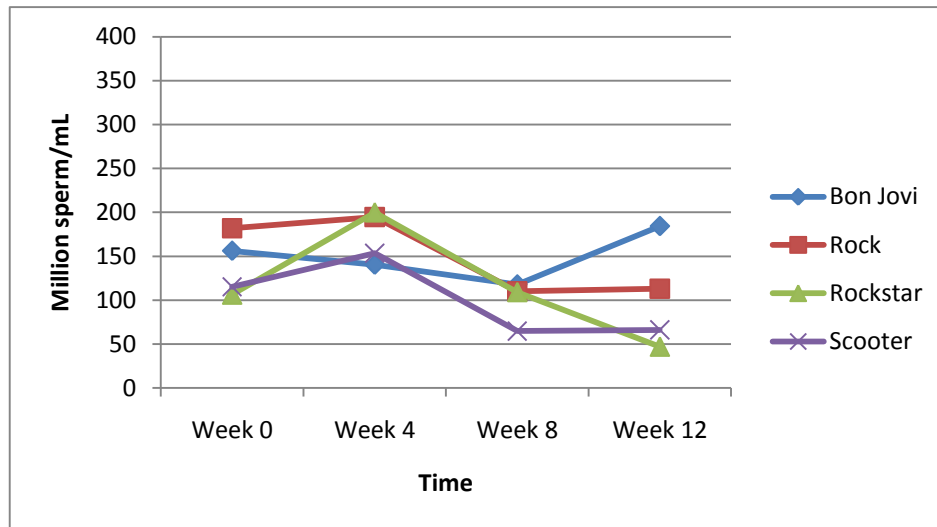


FIGURE E.1 Variations in mean gel-free volume (mL) of individual stallions within treatment groups: (a) non-exercised (control; n=4) and (b) exercised (treatment; n=4).

a)



b)

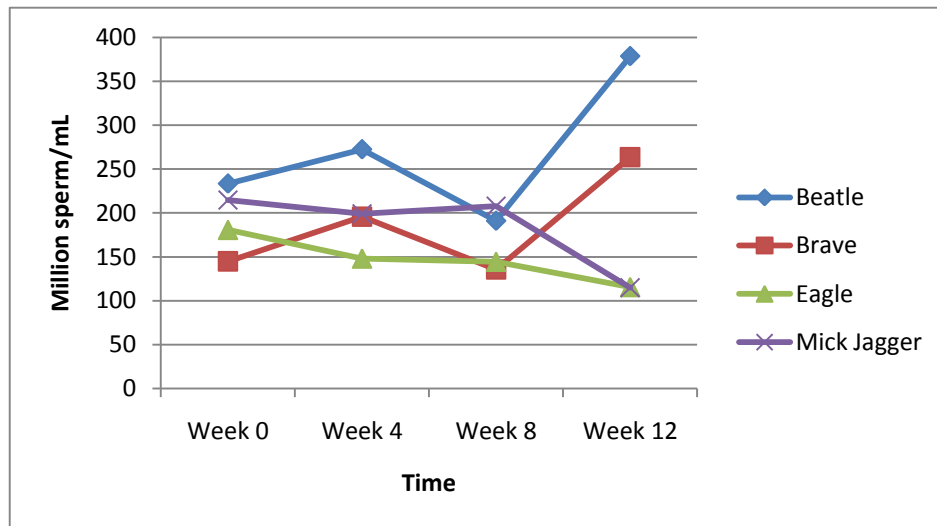
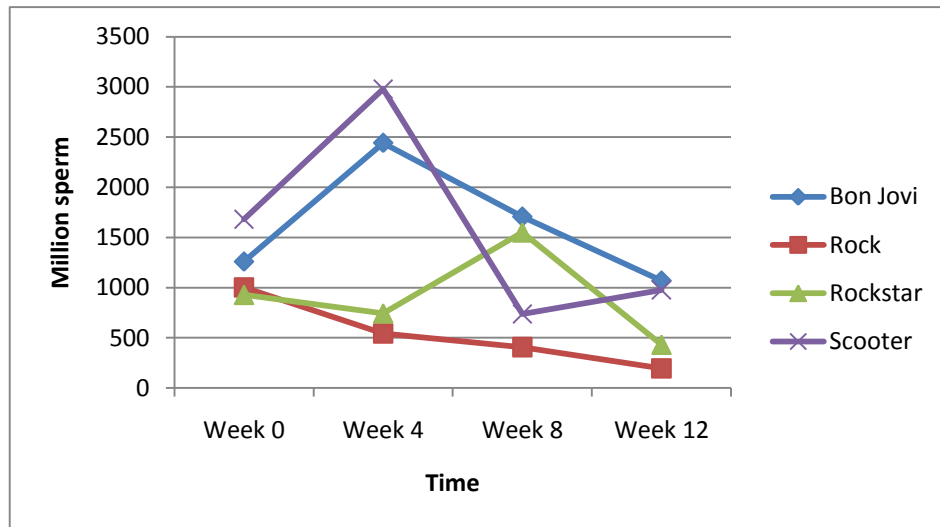


FIGURE E.2 Variations in mean spermatozoal concentration (million spermatozoa/mL) of individual stallions within treatment groups: (a) non-exercised (control; n=4) and (b) exercised (treatment; n=4).

a)



b)

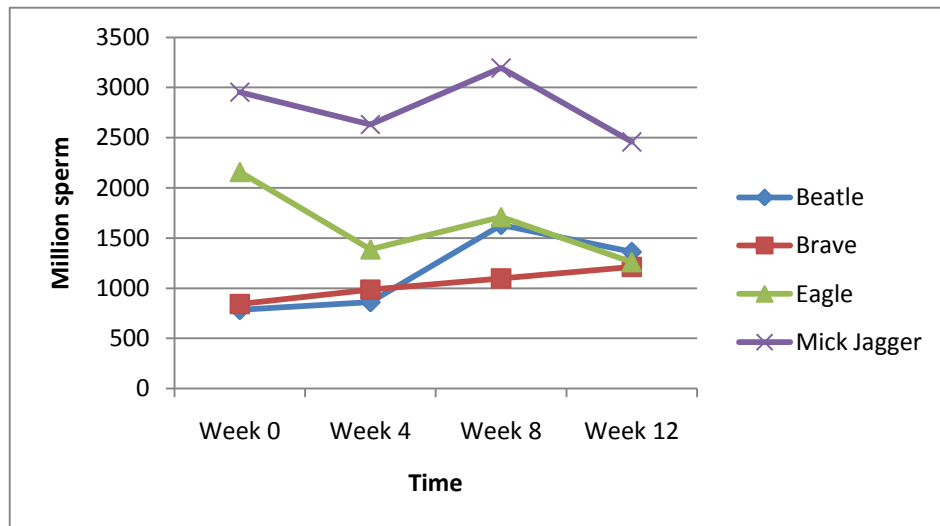
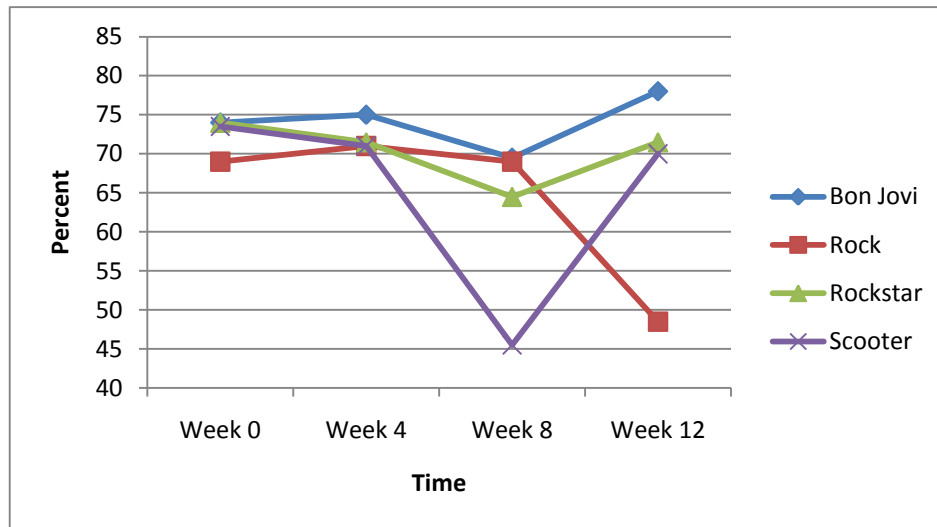


