INTRAUTERINE TEMPERATURES OF MARES UNDER DIFFERENT

MANAGEMENT CONDITIONS

A Thesis

by

LYNN FRANCES COMMAILLE

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 2008

Major Subject: Animal Science

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Approved by:

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ABSTRACT

Intrauterine Temperatures of Mares Under Different Management Conditions. (August 2008)

Lynn Frances Commaille, B.S., University of Vermont Chair of Advisory Committee: Dr. Martha M. Vogelsang

The objective of this study was to determine whether exercise-induced hyperthermia results in an increase in uterine temperature, as measured by an iButton temperature-measurement device inserted into the uterus, comparable to temperatures measured by a rectal thermometer or microchip with temperature-recording capability implanted in the neck. The 3 methods of measurement were examined under 4 different management conditions. The Control-pasture (Cont P) group was maintained in a pasture without man-made shelter, with the intent to measure effects of ambient temperature. The Control- No device (Cont N) group was under the same management conditions, but did not have intrauterine temperature measurement devices implanted. The Control-stall (Cont S) group was housed in individual stalls with fans in an effort to minimize the effect of environmental temperature. The Exercise (EXE) group was also housed in stalls with fans, similar to Cont S but was also subjected to 45 min of exercise each day.

The results of this study indicate that the 3 methods of temperature measurement are equally repeatable when evaluating temperatures during exercise (P<0.001). Among

the treatment groups, rectal temperatures were lowest in the Cont N and Cont P groups (P<0.05). In contrast, for both the microchip and iButton data, the mares in Cont S and EXE had the lowest core temperatures (P<0.001), indicating that horses under this management type underwent the least amount of heat stress as indicated by core temperature. While rectal temperatures did not show a diurnal effect, both core temperatures (microchip and iButton) showed significant differences between times (P<0.05 and P<0.005, respectively), demonstrating a diurnal temperature effect.

DEDICATION

For all the "four-leggeds" who have touched my life...

Past

Present

&

Future

ACKNOWLEDGEMENTS

I would first like to thank my committee chair, Dr. Martha Vogelsang, for accepting me into the prestigious program at Texas A&M University to pursue a degree in something I truly love. I would also like to thank my committee members, Dr. Dennis Sigler and Dr. Katrin Hinrichs, for their guidance and insight throughout my research. From my first visit to A&M, I have always been welcomed as an Aggie and know that I will be an Aggie from here on forward. Thank you for allowing me the opportunity to become part of something "so big".

I would like to thank Digital Angel for the generous donation of microchips and scanner for this project. In addition, I would like to thank Dr. Friend and his associates for the use of the iButtons. In particular, I would like to thank Boone Carter for all of his time and efforts in preparing and downloading the devices.

True and heartfelt thanks go out to Nikki Ferwerda, for her unwaivering support, help in the organization of this project and constant words of encouragement. Furthermore, thanks to Elena Eller, who is my "knight in shining LSU armor" when it came to help with statistics!

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I need to acknowledge the endless support I have had from the doctors and staff at VSSAH: Doc, Dr. Bay, Staci, Katy, Kate, Hil, Erik, Lori, Pam, Jess, Sara, Di, & Steph G. Every single one of you has made a sacrifice at one point or another to get me not only through this project, but through my entire master's program. You have become my family here in Texas and I cannot express my gratitude enough.

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To my roomie, Miss Tita Bonita! You helped me get through the last stretch! I truly appreciate you for ignoring me (as hard as it was!) so that I could write, and write, and write...

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"Do not go where the path may lead; Go instead where there is no path and leave a trail."

- R. W. Emerson

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CHAPTER I

INTRODUCTION

Horses represent a popular recreational activity in the United States and the horse industry has a significant financial impact. Although there has been an increasing demand for information on reproductive efficiency in many livestock species, relatively little attention has been paid to the horse. With the heightened importance of many horse owners' interest in maintaining mares in competition and training while obtaining foals from them, assisted reproductive technologies have become important management tools.

Embryo transfer is a technique that has had a significant impact on the equine industry. With the 2002 ruling of the American Quarter Horse Association allowing registration of multiple foals per year resulting from embryo transfer, this technology has become more popular in the performance horse industry. Embryo transfer allows mares who are in training or competing to still remain reproductively active.

Heat stress has a negative effect on early embryonic development in dairy cattle (Dunlap and Vincent, 1971; Monty and Wolff, 1974; Biggers et al., 1987); however, this area has not been comparably researched in the horses. Recently, Mortensen et al. (2008) reported that exercise had an adverse effect on embryo recovery and embryo quality in horses, similar to reported effects of heat stress in cattle (Gordon et al., 1987; Putney et al., 1988; Monty and Racowsky, 1987). This could have implications in the performance horse industry and deserves further investigation.

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

The objective of this study was to determine whether exercise results in an increase in uterine temperature comparable to the temperature measured by rectal thermometer, or microchip with temperature-recording capability implanted in the neck.

CHAPTER II

REVIEW OF LITERATURE

Heat Stress

In today's equine industry, the breeding season and show season occur concurrently, leading many horse owners to keep their mares in training or competition, while concurrently trying to breed their mares to obtain embryos. Embryo transfer has become a very popular and viable option for obtaining offspring from performance mares since the research done by Sertich (1989).

Embryo transfer was first described by Oguri and coworkers in 1972 and became commercially available in the early 1980's (Imel et al., 1981). At that time, its popularity was limited in the United States because most major breed registries did not recognize or permit the registry of offspring produced by the procedure. In 2002, the largest horse registry in the United States, the American Quarter Horse Association, changed the rule that governed the number of embryos transferred per mare per year (AQHA, 2004). It also retrospectively registered foals that had been born through multiple embryos transfers from a single mare within a year. Other breed registries have followed suit, allowing embryo transfer to become a technology with potential for much greater commercial use.

Many studies have been published on the recovery rate of embryos in horses, with Vogelsang et al. (1989) reporting up to a 61% recovery rate and a 70% pregnancy rate after embryo transfer in a commercial setting. In performance mares, Sertich (1989) reported a 40.5% recovery rate. Squires et al. (2003) at Colorado State University reported an average of a 50% embryo recovery rate per cycle per single ovulating mare in the school's program. This has become an accepted approximate recovery rate for commercial breeding programs.

According to Kohn et al. (1999), horses that are being exercised, especially in a hot and humid environment, have physiological adaptations which allow them to deal with induced hyperthermia. An increase in body heat is a normal physiologic response to exercise secondary to the inefficient chemical processes which create energy sources for muscles. The importance of this to reproduction was suggested in the research done by Sertich (1989) in which performance mares had a relatively low embryo recovery rate (40.5%). However, in that study there were sedentary mares to serve as controls. Recently, Mortensen et al. (2008) clearly showed that exercise negatively effects mare reproductive efficiency.

In the study done by Mortensen et al. (2008), 16 mares were either subjected to daily exercise or not throughout a period of 2 estrous cycles including embryo collection attempts on d 7 post-ovulation. The study was conducted over a 2-yr period with similar results being obtained each year. The mares were maintained in a climate in which the temperature was about 30°C and humidity was greater than 50%. The mares each participated in both treatment groups, serving as controls or exercised mares for 2 cycles and then being switched to the opposite group. The exercise group was trotted for 30 min per day; control mares were not exercised. Rectal temperatures were monitored throughout the exercise and recovery. The embryo recovery rate in control mares was 22/35 (63%). The embryo recovery rate in exercised mares was significantly lower

(11/32; 34%). After recovery, all embryos were graded using a scale of 1 (normal) to 4 (degenerating), a standard in the industry (McKinnon and Voss, 2005). A higher percentage of the embryos were categorized as grade 1 from the control group than from the exercised group (74% vs. 36%, respectively).

Research has also evaluated how oocytes respond to increased temperatures in vitro. Mortensen et al. (2008) found that oocytes were less capable of blastocyst formation than controls when exposed to elevated temperatures (42°C) for 4 h post-maturation before in vitro fertilization through intracytoplasmic sperm injection (ICSI). These authors concluded that there was an effect of exercise on mare reproductive efficiency, and concluded from the in vitro oocyte work that this may be related to the increased temperature during exercise. Mortensen et al. (2008) measured temperature rectally; the actual temperature achieved in the reproductive tract was unknown.

Additional studies are needed to distinguish among several factors that could affect reproductive efficiency after exercise in a hot, humid environment. The first factor that needs to be investigated is whether the changes that were occurring in the mare's reproductive system were due to heat from exercise in combination with a hot environment, or whether exercise in any environment would achieve the same result. This information would be important to the equine industry, especially in the southern part of the United States where horse owners routinely experience excessive environmental heat during the breeding season. Such data could clarify the interplay of exercise and environmental temperature in the process of equine reproduction. The experiment done by Mortensen et al. (2008) was the first of its kind in regard to heat stress and reproductive efficiency in the horse. However, there has been more extensive research done in other species. Dutt (1964) found that 55.6% of ova were abnormal in heat-exposed ewes, in comparison with only 3.8% in control sheep. They also found that embryo mortality was significantly higher in the ewes exposed to 30°C temperatures. Dutt (1964) postulated that sheep zygotes are most affected by ambient temperature during the early stages of cleavage.