FIGURE E.3 Variations in mean total numbers (million spermatozoa) of individual stallions within treatment groups: (a) non-exercised (control; n=4) and (b) exercised (treatment; n=4).

a)



b)

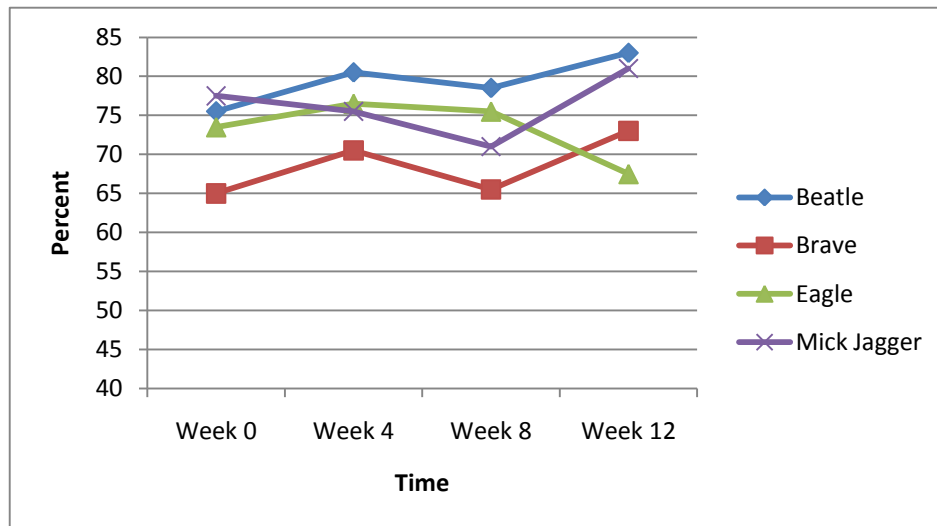
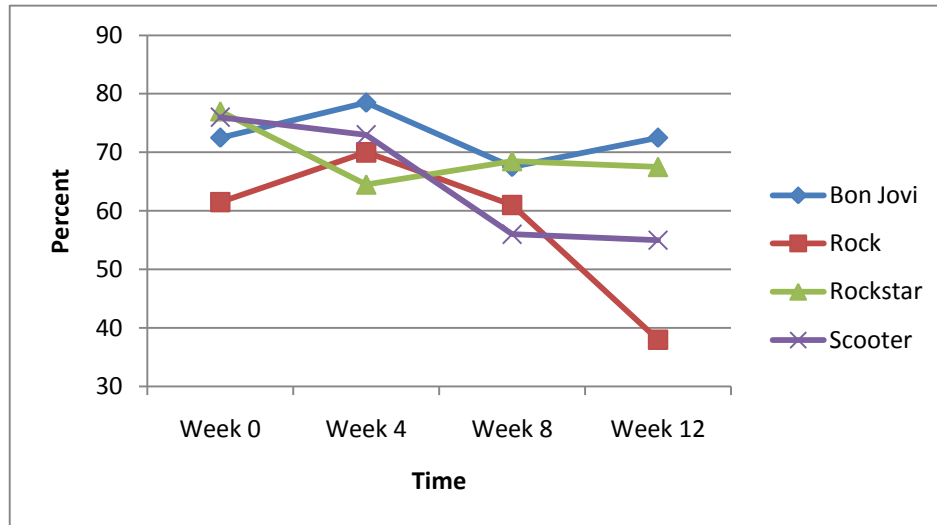


FIGURE E.4 Variations in mean viability (%) of individual stallions within treatment groups: (a) non-exercised (control; n=4) and (b) exercised (treatment; n=4).

a)



b)

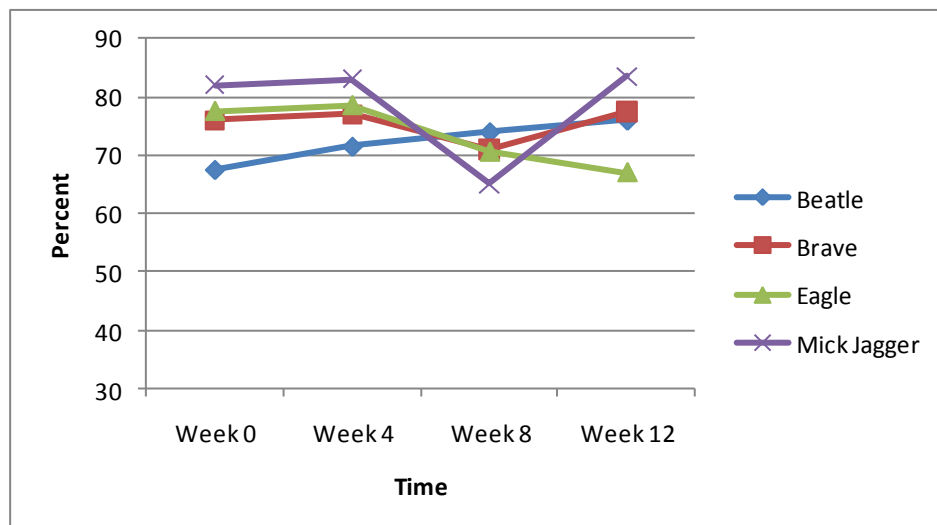
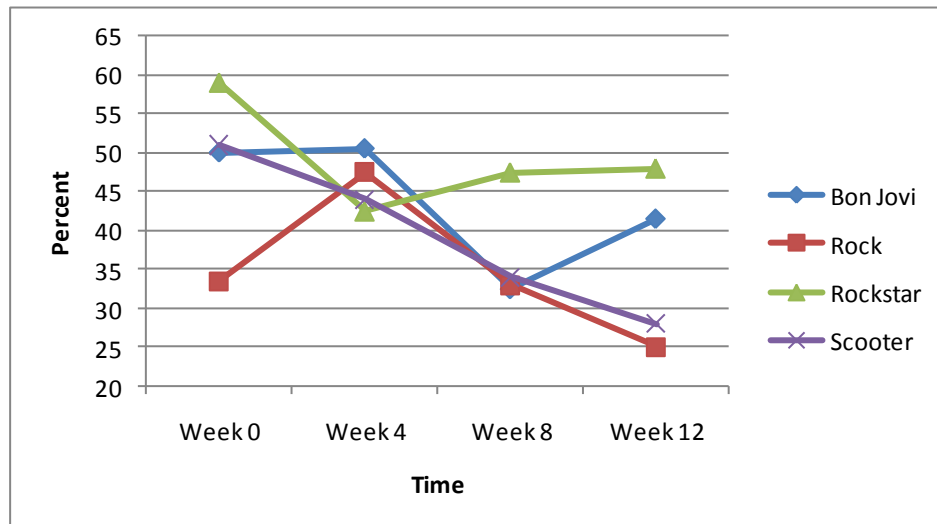


FIGURE E.5 Variations in mean total motility (%) of individual stallions within treatment groups: (a) non-exercised (control; n=4) and (b) exercised (treatment; n=4).

a)



b)

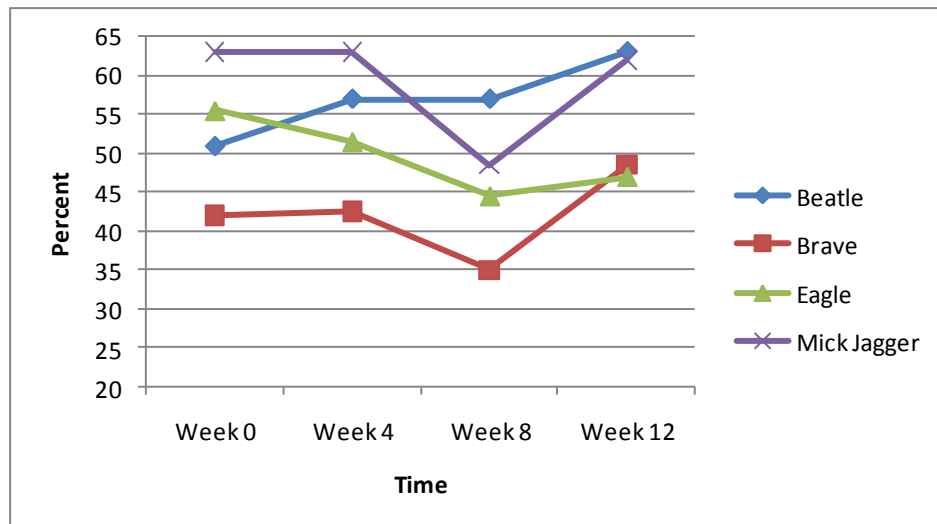
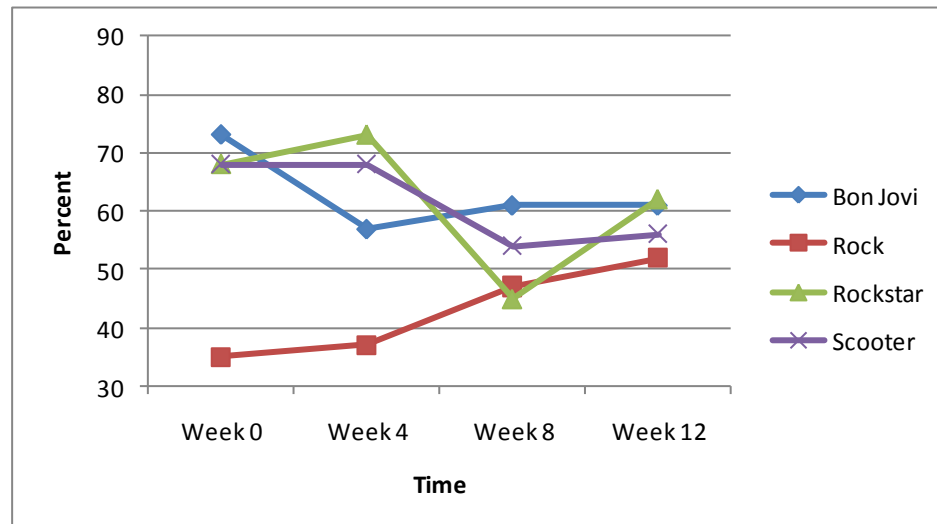


FIGURE E.6 Variations in mean progressive motility (%) of individual stallions within treatment groups: (a) non-exercised (control; n=4) and (b) exercised (treatment; n=4).

a)



b)

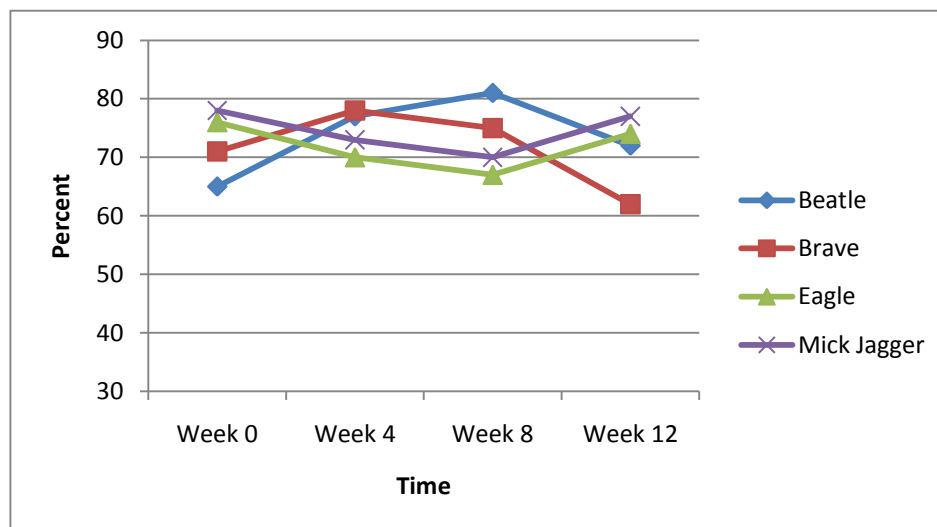


FIGURE E.7 Variations in mean normal spermatozoa (%) of individual stallions within treatment groups: (a) non-exercised (control; n=4) and (b) exercised (treatment; n=4).

APPENDIX F

MORPHOLOGICALLY ABNORMAL SPERMATOZOA BETWEEN GROUPS

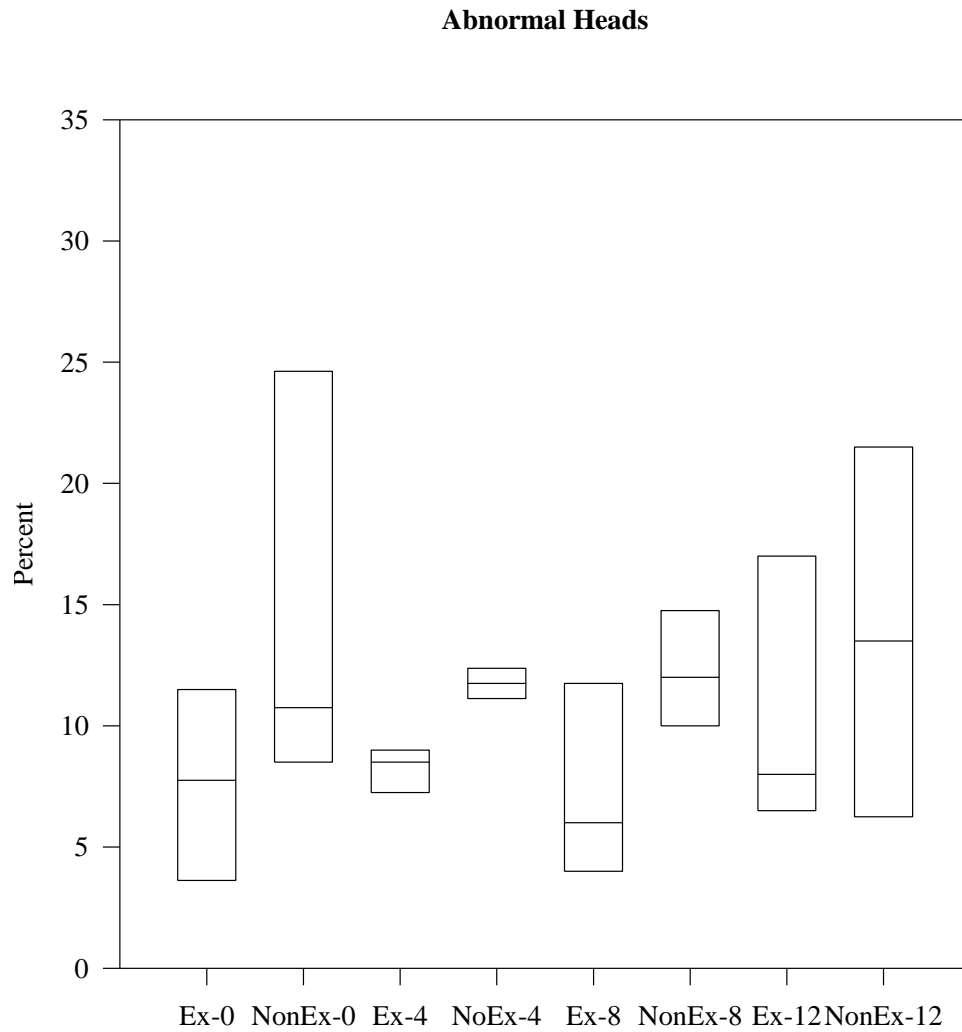


FIGURE F.1 Box plots representing mean percentages of abnormal heads among non-ex (control) and ex (treatment) groups at weeks 0, 4, 8, and 12 (n=4/group).