In an experiment done by Dunlap and Vincent (1971), which used a more controlled environment to investigate reproductive efficiency, 43 heifers were split into 2 groups. Group I was exposed to 32.2°C and 65% humidity for 72 h immediately following breeding. Group II was exposed to 21.1°C and the same humidity during this time period. They found that there was a 0% conception rate in the heat-stressed groups, while heifers exposed to cooler temperatures had 48% conception rate. They also found a strong negative correlation between conception rate and rectal temperature in the control cows. This study suggested a strong relationship between high rectal temperature and failure to establish pregnancy.

In 1974, Monty and Wolff reported that during the months of July, August and September in Arizona, fertility rates dropped off by 20% in Holstein-Friesian cows. In a previous study, they also noted that intrauterine temperature in heat-stressed cattle was higher than rectal temperature.

Edwards et al. (1968) investigated whether environmental heat stress prior to breeding and during early gestation had a negative impact on reproductive performance in gilts. Although Edwards found that high ambient temperature had no influence if the gilt was exposed prior to conception, heat stress during the first 15 d post partum was detrimental to productivity. Exposure to high ambient temperature during the first 15 d after mating led to lower efficiency than did exposure to ambient heat at 15 to 30 d post-breeding. This indicated that embryos were more susceptible to high ambient temperatures during the first 2 w post-breeding.

Work with rabbits further supported this evidence. Wolfenson and Blum (1988) heat stressed rabbits during two specific stages of pregnancy: during the early stages of blastocyst formation (d 3 - 5 post mating) and during the implantation period (d 6 - 8 post mating). Embryos were more susceptible to heat stress during the blastocyst stage formation than during the implantation phase.

Tompkins et al. (1967) subjected pregnant sows to elevated temperatures in early gestation. They measured the number of viable embryos per 100 corpora lutea. Their results indicated that there were adverse effects on early embryonic survival if exposure to heat occurred from days 1 - 5 of gestation; however, in sows that were exposed to heat from days 25 - 30 of pregnancy, there were no adverse effects on embryonic survival, further indicating that heat exposure causes a detrimental effect on reproductive efficiency when gilts are exposed during the first few days to weeks of early pregnancy.

Biggers et al. (1987) at the Oklahoma Agricultural Experiment Station investigated whether periods of high ambient temperatures with high humidity were correlated with seasonal depressions of pregnancy rate in domestic cattle. The study concentrated on conceptus development and survival during the second and third weeks of pregnancy. This group did not find that pregnancy rate was affected by ambient conditions; however, they saw a decreased conceptus weight, implying that early embryonic development had been altered.

Gordon et al. (1987) elucidated not only the quantity but also the quality of bovine embryos recovered under elevated thermal conditions. This work was done with Holstein cattle in Saudi Arabia. Embryo recovery was attempted at d 7 post-ovulation. A total of 146 cows were flushed during mid-summer, while 97 cows were flushed in the winter/early-spring period (periods of lower ambient temperature). All embryos were recovered non-surgically and were graded on a standardized four-point scale. The percentage of embryos recovered was significantly lower during the summer period (44%) in comparison to the winter period (59%). There was a lower percentage of embryos that were classified as transferable (46% in the summer group; 73% in the control) and freezable (11% vs. 20%, respectively) during the summer period. This work not only confirmed prior data relative to the decreased number of embryos recovered during a period of high ambient temperature, but also shed light on the quality of embryos that were recovered.

More work on embryo quality was done at the University of Florida by Putney et al. (1988). Two groups of Holstein heifers were superovulated and inseminated. One group was maintained in a thermoneutral environment, while the other was maintained under hyperthermic conditions. At d 7 post-estrus, embryos were non-surgically removed and were evaluated for stage of development and quality. The researchers classified the embryos as normal, abnormal, retarded, or as unfertilized. Out of 82 embryos recovered from the stressed heifers, only 20% were classified as normal, in comparison to 51.5% of the 68 embryos recovered from the non-stressed animals. Heifers that were thermally challenged also had a higher incidence of abnormal and retarded embryos with nonviable blastomeres. This provides more evidence that low apparent conception rates may be an indirect result of modified embryo quality during early development under hyperthermic conditions.

Monty and Racowsky (1987) performed field studies in dairy cattle that also identified early embryonic development as being compromised by heat stress. They found that embryos collected in Arizona during the time period of June through September (highest heat and humidity) were less likely to develop in culture than similar embryos collected from superovulated cows in October through May. They speculated that the increased intrauterine temperatures were compromising either ova or embryo development, thus resulting in an overall decreased reproductive efficiency.

In many of the above studies, the effect of heat on the oocyte vs. on the developing embryo was not determined. Some of the above mentioned studies subjected animals to heat stress only after ovulation, demonstrating a direct effect of heat on the developing conceptus. However, heat has also been shown to affect oocyte development competence. Al-Katnanai et al. (2002) found that oocyte competence is compromised in Holstein cows during times of heat stress. While doing in vitro research, this group found that cleavage rates after in vitro fertilization were similar between a control and heat-stressed groups, but none of the cultured oocytes developed to the blastocyst stage in the heat-stressed cows.

Hormonal Effects of Stress

The mechanisms behind the effect of heat on oocyte and embryo viability are not well understood. In dairy cows, there is evidence that heat stress has an effect on the hypothalamic-hypophyseal-ovarian axis, which in turn has an effect on folliculogenesis. The main hormones that are involved in regulating ovarian function, and therefore ovulation, are gonadotropin-releasing hormone (GnRH) from the hypothalamus, and luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary. Some reported studies have evaluated the effects of heat stress and exercise on these parameters. The studies on LH, which is responsible for ovulation and the maintenance of the corpus luteum, have been inconsistent. Gwazdauskas et al. (1981) reported that in heat-stressed heifers, peripheral blood concentration remained unchanged, while others have seen a decrease in LH levels as a result of heat stress (Madam and Johnson, 1993; Gilad et al. 1993). Gilad et al. (1993) also found differences in LH secretion patterns as a result of heat stress, with decreases in the pulsatile amplitude. However, Gilad et al. (1993) also found the amplitude of LH pulse surges before ovulation are decreased in cows with low estradiol plasma concentrations during times of heat stress, but not in those with high plasma estradiol concentrations, so the specific mechanism remains unclear.

In heat-stressed cows, Wolfensen et al. (1995) reported that plasma inhibin concentrations decrease, possibly due to reduced folliculogenesis, as the majority of inhibin is secreted from small to mid-sized follicles. This phenomenon has been correlated with higher concentrations of FSH during the preovulatory period. Roth et al. (2000), in his work with cows, postulated when the dominant follicle develops in an environment that has low LH levels, the estrogen level is decreased, leading to poor expression of estrus, and therefore lowered fertility. They determined that FSH levels increase with heat stress, which may be a result of a decreased plasma concentration of inhibin due to the compromised follicles. This leads to a disruption in folliculogenesis in which the dominant follicle is suppressed. Badinga et al. (1993) studied follicular traits in Holstein cows that were either provided shade or no shade. Between these groups, there was a significant difference in mean rectal temperature. The results of the study described the disruption of folliculogenesis, in that it appeared to alter the efficiency of follicular selection and dominance, possibly resulting in the decreased quantity and quality of the recovered embryos.

It has been documented by Williams et al. (2002) that exercise, especially in hot, humid environments, causes an increase in plasma cortisol and β -endorphin levels in horses. Gilad et al. (1993) saw similar hormone changes in heat-stressed cattle. These workers have also shown that increased cortisol levels have an inhibitory effect on GnRH and LH. This, in turn, would interrupt folliculogenesis, which as shown by Badinga et al. (1993) affects follicular selection and dominance.

Recording Temperatures

There are many challenges inherent in the ability to continuously monitor core temperatures in the horse. Rectal temperature is the most common method used in studies involving horse transport and exercise. While the use of rectal thermometers is easy and convenient for intermittent use, frequent defecation inhibits their use for long periods of time or during times of increased movement (i.e., exercise). One study reports the use of a harness with a long metal probe to gather deep rectal temperatures in beef cattle (Brown-Brandl et al., 2003). This procedure, however, was not feasible for a prolonged period of time due to the potential risk of infection.

Pulmonary artery temperature (PAT) is another way to measure the temperature of the circulatory system in the horse, especially in studies involving exercise. This is done by feeding the temperature sensor through the heart via the jugular vein, and then through the pulmonary artery (Kohn et al., 1999). While this is a good measure of core temperature, the procedure must be done by qualified personnel under aseptic conditions and is not practical for every-day use.

Research has been done on recording temperatures using remote telemetry devices in order to be able to continuously monitor core temperature from a distance. This work was first done by Cross et al. (1991) by placing a sensor internally within the animal which transmits by radio or electromagnetic frequency (Hewlett-Packard Vectra ES/12 AT computer) to a remote receiver. In horses, the sensor was surgically placed in the flank. In cattle, the sensors were placed in the omental sling of the peritoneal cavity (Brown-Brandl et al., 2003). Although this method is convenient in regards to remote access, a non-surgical approach has not been developed.