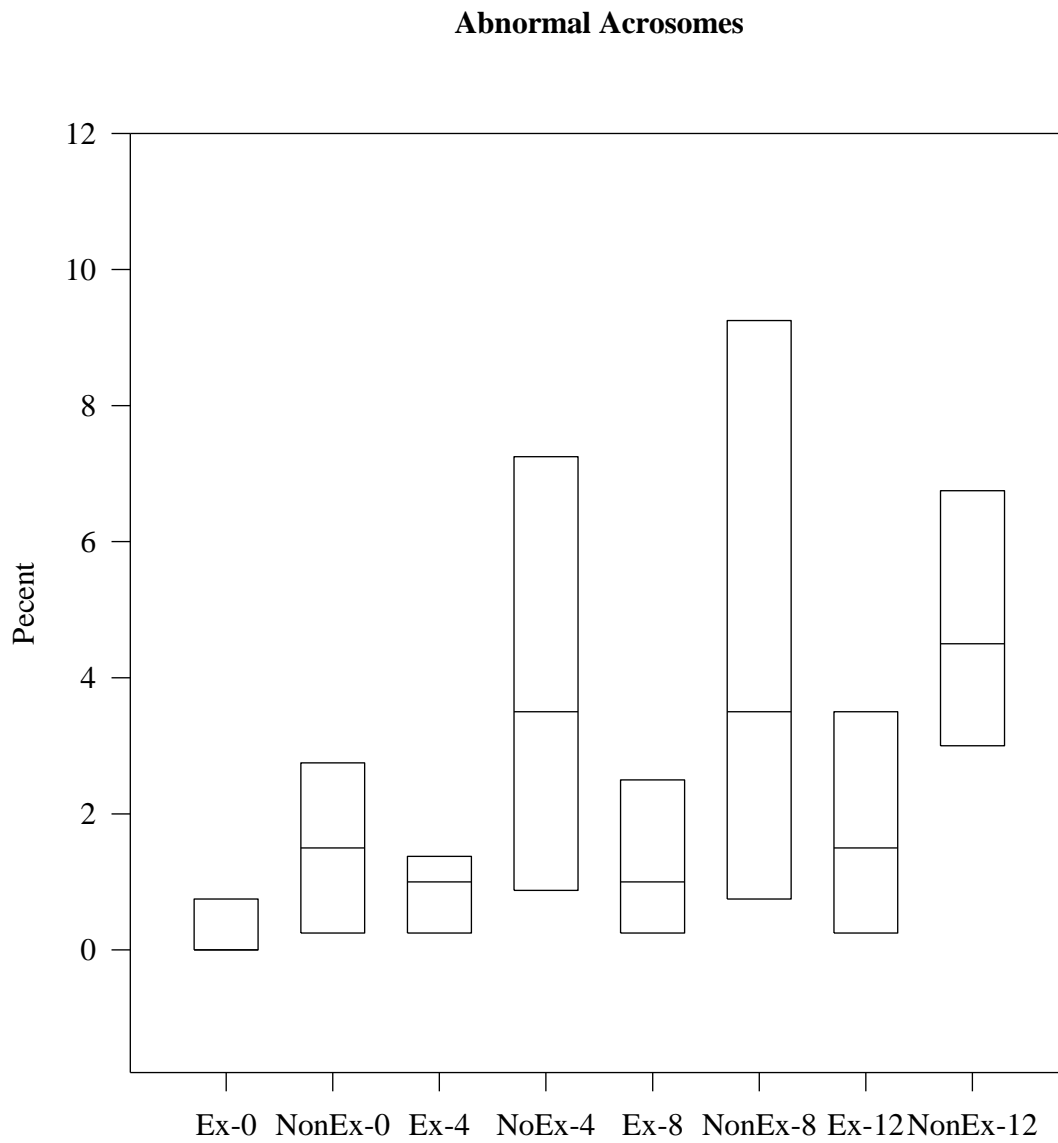


FIGURE F.2 Box plots representing mean percentages of abnormal acrosomes among non-ex (control) and ex (treatment) groups at weeks 0, 4, 8, and 12 (n=4/group).

Detached Heads

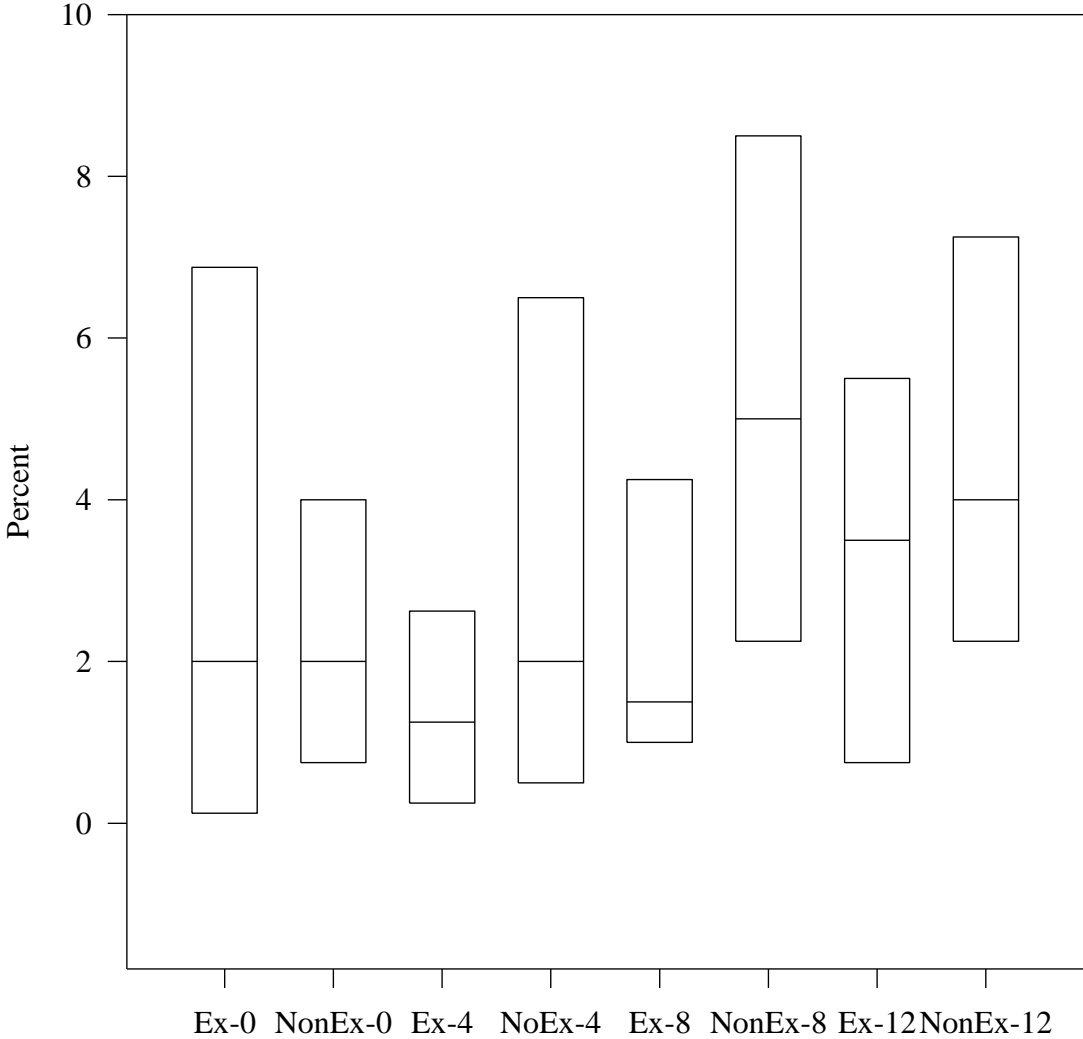


FIGURE F.3 Box plots representing mean percentages of detached heads among non-ex (control) and ex (treatment) groups at weeks 0, 4, 8, and 12 (n=4/group).

Proximal Droplets

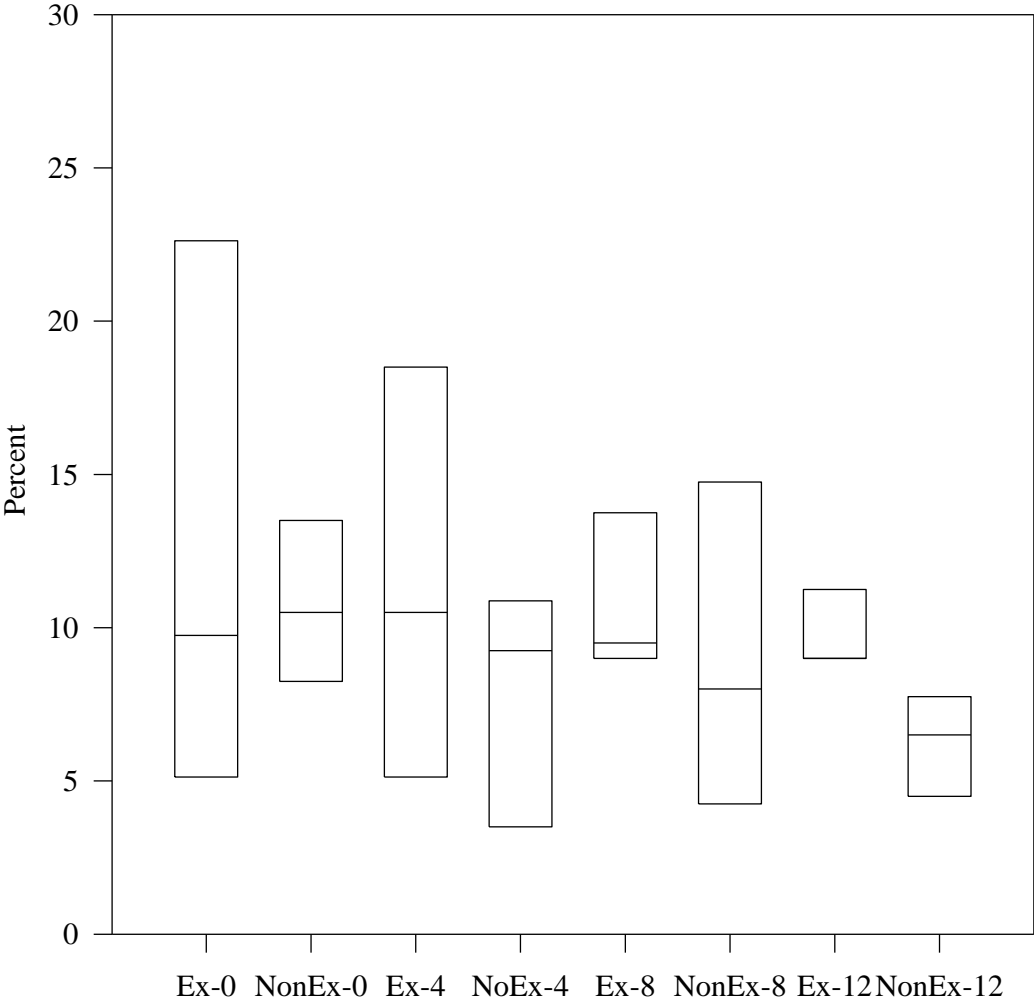


FIGURE F.4 Box plots representing mean percentages of proximal droplets among non-ex (control) and ex (treatment) groups at weeks 0, 4, 8, and 12 (n=4/group).

Distal Droplets

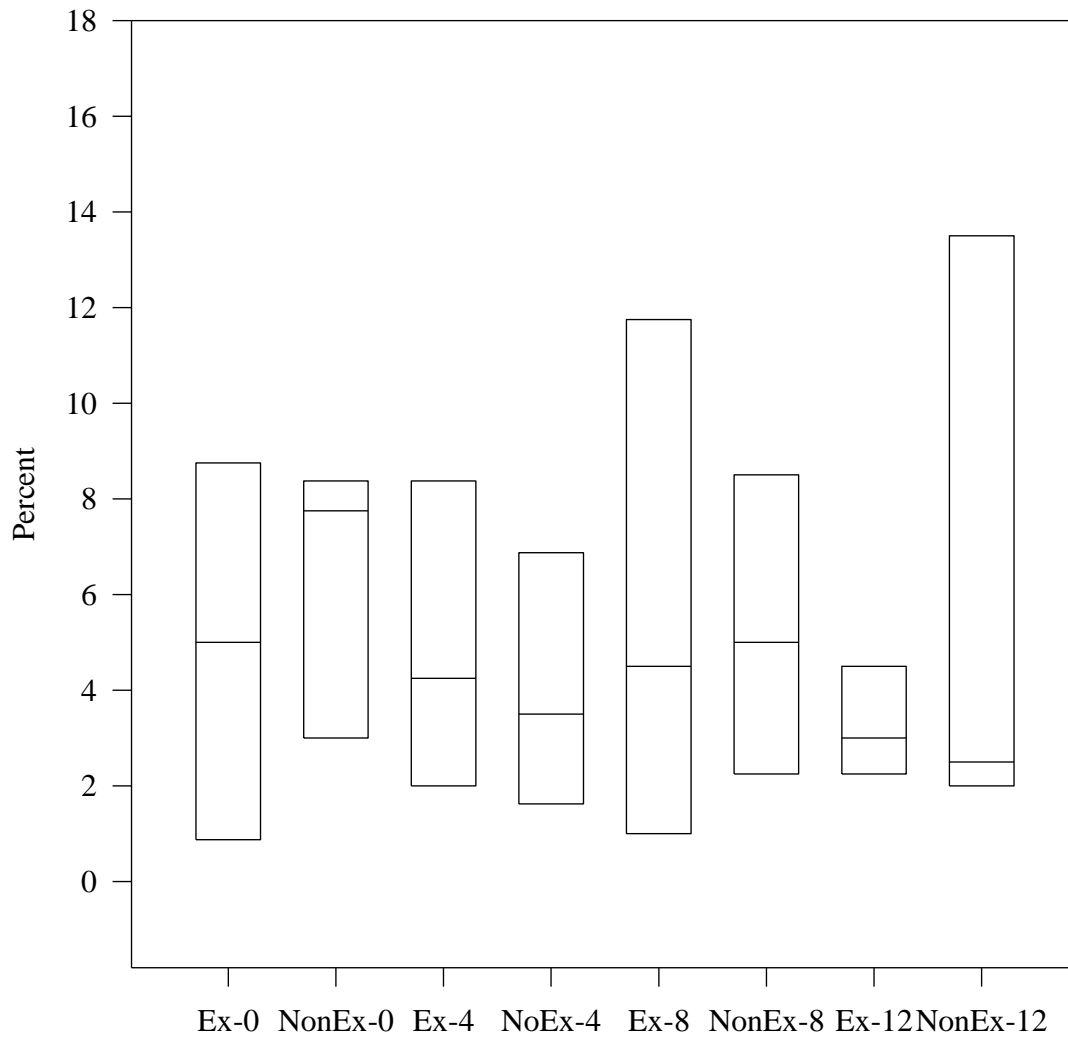


FIGURE F.5 Box plots representing mean percentages of distal droplets among non-ex (control) and ex (treatment) groups at weeks 0, 4, 8, and 12 (n=4/group).