To find a method that did not require surgical implantation, recent work was done in Kentucky using telemetry-based gastrointestinal temperature (Green et al., 2004). They found that this system was a viable method to determine equine core body temperature. Although this approach is non-surgical, it still requires a veterinarian to place the telemetry sensor in the horse's stomach via nasogastric wash. No research has been published that examine whether specific diet and/or the heat produced by digestion could have an effect on this method. The results of this work did determine that these core temperature readings were an average of 0.5° C greater than rectal temperatures.

Roa et al. (2006) used a novel IC thermometer (infrared tympanic thermometer) to measure bovine uterine temperature and compared it with rectal and vaginal temperatures. The IC was installed in the left uterine horn near the uterotubal junction of eleven Japanese Black cows via trans-lumbar laparotomy. In all cows, a diurnal rhythm was observed. However, fluctuation of uterine temperature seemed less variable when compared to rectal and vaginal temperatures. On average, the uterine temperature tended to be lower than the rectal or vaginal temperatures, which conflicts with many reports that core temperatures tend to be slightly higher than those recorded by rectal thermometers.

An alternative non-surgical approach is to place the telemeters in the uterus of the mare. A recent study placed a coated thermometer logger (the Thermochron iButton) in the uterus of nine mares that were subjected to endurance-type exercise for a range of time periods (Smith et al., 2006). Rectal temperatures were recorded to correlate the 2

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methods of temperature logging. The results indicated a high correlation between the 2 methods of recording temperature. The iButtons placed in the uterus provided a way to record core body temperature over an extended period of time and during different types of exercise.

CHAPTER III

MATERIALS AND METHODS

Horse Management

The 20 mares in this research project were owned by the Texas A&M University Department of Animal Science, and were maintained similarly to one another in regards to vaccination, de-worming and hoof care (Appendix 1). The mares were randomly assigned to one of four groups. The mares used in this study were maintained and used within approved guidelines of the Institutional Agricultural Care and Use Committee (AUP #2006-246). The mares maintained on pasture were supplemented twice daily with a commercially produced 13% crude protein pellet (Producers Cooperative Association, Bryan, Texas 77606) that fulfilled the maintenance requirement as outlined by the National Research Council (2007) and had access to Coastal bermudagrass coastal hay. All horses had ad libitum access to water. The horses maintained in stalls were fed similarly, with the exception that they were fed Coastal bermudagrass hay from square bales. Horses were maintained on pasture and in stalls at both the Texas A&M Horse Center and the N.W. "Dick" Freeman Arena facility.

Treatment Groups

Control No Device (Cont N). The 5 mares in this group were maintained on pasture. They were implanted in the nuchal ligament with a microchip with temperature sensing capability as outlined below. They did not have an iButton inserted into the

utuerus and uterine temperature was not recorded. This group served as a control for the effect of placement of an intrauterine device on rectal and nuchal temperatures.

Control Pasture (Cont P). The 5 mares in this group were also maintained on pasture. In addition to being implanted in the nuchal ligament with a microchip, these mares also had a temperature recording device (iButton) inserted into the uterus via the cervix.

Control Stall (Cont S). Four mares were used in this treatment group. They were housed in 3.6 x 3.6 m stalls equipped with commercially-available window fans for increased ventilation. These mares had both an implanted temperature sensor in the nuchal ligament and an intrauterine temperature recorder. Five mares were originally assigned to this group but 1 mare was removed due to an infectious illness unrelated to the study before implantation of the uterine temperature recording device.

Exercise (EXE). Five mares were assigned to this treatment group. They were maintained similarly to Cont S group. Mares were exercised at a long trot or canter, depending on stride length, for 45 min at approximately 1400 h every day for 5 d. Rectal and microchip temperatures were taken before the onset of exercise, at 10 min, 20 min, 30 min during exercise and at the end of exercise. Intrauterine temperatures were preprogrammed to take temperatures every 15 min. In addition, temperatures were monitored post-exercise at 15 min, 30 min, 1 h, and 2 h.

Recording Temperatures

Rectal Temperatures. Temperatures were recorded with a 60 sec flexible tip digital thermometer (Mabis Healthcare Inc., Waukegan, Illinois 60085) at 0600, 1400, 1800 and 2200 h for 5 d. These thermometers had a variance of $\pm 0.01^{\circ}$ C.

Core Temperatures (Microchip). At least one month before the onset of data collection, 18 horses were implanted in the nuchal ligament on the left side of the neck via syringe with a Bio-Thermo implantable RFID microchip (Digital Angel Corporation, South St. Paul, Minnesota 55075) (Appendix 1). The microchip has a unique numeric identification code and core temperature sensing capabilities. Two mares already had this device implanted in their neck for a previous study. All mares were scanned at 0600, 1400, 1800 and 2200 h for 5 d to record temperature. A scanner (Destron Technologies, division of Digital Angel Corporation, South St. Paul, Minnesota 55075) used RDIF technologies to identify the animal by alpha-numeric identification codes and would also access core temperatures as measured by the microchip.

Uterine Temperatures (iButton). Horses in groups Cont P, Cont S, and EXE had a coated temperature logger, the DS19221H Thermochron iButton (Maxim Integrated Products, Inc. Sunnyvale, CA 94086), inserted into the uterus after ovulation when the cervix was closing (see below for follicular monitoring techniques). The iButtons were preset to begin logging temperatures at $1/8^{\circ}$ C increments with \pm 1°C accuracy every 15 min to an on-board memory system. These iButtons were provided from the laboratory of Dr. Ted Friend at Texas A&M University and information was downloaded with a special program (the Multidrop controller for 1-Wire net) at the end of the data collection period.

The iButtons were mounted on a fob (Maxim Integrated Products, Inc. Sunnyvale, CA 94086) and had a 1.22 m piece of Braided Nylon Polyamid Non-absorbable suture (RXVeterinary, Grapevine, Texas, 76051) tied to the fob. This unit was soaked in a 0.05% chlorhexadine solution for 10 min before being inserted into the mare's uterus.

To determine stage of estrous, mares that were to receive an iButton were evaluated by transrectal ultrasonography (Medison Sonovet 600, Universal Medical Systems, Inc., Bedford Hills, New York, USA). Any mare with a corpus luteum was short-cycled with a 10mg/kg/BW IM injection of dinoprost tromethamine (Lutalyse, Pfizer Animal Health Division, New York, New York, 10017), while mares with growing follicles did not receive treatment. Follicular growth on the ovary was monitored daily via transrectal ultrasonography once the follicle had reached a diameter of 35 mm. When the mare had ovulated, the iButton, fob and suture was placed aseptically via gentle digital dilation of the cervix into the body of the uterus, with the suture hanging out of the vulva. Prior to this, the mare's vulva and perineal area had been washed with a diluted Betadine scrub (Purdue Pharma, L.P., Stamford, Connecticut, 06901). Temperatures were recorded by the uterine device for 5 d; if at any point the iButton came out of the uterus, it was immediately replaced and the intermediate data points were discarded.

Statistical Analyses

Temperatures measured by the three different methods were modeled to examine treatment, time, and day effects using Generalized Least Squares with a Gaussian family and identity link function. Maximum likelihood estimates were evaluated using joint Ftests and a Bonferroni Means Comparison Test. Data collected during exercise was evaluated using pairwise correlations. All statistical analysis was performed using Stata Statistical Software (Stata Corp, College Station, TX).

CHAPTER IV RESULTS

Cont N

Cont N was established as a means to compare with the mares in the Cont P group. These mares were maintained under the same management condition as Cont P with the exception that they did not receive an intrauterine device (iButton). If the mares with the iButtons showed an increase in rectal and microchip temperatures, it would remain unclear whether the increase had been caused by the environment or as an inflammatory response due to the iButton in the uterus. There was no statistical difference in rectal temperature or microchip temperature between the groups over all time periods (P>0.1), indicating the uterine devices did not cause a subsequent increase in overall body temperature as measured via rectal and microchip temperature. It was noted, however, that 4 out of 14 mares (28.6%) had uterine discharge when the iButtons were removed. A uterine culture and cytology was performed on the mares at the conclusion of the study to establish if bacteria, in addition to inflammatory components, were present. All mares that had uterine discharge had negative culture results, indicating there was no infection or organism present (Appendix 2A, Appendix 3).

Rectal Temperatures

There was a significant effect of treatment group for rectal temperatures (P<0.001) as horses living outside on pasture (Cont P) had lower rectal temperatures than both the Cont S group and the EXE group during the resting temperatures only (P<0.05 and P<0.001, respectively). There was no statistical difference between Cont N and Cont P (Table 1).

Treatment Group Rectal Temperature (°C) Cont P $37.5 \pm .05^{b}$ Cont N 37.6 ± 04^{b} Cont S $37.7 \pm .03^{a}$ $37.7 \pm .05^{a}$ EXE ^{a,b} Groups lacking common superscripts differ (P<0.05)

Table 1. Mean (\pm SE) rectal temperature (°C) in mares kept in different environments

No day effect was seen in rectal temperatures which can most likely be attributed to the fact that d 1 was different for all horses in the trial as starting date was based on the first day after ovulation. There were no differences in rectal temperature by time of day.