Abnormal Midpieces

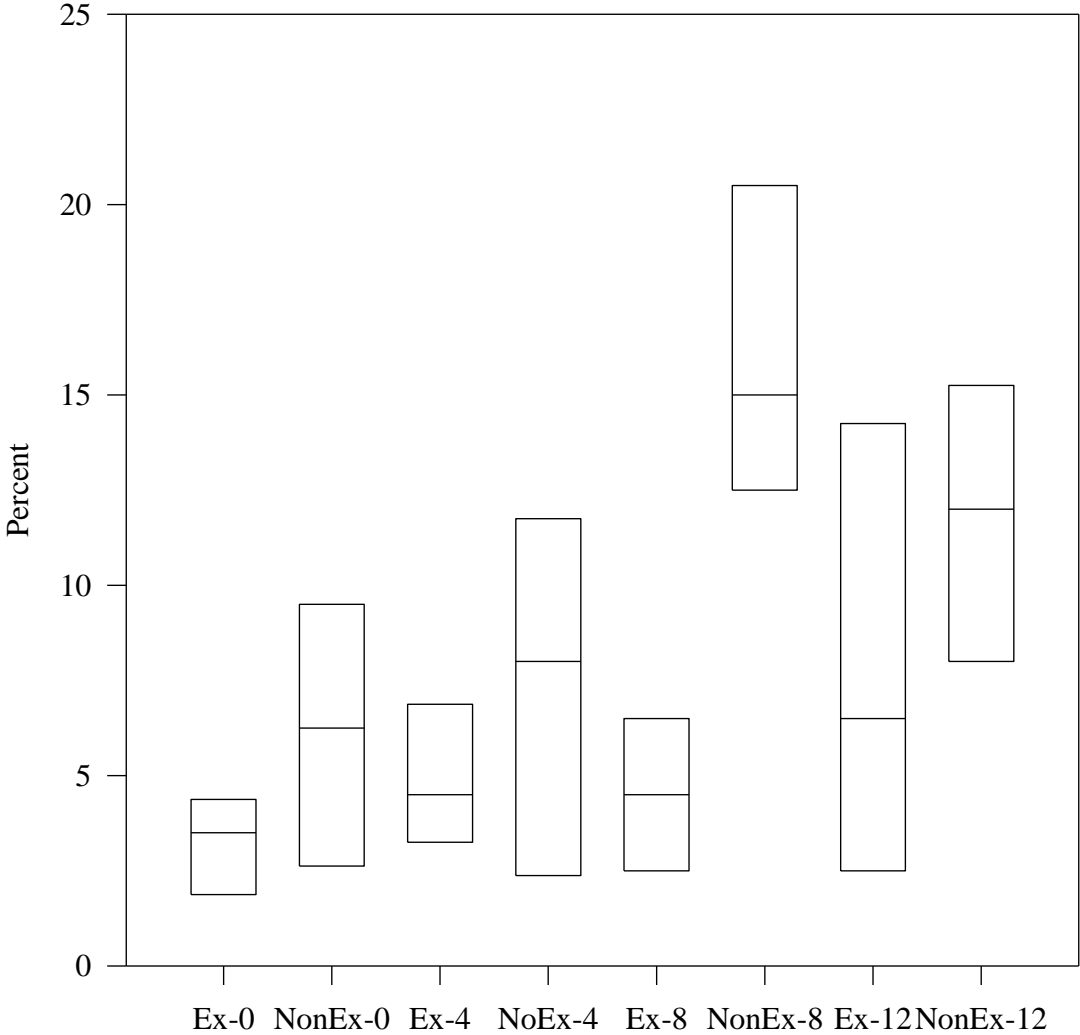


FIGURE F.6 Box plots representing mean percentages of abnormal midpieces among non-ex (control) and ex (treatment) groups at weeks 0, 4, 8, and 12 (n=4/group).

Bent Midpieces

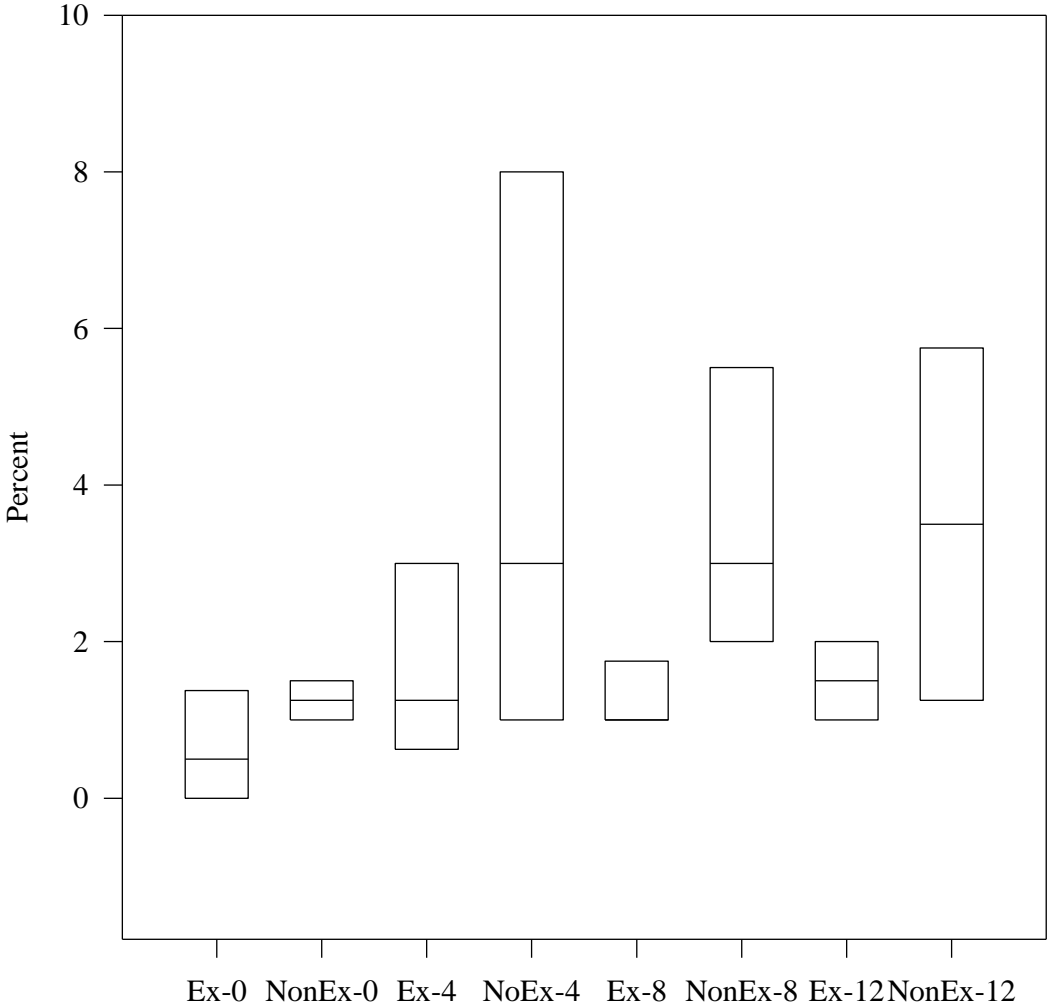


FIGURE F.7 Box plots representing mean percentages of bent midpieces among non-ex (control) and ex (treatment) groups at weeks 0, 4, 8, and 12 (n=4/group).