Core Temperatures (Microchip)

Temperatures as measured by microchip were significantly different by treatment (P<0.001), time (P<0.001), and day (P<0.01). Mares that were living inside with fans (Cont S) had a significantly lower core temperature than the mares managed in the same way, but with forced exercise (EXE) (P<0.001) (Table 2). There was no significant

difference seen between the Cont P group and the EXE group. However, there was a significant difference seen between the Cont N and Cont S groups (P<0.005)

Treatment Group	Microchip Temperature (°C)	
Cont P	$37.5 \pm .06^{a,b}$	
Cont N	$37.5 \pm .05^{a}$	
Cont S	$37.3 \pm .05^{b}$	
EXE	$37.6 \pm .05^{a}$	
ab c 1 1.	(1, 1)	

Table 2. Mean (\pm SE) microchip temperature (°C) in mares kept in different environments

^{a,b} Groups lacking common superscripts differ (P<0.05)

There was a significant difference (P<0.05) among almost all time points, with the exception of 1400, which was not significantly different from 1800 and 2200 (Table 3). This describes a diurnal effect on temperature. Temperatures in all groups were lowest at 0600 with a mean of $37.2 \pm .06$ °C and highest at 1800, with mean temperatures reaching 37.6 ± 0.5 °C.

1 uei e : 110uii (
Time of Day	Microchip Temperature (°C)
0600	$37.2 \pm .06^{a}$
1400	$37.6 \pm .05^{b,c}$
1800	$37.7 \pm .05^{b}$
2200	$37.5 \pm .06^{\circ}$
a,b,c Crosses lo alsin	1:65 m (D <0.05)

Table 3. Mean (± SE) microchip temperature (°C) in mares at four different times of day

^{,o,c} Groups lacking common superscripts differ (P<0.05)

Mean temperature on d 1 was higher (P<0.05) than on days 3, 5, 6, and 7. It is difficult to account for this because d 1 was staggered in all horses and the microchip had

been implanted at least one month in advance to prevent local inflammation and the potential for a secondary increase in temperature. The difference by day was accounted for in the statistical analysis, but cannot be explained by experimental design.

Uterine Temperatures (iButton)

Core temperature as measured with the iButtons was significantly different by both treatment (P< 0.0001) and time (P<0.001) (Table 4). The mares in stalls with fans (Cont S) had a significantly lower temperature than the mares under the same management conditions but with the addition of exercise (EXE) (P<0.005). These results are consistent with those for core temperature via microchip. There was no significant difference between the EXE and Cont P groups.

Table 4. Mean (\pm SE) iButton temperature (°C) in mares kept in different environments

Treatment Group	iButton Temperature (°C)	
Cont P	$37.6 \pm .04^{b}$	
Cont S	$37.3 \pm .02^{a}$	
EXE	$37.5 \pm .05^{b}$	
ab C 1 1.	(1, 1, 1, 0) (D (0, 0.5))	

^{a,b} Groups lacking common superscripts differ (P<0.05)

Also consistent with the data obtained from the microchips, temperatures measured with iButtons were different by time of day (P<0.005) (Table 5).

		/
Time of Day	iButton Temperature (°C)	
0600	$37.3 \pm .04^{a}$	
1400	$37.4 \pm .05^{b}$	
1800	$37.5 \pm .05^{\circ}$	
2200	$37.6 \pm .04^{a}$	
a,b,c Crosses lostring some	$a_{\rm con}$ give any animate different (D < 0.001)	

Table 5. Mean (\pm SE) iButton temperature (°C) in mares at four different times of day

^{a,b,c} Groups lacking common superscripts differ (P<0.001)

EXE Group

Within the exercised group, statistical analysis by Pairwise Correlation showed that all 3 ways of measuring temperature were correlated to one another at all time points during exercise and recovery (P<0.001) (Figure 1).



Figure 1. Mean temperature (°C) of mares during exercise and recovery
Overall

Table 6. Mean (\pm SE) temperature (°C) in mares at four different times of day

Time of Day	Microchip	Rectal	iButton
0600	$37.2 \pm .06^{a}$	$37.6 \pm .04$	$37.3 \pm .04^{e}$
1400	$37.6 \pm .05^{b}$	$37.7 \pm .04$	$37.4 \pm .05^{e}$
1800	$37.7 \pm .05^{b,c}$	$37.7 \pm .04$	$37.5 \pm .05^{f}$
2200	$37.5 \pm .06^{b,d}$	37.6 ± 0.5	$37.6 \pm .04^{g}$
ahai ii	•	1.00 (D 0.01)	

^{a,b} Columns lacking common superscripts differ (P<0.01)

^{c,d} Columns lacking common superscripts differ (P<0.05)

^{e,f,g} Columns lacking common superscripts differ (P<0.01)

Table 7. Mean (\pm SE) temperature (°C) in mares obtained by three methods under three management conditions

Treatment	Microchip	Rectal	iButton
Cont P	$37.5 \pm .06^{a}$	$37.5 \pm .05^{\circ}$	$37.6 \pm .04^{e}$
Cont S	$37.3 \pm .05^{b}$	$37.7 \pm .03^{d}$	$37.3 \pm .02^{f}$
EXE	$37.6 \pm .05^{a}$	$37.7 \pm .05^{d}$	$37.5 \pm .05^{e}$
a h			

^{a,b} Columns lacking common superscripts differ (P<0.05) ^{c,d} Columns lacking common superscripts differ (P<0.001) ^{e,f,} Columns lacking common superscripts differ (P<0.0005)

Tables 6 and 7 show an overall comparison of data points by time of day and by

treatment groups.

CHAPTER V DISCUSSION

The first purpose of this study was to establish the relationship of uterine temperature measured via use of the iButton to those measured by microchip or rectal temperature. The second aim was to establish the effect of different management systems on mare core and uterine temperature.

The Cont N group was established with the intent to distinguish whether a potential inflammatory response by the iButton would elicit a temperature increase that could lead to confounding data. The results showed that there was no difference in core body temperature (by rectal thermometer or by microchip) between group Cont N and Cont P. This led us to the conclusion that the iButton in the mares' uterus did not cause an inflammatory response leading to an increase in temperature. Therefore, it was assumed that the three methods for measuring temperature were providing physiological temperature readings based on the treatment or environment for each group (Cont P, Cont S, and EXE).

There were no statistical differences in rectal temperature based on time of day, which is in contrast to the results of other studies. Ammons et al. (1989) showed that rectal temperature in mares has a significant diurnal pattern. This was further supported in the study by Bowman et al. (2006) regarding the diurnal effect of core temperatures measured via microchip. Work in other species, specifically bovine, further demonstrates the diurnal pattern of body temperature (Roa et al., 2006). Although rectal temperature is the most conventional method of taking temperatures in most livestock species, there are many factors that could affect the reliability of this method. The composition of fecal matter and gas at the lower end of the colon could affect measurement readings, as well anal tone. All of these could lead to falsely lowered temperatures.

As shown by Bowman et al. (2006), microchips implanted in the nuchal ligament show a diurnal variation on body temperature. While the study by Bowman (2006) showed this diurnal effect, it is important to note that these sensors are located only approximately 2.54 cm below the skin. Thus, the microchip may be more sensitive to direct environmental conditions like sunshine than previously anticipated. All horses in the current study had access to sunshine during the day, whether from living outside or through windows in the stall. Regardless, these results show that the microchips are sensitive indicators of temperature change.

Smith et al. (2006) showed the same correlation between rectal and uterine temperature with iButtons placed in the uteri of mares that were then subjected to a variety of exercise protocols. The results of the current study provide an additional correlation of microchip temperature to both of these measurement devices. Although the 3 methods of measuring temperature seemed to give equivalent results in this study during exercise, the practicality of each method has certain implications. In some areas of research such as exercise physiology, the capability to measure core temperature without stopping exercise, either with the microchip or iButton, could prove to be useful. In the current study, mares living outside (Cont P) had the lowest mean rectal temperatures. However, temperatures taken both by iButton and microchip were lowest in horses kept in stalls without forced exercise. The results of this study suggest that the horses living in stalls with fans underwent the least amount of core temperature heat stress. It is interesting to note that the mean low rectal temperature is still higher than the mean low temperature for the microchip and iButton.

Although a difference by time of day is shown with the iButton temperatures, there is less variability than with the microchip temperatures. This may provide evidence that uterine temperatures are less affected by the environment (as compared with the microchip) and could be a more precise indicator of true core temperature. Roa et al. (2006) found that uterine temperatures were more stable when compared to rectal temperatures in cattle.

Between the Cont P mares and the EXE mares, there was no significant difference for both microchip and iButton temperatures. This may indicate that there is no difference between heat-stress induced by the environment versus heat stress induced by exercise.

In order to clarify whether there had been local inflammation or infection, a uterine culture and cytology was performed on all mares at the conclusion of the study. The 4 mares that had shown signs of discharge had a clean cytology, indicating that there had been no inflammation, and clean cultures, showing there were no signs of infection. The 4 mares that did have a positive culture or cytology did not show signs of discharge when the iButtons were removed. The discharge present in the 4 mares and the 4 mares that had positive culture and cytology can not be limited or strictly caused by the iButtons. The reproductive history on several of these mares was unknown, so in the future, it would be best to do a reproductive wellness exam prior to the onset of a similar study to determine the individual mare's reproductive status (Appendix 2A).

CHAPTER VI SUMMARY

It was shown in this study that core temperature, measured via microchip and iButton, followed similar trends, but that these differed from rectal temperatures in that rectal temperatures did not show diurnal variation, and were overall higher than were core temperatures. Previous studies have also indicated a difference between core and rectal temperatures (Green et al., 2004). In contrast, this study showed core temperatures to be 0.5°C higher than rectal temperatures.

The different management conditions set up in this study were intended to differentiate exercise-induced hyperthermia from environmentally-induced hyperthermia. However, although the fans in the stalls may have helped to reduce the severity of hyperthermia, it did not completely eliminate it. Further work needs to be done to distinguish between exercise-induced heat stress and environmental heat stress, as well as to delineate any synergism that may exist. Conducting a study in an environment in which temperature and humidity are strictly controlled would eliminate the conflicting environmental heat stress and thereby confirm that exercise by itself can have an effect on the reproductive environment.

In the study done by Mortensen et al. (2008), oocytes were heat shocked in vitro for 2h and 4h at 42°C. These researchers found that this relatively short period of heat shock at the final stages of oocyte maturation had a detrimental effect on early embryo development into the blastocyst stage. While mares in the in vivo portion of Mortensen's study had elevated temperatures during exercise, they never reached a comparable temperature to that achieved in vitro (42°C). In the present study, the average highest temperature for all 3 different types of measurements was at the end of exercise (45 min). The highest was the microchip temperature (39.8°C \pm 0.1), followed by the rectal temperature (39.6°C \pm .09), and finally the iButton temperature (38.9°C \pm 0.1). In the portion of the study done in vivo, Mortensen et al. (2008) exercised horses to a high mean rectal temperature of 39.9°C at the end of exercise (30 min). Other reports on increased rectal temperatures after 30 min exercise in a hot environment include 40.5°C (Geor et al., 1995) and 41°C (Kohn et al., 1999). In all of these studies, none approached the temperature of the heat shock done in vitro (42°C) that caused the early blastocyst formation to become altered. More research needs to be done in the area of in vitro heat shock at temperatures that have a better relationship with actual peak rectal temperatures during exercise in a hot and humid environment.

The current study demonstrated that uterine temperatures were similar to those obtained by the microchip. There was no significant difference in uterine temperatures during exercise when compared to rectal and microchip temperatures, but there were trends for the uterine temperature to not get as high, with a quicker recovery period. More work on this subject needs to be done to further clarify this information.

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APPENDICES

HORSE NAME	GROUP	MICROCHIP NUMBER	BREED	AGE
Blue	-	985140000459000	QH	3
Oakla	Cont N	985140000452434	QH	2
Rocky	Cont N	985140000459222	QH	2
Girly	Cont N	985140000506295	QH	2
Trinket	Cont N	985140000447443	QH	2
Destiny	Cont N	985140000452955	QH	2
Dee	Cont P	985140000394765	QH	3
Faces	Cont P	985140000513917	QH	3
Missa	Cont P	460A3C7CIF	QH	17
Sugar	Cont P	985140000534983	Ar / Std.	9
Prissy	Cont P	985140000399051	QH	3
Shaggy	Cont S	985140000456137	QH	9
Beauty	Cont S	985140000398541	QH	17
Lena	Cont S	985140000507951	QH	6
Fancy	Cont S	460A2D_5422	QH	15
Slimer	EXE	985140000515215	QH	3
Miss Lectric	EXE	985140000458586	QH	9
Marla	EXE	985140000512790	QH	3
Scoot	EXE	985140000452345	QH	3
Sun	EXE	985140000528886	QH	3

APPENDIX 1. OVERVIEW OF HORSES WITH MICROCHIP NUMBER

APPENDIX 2A. CULTURE AND CYTOLOGY RESULTS

HORSE NAME	UTERINE CULTURE RESULTS	ENDOMETRIAL SWAB RESULTS
Dee	Corynebacterium sp.	No neutrophils or bacteria seen on slide
Faces	Contaminated	No neutrophils or bacteria seen on slide
		Deg, neutrophils, active phago. low num.
Missa	Contaminated	bacteria
Sugar	No bacteria seen	No neutrophils or bacteria seen on slide
Prissy	Contaminated	No neutrophils or bacteria seen on slide
Shaggy	Beta-strept. sp.	low neutrophils, no bacteria
Beauty	Strept. e, corynebacterium sp.	No neutrophils or bacteria seen on slide
Lena	No bacteria seen	moderate leukocytes, no neutrophils
Fancy	Contaminated	No neutrophils or bacteria seen on slide
Slimer	No bacteria seen	No neutrophils or bacteria seen on slide
Miss Lectric	No bacteria seen	No neutrophils or bacteria seen on slide
Marla	Streptococcus equisimilis	No neutrophils or bacteria seen on slide
Scoot	No bacteria seen	No neutrophils or bacteria seen on slide
Sun	No bacteria seen	No neutrophils or bacteria seen on slide

APPENDIX 2B. CONCLUSIONS AND DISCHARGE PRESENT WITH IBUTTON REMOVAL

HORSE NAME	CONCLUSION	DISCHARGE PRESENT
Dee	No evidence of infectious disease process	No
Faces	No evidence of infectious disease process	No
Missa	septic suppurative endometritis, inflamm. to bacteria	No
Sugar	No evidence of infectious disease process	No
Prissy	No evidence of infectious disease process	Yes
Shaggy	Mild suppurative inflammatory response	No
Beauty	No evidence of infectious disease process	No
Lena	Mild suppurative inflammatory response	Yes
Fancy	No evidence of infectious disease process	No
Slimer	No evidence of infectious disease process	Yes
Miss Lectric	No evidence of infectious disease process	Yes
Marla	No evidence of infectious disease process	No
Scoot	No evidence of infectious disease process	No
Sun	No evidence of infectious disease process	No

APPENDIX 3. ANOVA MEANS TABLE (±SE) OF 3 WAYS OF MEASURING TEMPERATURES (°C) IN MARES IN DIFFERENT MANAGEMENT CONDITIONS

Treatment	Microchip	Rectal	iButton	
Cont N	37.53241	37.62327	-	
	0.0481434	0.0378832	-	
EXE	37.64658	37.77362	37.48955	
	0.0524466	0.046087	0.0496365	
Cont P	37.52386	37.50948	37.58651	
	0.0610691	0.0474085	0.0365314	
Cont S	37.25643	37.73349	37.30833	
	0.0532702	0.0334397	0.0231881	

Dav	Microchip	Rectal	iButton	
1	37.76	37.48077	-	
	0.1078223	0.1712228	-	
2	37.504	37.728	-	
	0.1701507	0.0688117	-	
3	37.517	37.5585	-	
	0.0859716	0.0857546	-	
4	37.533	37.705	-	
	0.1068745	0.0685775	-	
5	37.4735	37.60263	-	
	0.096756	.0910974	-	
6	37.47133	37.61067	-	
	0.1078618	0.0918979	-	
Total	37.53241	37.62327	-	
	0.0481434	0.0378832	-	

APPENDIX 4A. ANOVA MEANS TABLE (±SE) OF TEMPERATURES (°C) IN MARES SEPARATED BY DAY OF TRIAL: CONT N GROUP

APPENDIX 4B. ANOVA MEANS TABLE (±SE) OF TEMPERATURES (°C) IN MARES SEPARATED BY DAY OF TRIAL: EXE GROUP

Day	Microchip	Rectal	iButton	
1	38.245	38.05	37.96857	
	0.1462819	0.2262201	0.1345084	
2	37.87105	37.9105	37.57563	
	0.1239433	0.1216779	0.1371616	
3	37.51211	37.742	37.64833	
	0.1661035	0.1162634	0.1801466	
4	37.5575	37.7635	37.5375	
	0.1529188	0.1394335	0.1152226	
5	37.5735	37.7105	37.40733	
	0.0668158	0.0785844	0.0867259	
6	37.48278	37.66389	37.29533	
	0.079803	0.0644085	0.0626469	
7	37.5	37.57714	37.06286	
	0.081504	0.0742816	0.0405743	
Total	37.64658	37.77362	37.48955	
	0.0524466	0.046087	0.0496365	

Day	Microchip	Rectal	iButton	
1	37.746	37.852	37.57231	
	0.1226215	0.0870315	0.1354723	
2	37.6115	37.5325	37.66056	
	0.1521223	0.0927501	0.0914105	
3	37.464	37.5695	37.678	
	0.1349181	0.1152628	0.0804219	
4	37.571	37.3795	37.639	
	0.1466359	0.1331332	0.0809448	
5	37.46842	37.349	37.494	
	0.1559245	0.0869299	0.0788015	
6	37.47357	37.31643	37.50357	
	0.1886901	0.1604616	0.1023225	
7	37.01167	37.795	37.3325	
	0.3430683	0.2279001	0.1379236	
Total	37.52386	37.50948	37.58651	
	0.0610691	0.0474085	0.0365314	