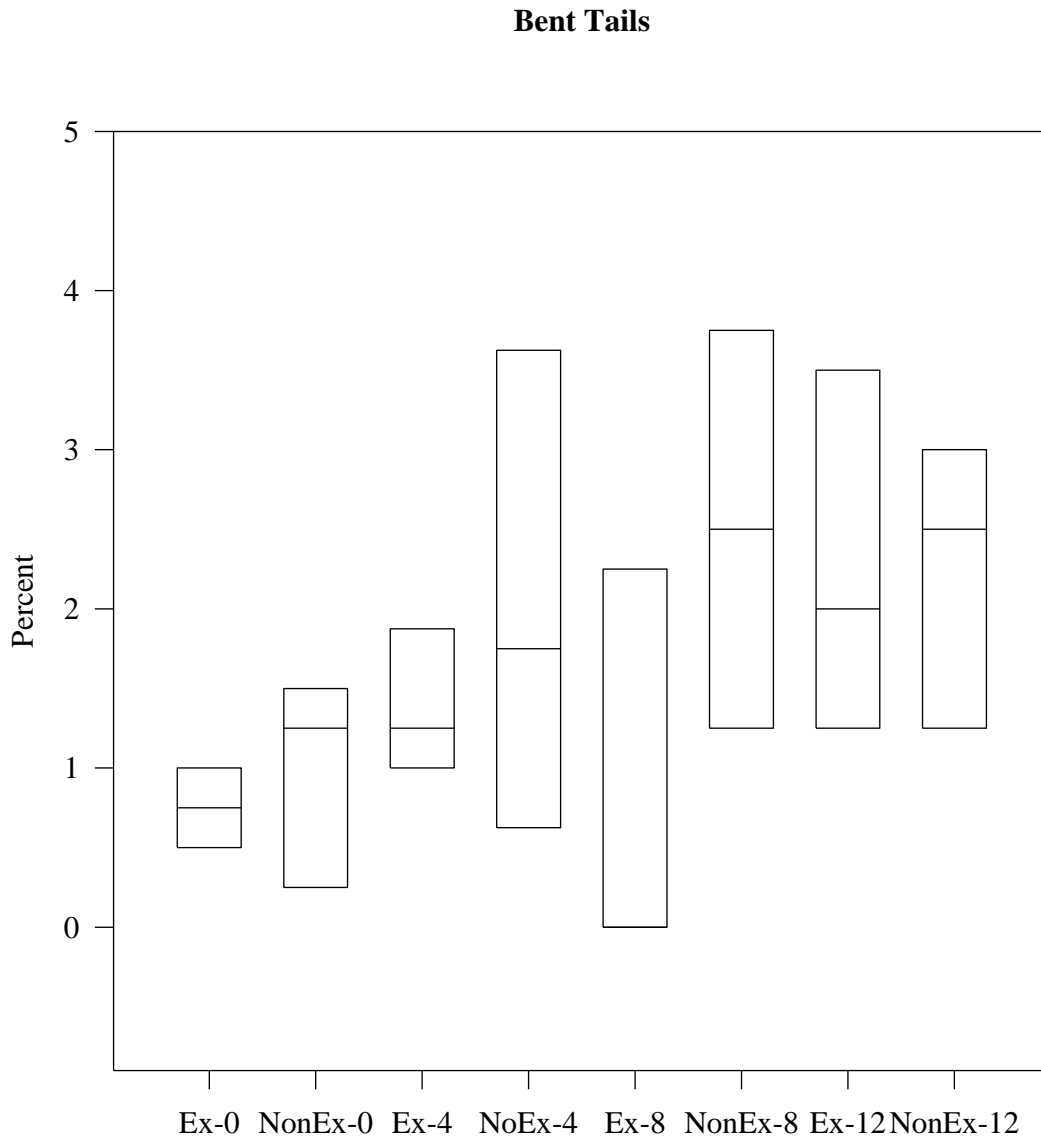


FIGURE F.8 Box plots representing mean percentages of bent tails among non-ex (control) and ex (treatment) groups at weeks 0, 4, 8, and 12 (n=4/group).

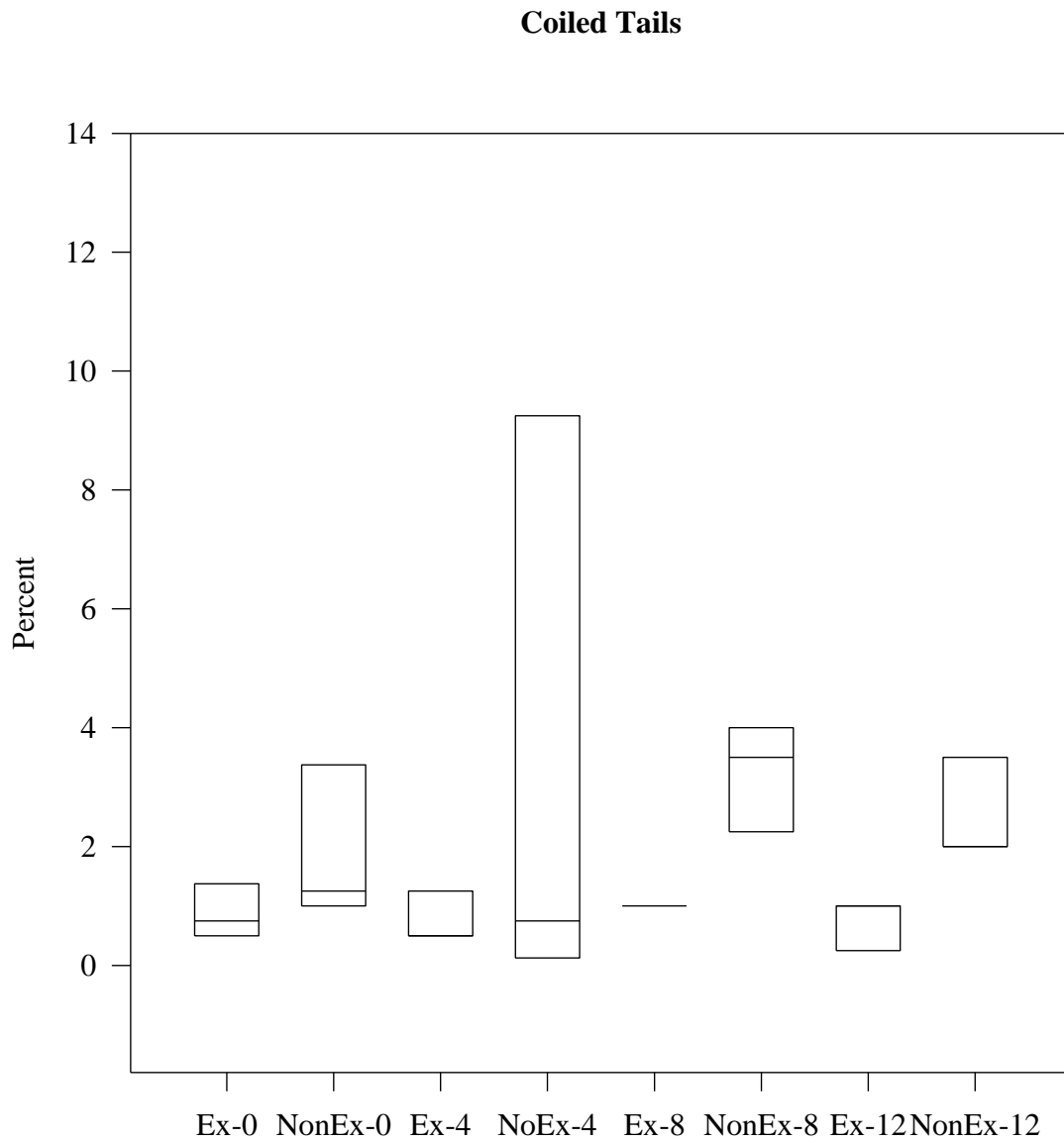


FIGURE F.9 Box plots representing mean percentages of coiled tails among non-ex (control) and ex (treatment) groups at weeks 0, 4, 8, and 12 (n=4/group).