APPENDIX 4C. ANOVA MEANS TABLE (±SE) OF TEMPERATURES (°C) IN MARES SEPARATED BY DAY OF TRIAL: CONT P GROUP

APPENDIX 4D. ANOVA MEANS TABLE (±SE) OF TEMPERATURES (°C) IN MARES SEPARATED BY DAY OF TRIAL: CONT S GROUP

Dav	Microchip	Rectal	iButton	
1	37.40222	37.67889	37.33333	
	0.2342288	0.1040493	0.0501385	
2	37.24875	37.72875	37.24625	
	0.1352311	0.0885197	0.0517757	
3	37.244	37.6975	37.25688	
	0.1157124	0.0695731	0.0499976	
4	37.212	37.77688	37.3325	
	0.1120511	0.0838943	0.0522693	
5	37.26625	37.7775	37.36688	
	0.1268655	0.0743219	0.0683904	
6	37.27273	37.70727	37.33273	
	0.1136189	0.1206354	0.0576681	
7	36.92	37.75	-	
	0.3599987	0.0299988	-	
Total	37.25643	37.73349	37.30833	
	0.0532702	0.0334397	0.0231881	

Time	Microchip	Rectal	iButton	
0600	37.2333	37.61735	37.33265	
	0.0595576	0.038533	0.0350533	
1400	37.64376	37.67073	37.39486	
	0.0505977	0.0394949	0.0498123	
1800	37.67209	37.72609	37.54932	
	0.0467915	0.0421398	0.0500538	
2200	37.45245	37.6034	37.6062	
	0.0570535	0.0543239	0.0407655	
Total	37.50588	37.6559	37.47305	
	0.0279668	0.022019	0.0231485	

APPENDIX 5. ANOVA MEANS TABLE (±SE) OF TEMPERATURES (°C) IN MARES AT 4 DIFFERENT TIMES OF DAY: ALL GROUPS AVERAGED

APPENDIX 6A. ANOVA MEANS TABLE (±SE) OF TEMPERATURES (°C) IN MARES AT 4 DIFFERENT TIMES OF DAY: CONT N GROUP

Time	Microchip	Rectal	iButton	
0600	37.34	37.65958	-	
	.0994603	0.0547738	-	
1400	37.7475	37.69214	-	
	0.01011621	0.0816892	-	
1800	37.561	37.62367	-	
	0.0693506	0.0507609	-	
2200	37.4496	37.5108	-	
	0.1041721	0.1068799	-	
Total	37.53241	37.62327	-	
	0.0481434	0.0378832	-	

APPENDIX 6B. ANOVA MEANS TABLE (±SE) OF TEMPERATURES (°C) IN MARES AT 4 DIFFERENT TIMES OF DAY: EXE GROUP

Time	Microchip	Rectal	iButton	
0600	37.50828	37.72759	37.37476	
	0.103935	0.0785736	0.0708237	
1400	37.59643	37.53214	37.277	
	0.093196	0.0877335	0.1107204	
1800	37.78267	37.90433	37.60875	
	0.1103641	0.0962453	0.1162695	
2200	37.69593	37.91759	37.64792	
	0.1084248	0.0903968	0.0730519	
Total	37.64658	37.77362	37.48955	
	.0524466	0.045087	0.0496365	

Time	Microchip	Rectal	iButton	
0600	36.96714	37.46786	37.38037	
	0.1305968	0.0797572	0.0616753	
1400	37.91161	37.75129	37.62276	
	0.0766575	0.0809563	0.0718216	
1800	37.80929	37.51929	37.64593	
	0.0781007	0.0941054	0.0719474	
2200	37.36	37.27357	37.69846	
	0.1114973	0.1056864	0.0745339	
Total	37.52386	37.50948	37.58651	
	0.0610691	0.0474085	0.0365314	

APPENDIX 6C. ANOVA MEANS TABLE (±SE) OF TEMPERATURES (°C) IN MARES AT 4 DIFFERENT TIMES OF DAY: CONT P GROUP

APPENDIX 6D. ANOVA MEANS TABLE (±SE) OF TEMPERATURES (°C) IN MARES AT 4 DIFFERENT TIMES OF DAY: CONT S GROUP

Time	Microchip	Rectal	iButton	
0600	37.08143	37.61619	37.224	
	0.1060061	0.0816102	0.0357726	
1400	37.19455	37.70636	37.19238	
	0.0850535	0.0367426	0.0342463	
1800	37.49818	37.88591	37.36591	
	0.1032956	0.0523998	0.0464154	
2200	37.24158	37.71952	37.44429	
	0.1181713	0.0796465	0.046005	
Total	37.25643	37.73349	37.30833	
	0.0532702	0.0334397	0.0231881	

Day	Microchip	Rectal	iButton	
1	37.79021	37.7583	37.59379	
	0.0813607	0.0792835	0.0815768	
2	37.5712	37.72474	37.5008	
	0.0770312	0.0495678	0.0616149	
3	37.44608	37.63895	37.53021	
	0.0649321	0.0506698	0.0636546	
4	37.48547	37.64987	37.51346	
	0.0677181	0.0581231	0.0520332	
5	37.45467	37.60107	37.42863	
	0.0574106	0.0453028	0.0452227	
6	37.43776	37.57448	37.3785	
	0.061889	0.0566766	0.0469677	
7	37.22733	37.68733	37.16091	
	0.1551879	0.0964637	0.0662291	
Total	37.50588	37.6559	37.47305	
	0.0279668	0.022019	0.0231485	

APPENDIX 7. ANOVA MEANS TABLE (±SE) OF TEMPERATURES (°C) IN MARES AT 4 DIFFERENT TIMES OF DAY: ALL GROUPS AVERAGED TOGETHER

APPENDIX 8A. ANOVA TABLE FOR MEAN COMPARISON TEST OF RECTAL TEMPERATURE (°C) IN MARES BY TREATMENT, TIME AND DAY

Source	Partial SS	df	MS	F	Prob > F
Model	7.50049506	13	0.576961159	2.98	0.0003
Treatment	4.97042334	3	1.65680778	8.55	0.0000
Time	1.05002027	3	0.350006756	1.81	0.1454
Day	1.77417932	7	0.253454189	1.31	0.2449
Residual	79.4555744	410	0.193794084		
Total	86.9560695	423	0.205569904		

APPENDIX 8B. BONFERRONI MEANS COMPARISON TEST COMPARING RECTAL TEMPERATURE (°C) IN MARES BY DIFFERENT MANAGEMENT CONDITIONS

	Cont N	EXE	Cont P	
EXE	0.15035			
	0.070			
Cont P	- 0.113793	- 0.264142		
	0.337	0.000		
Cont S	0.110217	- 0.040132	0.22401	
	0.517	1.000	0.003	

Source	Partial SS	df	MS	F	Prob > F	
Model	25.6913262	13	1.97625586	7.17	0.0000	
Treatment	8.33084174	3	2.77694725	10.07	0.0000	
Time	11.19981	3	3.73327	13.54	0.0000	
Day	4.95855815	7	0.70836545	2.57	0.0134	
Residual	111.950487	406	0.275740117			
Total	137.641814	419	0.328500749			

APPENDIX 9A. ANOVA TABLE FOR MEAN COMPARISON TEST OF MICROCHIP TEMPERATURE (°C) IN MARES BY TREATMENT, TIME AND DAY

APPENDIX 9B. BONFERRONI MEANS COMPARISON TEST COMPARING MICROCHIP TEMPERATURE (°C) IN MARES BY DIFFERENT MANAGEMENT CONDITIONS

	Cont N	EXE	Cont P	
EXE	0.114171			
	0.775			
Cont P	- 0.008548	- 0.122719		
	1.000	0.590		
Cont S	- 0.275979	- 0.39015	- 0.267431	
	0.005	0.000	0.006	

APPENDIX 9C. BONFERRONI MEANS COMPARISON TEST COMPARING MICROCHIP TEMPERATURE (°C) IN MARES BY 4 DIFFERENT TIMES OF DAY

	1400	1800	2200	
1800	0.028329			
	1.000			
2200	- 0.191313	- 0.219642		
	0.074	0.024		
0600	- 0.41046	- 0.43879	- 0.219148	
	0,000	0.000	0.029	

	1	2	3	4	5	6	7
2	- 0.219013						
	1.000						
3	- 0.344132	- 0.125119					
	0.033	1.000					
4	- 0.304746	- 0.085733	0.039386				
	0.110	1.000	1.000				
5	- 0.335546	- 0.116533	0.008585	- 0.0308			
	0.042	1.000	1.000	1.000			
6	- 0.352454	- 0.133441	- 0.008322	- 0.047708	- 0.016908		
	0.045	1.000	1.000	1.000	1.000		
7	- 0.562879	- 0.343866	- 0.218747	- 0.258133	- 0.227333	- 0.210425	
	0.024	0.895	1.000	1.000	1.000	1.000	
8	- 0.620214	- 0.401201	- 0.276083	- 0.315468	- 0.284668	- 0.26776	- 0.057335
	1.000	1.000	1.000	1.000	1.000	1.000	1.000

APPENDIX 9D. BONFERRONI MEANS COMPARISON TEST COMPARING MICROCHIP TEMPERATURE (°C) IN MARES BY DAY OF TRIAL

APPENDIX 10. ANOVA TABLE FOR MEAN COMPARISON TEST OF IBUTTON TEMPERATURE (°C) IN MARES BY TREATMENT, TIME AND DAY

Source	Partial SS	df	MS	F	Prob > F
Model	8.8902148	12	0.740851233	5.94	0.0000
Treatment	4.1817265	2	2.09086325	16.75	0.0000
Time	2.39208952	3	0.79736173	6.39	0.0000
Day	1.61206022	7	0.230294317	1.85	0.0789
Residual	33.5717397	269	0.124802006		
Total	42.4619545	281	0.151110158		

APPENDIX 11A. BONFERRONI MEANS COMPARISON TEST COMPARING IBUTTON TEMPERATURE (°C) IN MARES UNDER DIFFERENT MANAGEMENT CONDITIONS

	EXE	Cont P	
Cont P	0.906963		
	0.209		
Cont S	- 0.181217	- 0.27818	
	0.005	0.000	

	1400	1800	2200	
1800	0.154458			
	0.086			
2200	0.21134	0.056882		
	0.006	1.000		
0600	- 0.06221	- 0.216668	- 0.27355	
	1.000	0.004	0.0000	

APPENDIX 11B. BONFERRONI MEANS COMPARISON TEST COMPARING IBUTTON TEMPERATURE (°C) IN MARES BY 4 DIFFERENT TIMES

APPENDIX 12. ANOVA MEANS TABLE (±SE) OF TEMPERATURE (°C) IN MARES FOR TIME DURING EXERCISE FOR EXE GROUP

Time	Microchip	Rectal	iButton	
0	37.60423	37.54961	37.57529	
	0.0932593	0.0985237	0.1298333	
10	38.33045	38.14731	38.28	
	0.2047293	0.0983972	-	
20	38.84954	38.71615	39.28	
	0.2024466	0.0892672	-	
30	39.256	39.17923	39.56	
	0.168227	0.1004895	-	
45	39.75423	39.64962	38.89737	
	0.1437071	0.0927403	0.100411	
60	39.35885	39.40231	38.66684	
	0.0855181	0.0780303	0.1174312	
75	38.82615	38.80461	38.3385	
	0.0960017	0.1092424	0.1558968	
105	38.04885	38.15462	37.7225	
	0.1166946	0.101795	0.1180184	
165	37.69885	37.87308	37.4995	
	0.1152688	0.0958489	0.1568632	
Total	38.63524	38.6085	38.14263	
	0.0663338	0.0546449	0.0732293	

APPENDIX 13. PAIRWISE CORRELATION COEFFICIENTS FOR EXERCISED MARES BY DIFFERENT MANAGEMENT CONDITIONS

	Microchip	Rectal	iButton	
Microchip	1.0000			
Rectal	0.8198	1.0000		
	0.0000			
iButton	0.8126	0.7596	1.0000	
	0.000	0.0000		

APPENDIX 14. TEMPERATURE RECORDS FOR ALL MARES

HORSE	ТХТ	DAY	TIME	M/C(°C)	RECTAL(°C)	I/B (°C)
Beauty	Cont S	1	1800	37.06	37.44	37.39
Beauty	Cont S	1	2200	36.06	37.28	37.22
Beauty	Cont S	2	0600	36.17	37.67	37.11
Beauty	Cont S	2	1400	37.17	37.72	37.00
Beauty	Cont S	2	1800	38.06	37.94	37.22
Beauty	Cont S	2	2200	37.56	36.67	37.39
Beauty	Cont S	3	0600	37.28	37.00	37.11
Beauty	Cont S	3	1400	37.17	37.50	37.11
Beauty	Cont S	3	1800	37.44	37.56	37.39
Beauty	Cont S	3	2200		37.89	37.50
Beauty	Cont S	4	0600	36.72	37.22	37.22
Beauty	Cont S	4	1400	36.67	37.56	37.11
Beauty	Cont S	4	1800	37.17	37.89	37.50
Beauty	Cont S	4	2200	37.28	37.72	37.61
Beauty	Cont S	5	0600	36.56	37.28	37.50
Beauty	Cont S	5	1400	37.17	37.61	37.22
Beauty	Cont S	5	1800	37.44	37.89	37.50
Beauty	Cont S	5	2200	37.06	37.89	37.61
Beauty	Cont S	6	0600	37.72	36.67	37.39
Beauty	Cont S	6	1400	37.06	37.50	37.22
Beauty	Cont S	6	1800	37.94	37.61	37.50
Fancy	Cont S	1	1400	38.06	37.44	37.39
Fancy	Cont S	1	1800	37.44	37.83	37.11
Fancy	Cont S	1	2200	36.56	37.61	37.11
Fancy	Cont S	2	0600	36.56	37.72	37.00
Fancy	Cont S	2	1400	37.06	38.00	37.00
Fancy	Cont S	2	1800	36.67	37.94	37.00
Fancy	Cont S	2	2200	36.67	37.67	37.11
Fancy	Cont S	3	0600	36.17	37.78	37.00
Fancy	Cont S	3	1400	36.67	37.67	36.89
Fancy	Cont S	3	1800	37.06	37.61	37.00
Fancy	Cont S	3	2200	37.06	37.78	37.39
Fancy	Cont S	4	0600	36.78	37.28	37.22
Fancy	Cont S	4	1400	37.61	37.94	37.00
Fancy	Cont S	4	1800	36.44	37.67	37.11
Fancy	Cont S	4	2200	37.06	37.50	37.39
Fancy	Cont S	5	0600	37.06	37.56	37.22
Fancy	Cont S	5	1400	36.56	37.94	37.11
Fancy	Cont S	5	1800	38.28	37.72	37.22
Fancy	Cont S	5	2200	37.17	37.39	37.39
Fancy	Cont S	6	0600	36.94	37.94	37.22
Fancy	Cont S	6	1400	36.67	37.72	37.39
Fancy	Cont S	6	1800	37.06	37.89	37.11
Fancy	Cont S	6	2200	37.44	37.89	37.61

HORSE	ТХТ	DAY	TIME	M/C(°C)	RECTAL(°C)	I/B (°C)
Fancy	Cont S	7	0600	37.28	37.78	
Fancy	Cont S	7	1400	36.56	37.72	
Lena	Cont S	1	1800	37.94	37.56	37.39
Lena	Cont S	1	2200	37.94	38.28	37.50
Lena	Cont S	2	0600	37.33	37.50	37.22
Lena	Cont S	2	1400	37.56	37.67	37.39
Lena	Cont S	2	1800	37.94	37.94	37.39
Lena	Cont S	2	2200	38.06	37.50	37.50
Lena	Cont S	3	0600	37.56	37.39	37.39
Lena	Cont S	3	1400	37.56	37.72	37.11
Lena	Cont S	3	1800	38.06	38.00	37.50
Lena	Cont S	3	2200	37.67	37.94	37.50
Lena	Cont S	4	0600	37.56	37.94	37.50
Lena	Cont S	4	1400	37.44	37.56	37.22
Lena	Cont S	4	1800	38.06	38.22	37.50
Lena	Cont S	4	2200	37.61	38.22	37.50
Lena	Cont S	5	0600	38.17	37.94	37.22
Lena	Cont S	5	1400	37.56	37.78	37.11
Lena	Cont S	5	1800	37.83	38.44	37.78
Lena	Cont S	5	2200	36.61	37.33	38.11
Lena	Cont S	6	0600	37.11	38.22	37.50
Lena	Cont S	6	1400	37.56	37.56	37.50
Shaggy	Cont S	1	1800	37.67	38.00	37.50
Shaggy	Cont S	1	2200	37.89	37.67	37.39
Shaggy	Cont S	2	0600	37.28	37.50	37.11
Shaggy	Cont S	2	1400	37.17	37.94	37.39
Shaggy	Cont S	2	1800	37.28	38.17	37.61
Shaggy	Cont S	2	2200	37.44	38.11	37.50
Shaggy	Cont S	3	0600	37.06	37.94	37.22
Shaggy	Cont S	3	1400	37.06	37.44	37.22
Shaggy	Cont S	3	1800	37.56	38.11	37.39
Shaggy	Cont S	3	2200	37.28	37.83	37.39
Shaggy	Cont S	4	0600	37.06	37.94	37.11
Shaggy	Cont S	4	1400	37.44	37.83	37.22
Shaggy	Cont S	4	1800	37.28	38.00	37.72
Shaggy	Cont S	4	2200		37.94	37.39
Shaggy	Cont S	5	0600	37.28	37.78	37.22
Shaggy	Cont S	5	1400	37.06	37.83	37.22
Shaggy	Cont S	5	1800	37.28	38.06	37.22
Shaggy	Cont S	5	2200	37.17	38.00	37.22
Shaggy	Cont S	6	0600	37.06	37.89	37.00
Shaggy	Cont S	6	1400	37.44	37.89	37.22
Faces	Cont P	1	1800	37.94	37.83	37.39
Faces	Cont P	1	2200	37.67	37.78	37.39