Premature Germ Cells

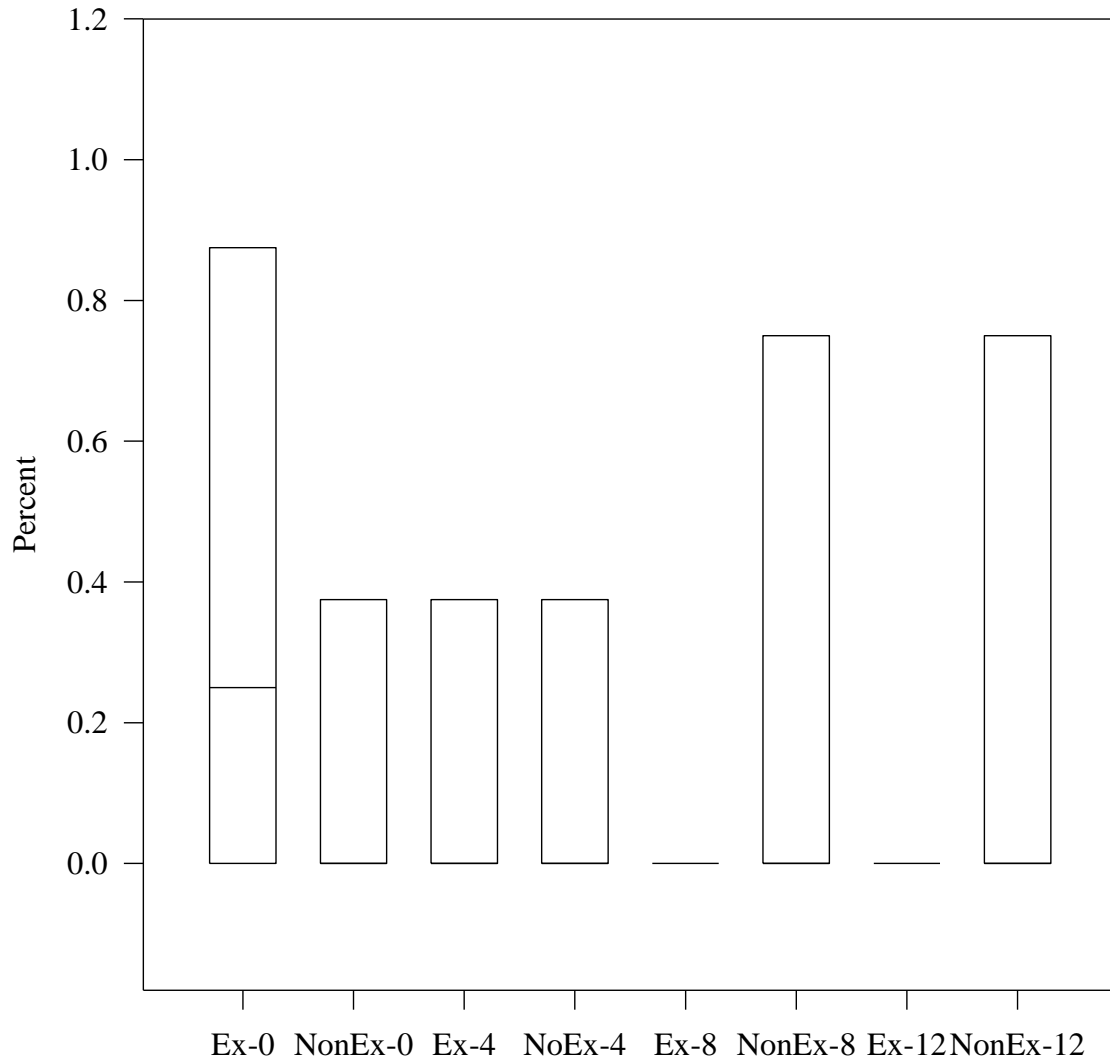


FIGURE F.10 Box plots representing mean percentages of premature germ cells among non-ex (control) and ex (treatment) groups at weeks 0, 4, 8, and 12 (n=4/group).

APPENDIX G

STANDARD EXERCISE TEST FIGURES

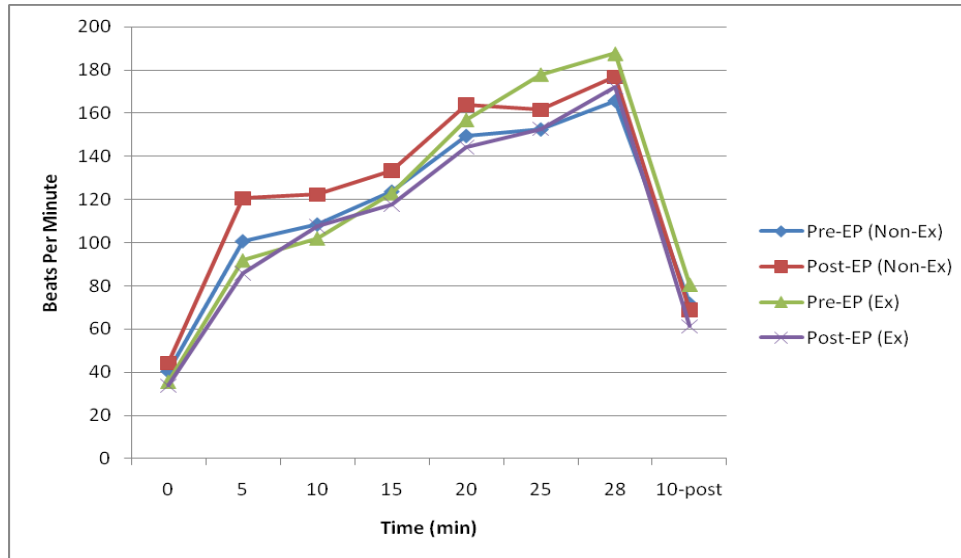


FIGURE G.1 Mean heart rates (beats per minute) of non-ex (control; n=4) and ex (treatment; n=4) stallions during standard exercise tests prior to (pre-EP) and following (post-EP) the exercise period.

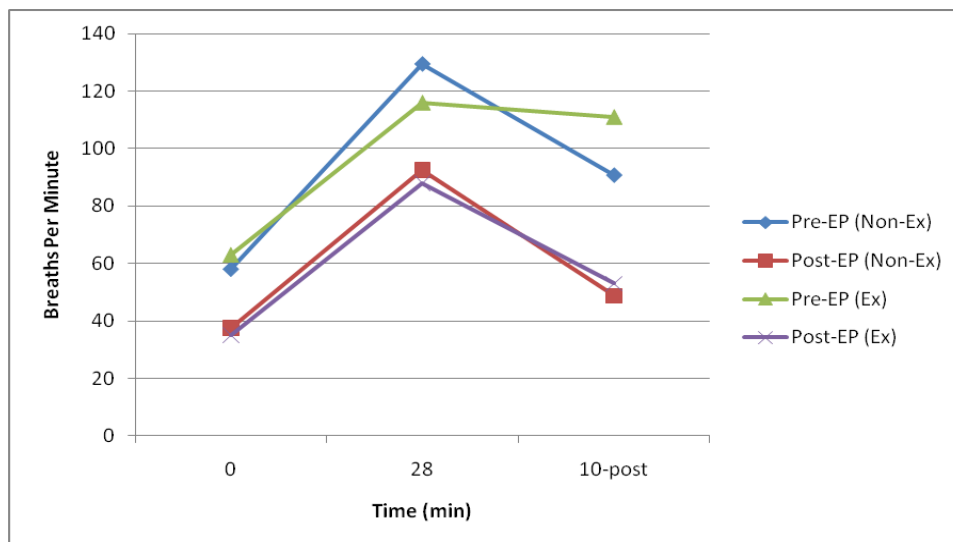


FIGURE G.2 Mean respiration rates (breaths per minute) of non-ex (control; n=4) and ex (treatment; n=4) stallions during standard exercise tests prior to (pre-EP) and following (post-EP) the exercise period.

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