HORSE	ТХТ	DAY	TIME	M/C(°C)	RECTAL(°C)	I/B(°C)
Faces	Cont P	2	0600	37.06	37.72	37.11
Faces	Cont P	2	1400	37.56	37.56	36.89
Faces	Cont P	2	1800	37.94	37.61	37.50
Faces	Cont P	2	2200	37.83	37.28	37.39
Faces	Cont P	3	0600	37.28	36.89	37.00
Faces	Cont P	3	1400	37.28	37.44	37.22
Faces	Cont P	3	1800	38.06	37.67	37.61
Faces	Cont P	3	2200	37.28	36.61	37.39
Faces	Cont P	4	0600	37.06	36.61	36.78
Faces	Cont P	4	1400	38.06	36.94	37.11
Faces	Cont P	4	1800	38.06	37.22	37.50
Faces	Cont P	4	2200	37.56	36.17	37.50
Faces	Cont P	5	0600	37.44	37.11	37.11
Faces	Cont P	5	1400	38.28	37.72	37.11
Faces	Cont P	5	1800	37.56	36.94	37.11
Faces	Cont P	5	2200		37.11	37.22
Faces	Cont P	6	0600	37.28	37.11	37.11
Faces	Cont P	6	1400	38.56	36.50	37.39
Faces	Cont P	6	1800	38.17	36.67	37.39
Faces	Cont P	6	2200	37.56	36.33	37.22
Faces	Cont P	7	0600	37.28	37.28	37.00
Faces	Cont P	7	1400	38.17	38.61	37.22
Dee	Cont P	1	0600	37.67	37.89	37.61
Dee	Cont P	1	1400	38.56	38.17	38.00
Dee	Cont P	1	1800	38.28	38 33	38.00
Dee	Cont P	1	2200	37.17	38.28	38.11
Dee	Cont P	2	0600	37.61	37.33	37.61
Dee	Cont P	2	1400	38.44	37 94	37.89
Dee	Cont P	2	1800	38.06	38.06	37.78
Dee	Cont P	2	2200	37 44	37 33	37 78
Dee	Cont P	3	0600	37.83	37.67	37.78
Dee	Cont P	3	1400	38.06	37.83	37.50
Dee	Cont P	3	1800	38.17	37.78	37.61
Dee	Cont P	3	2200	37.67	37.94	37.50
Dee	Cont P	4	0600	37.06	37.50	37.50
Dee	Cont P	4	1400	38.06	38.00	37.89
Dee	Cont P	4	1800	38.28	37.83	37.61
Dee	Cont P	4	2200	37.56	37.61	37 78
Dee	Cont P	5	0600	36.56	37.56	37.50
Dee	Cont P	5	1400	37 94	38.06	37.61
Dee	Cont P	5	1800	38 44	36.83	37.61
Dee	Cont P	5	2200	37.89	37.28	37 39
Dee	Cont P	6	0600	36 44	37 50	37.11
Dee	Cont P	6	1400	37.44	37.39	37.50

HORSE	ТХТ	DAY	TIME	M/C(°C)	RECTAL(°C)	I/B(°C)
Missa	Cont P	1	1400	38.44	37.89	37.00
Missa	Cont P	1	1800	38.06	38.00	37.11
Missa	Cont P	1	2200	38.06	37.50	37.22
Missa	Cont P	2	0600	37.67	37.83	37.00
Missa	Cont P	2	1400	38.94	38.17	37.78
Missa	Cont P	2	1800	38.17	37.28	37.61
Missa	Cont P	2	2200	37.94	38.00	37.61
Missa	Cont P	3	0600	37.94	37.67	37.22
Missa	Cont P	3	1400	38.06	37.94	37.78
Missa	Cont P	3	1800	38.06	38.11	37.39
Missa	Cont P	3	2200	38.06	37.39	37.39
Missa	Cont P	4	0600	38.06	38.00	37.39
Missa	Cont P	4	1400	37.94	38.44	37.50
Missa	Cont P	4	1800	38.06	37.89	37.39
Missa	Cont P	4	2200	38.06	37.11	37.50
Missa	Cont P	5	0600	37.89	37.61	37.00
Missa	Cont P	5	1400	38.06	37.94	37.22
Missa	Cont P	5	1800	37.61	37.61	37.22
Missa	Cont P	5	2200	37.72	36.94	37.00
Missa	Cont P	6	0600	37.28	37.94	37.00
Missa	Cont P	6	1400	37.94	37.78	37.11
Prissy	Cont P	1	1400	37.56	38.00	
Prissy	Cont P	1	1800	37.67	37.89	36.61
Prissy	Cont P	1	2200	36.94	38.00	
Prissy	Cont P	2	0600	35.67	37.33	
Prissy	Cont P	2	1400	38.06	37.94	
Prissy	Cont P	2	1800	37.44	36.89	38.11
Prissy	Cont P	2	2200	37.44	37.89	38.22
Prissy	Cont P	3	0600	36.67	38.06	38.00
Prissy	Cont P	3	1400	37.17	38.11	38.39
Prissy	Cont P	3	1800	37.56	38.00	38.11
Prissy	Cont P	3	2200	37.06	37.17	38.22
Prissy	Cont P	4	0600	35.44	37.39	37.39
Prissy	Cont P	4	1400	37.94	37.94	38.00
Prissy	Cont P	4	1800	37.94	37.00	37.89
Prissy	Cont P	4	2200	37.06	38.00	38.11
Prissy	Cont P	5	0600	36.83	37.44	37.61
Prissy	Cont P	5	1400	38.06	37.83	38.00
Prissy	Cont P	5	1800	37.28	37.44	37.89
Prissy	Cont P	5	2200	35.78	37.50	38.00
Prissy	Cont P	6	0600	36.22	36.94	37.61
Prissy	Cont P	6	1400	38.06	37.94	38.00
Prissy	Cont P	6	1800	38.28	38.33	38.11
Prissy	Cont P	6	2200	37.56	37.00	38.11

HORSE	ТХТ	DAY	TIME	M/C(°C)	RECTAL(°C)	I/B(°C)
Prissy	Cont P	7	0600	36.06	38.33	37.61
Prissy	Cont P	7	1400	37.56	37.33	37.50
Prissy	Cont P	7	1800	36.94	37.78	
Prissy	Cont P	7	2200	36.06	37.44	
Sugar	Cont P	1	1400	37.44	37.83	38.00
Sugar	Cont P	1	1800	37.56	37.28	38.00
Sugar	Cont P	1	2200	37.17	37.11	38.00
Sugar	Cont P	2	0600	36.67	37.44	37.50
Sugar	Cont P	2	1400	37.67	36.94	37.89
Sugar	Cont P	2	1800	37.56	37.44	38.00
Sugar	Cont P	2	2200	37.06	36.67	38.22
Sugar	Cont P	3	0600	36.17	37.89	37.89
Sugar	Cont P	3	1400	37.56	37.72	37.89
Sugar	Cont P	3	1800	37.06	37.28	37.89
Sugar	Cont P	3	2200	36.28	36.22	37.78
Sugar	Cont P	4	0600	36.94	36.61	37.61
Sugar	Cont P	4	1400	37.44	37.56	38.00
Sugar	Cont P	4	1800	37.28	36.94	38.11
Sugar	Cont P	4	2200	37.56	36.83	38.22
Sugar	Cont P	5	0600	36.44	37.28	37.61
Sugar	Cont P	5	1400	37.67	37.00	37.89
Sugar	Cont P	5	1800	37.17	36.61	37.89
Sugar	Cont P	5	2200	37.28	37.17	37.89
Sugar	Cont P	6	0600	36.56	37.17	37.61
Sugar	Cont P	6	1400	37.28	37.83	37.78
Marla	EXE	1	1400	38.06	37.44	
Marla	EXE	1	1800	38.28	37.17	37.78
Marla	EXE	1	2200	37.44	36.72	37.50
Marla	EXE	2	0600	37.56	37.44	37.11
Marla	EXE	2	1400	37.94	37.22	37.39
Marla	EXE	2	1800	38.06	37.44	37.50
Marla	EXE	2	2200	37.67	37.94	37.22
Marla	EXE	3	0600	37.33	37.33	
Marla	EXE	3	1400	37.67	37.17	
Marla	EXE	3	1800	37.67	37.89	37.50
Marla	EXE	3	2200	37.56	38.00	37 39
Marla	EXE	4	0600	37.56	37.50	37.22
Marla	EXE	4	1400	37.67	37.28	37.11
Marla	EXE	4	1800	37.67	37.94	37 39
Marla	EXE	4	2200	37.67	37.67	37 22
Marla	EXE	5	0600	37.17	37.44	37.11
Marla	EXE	5	1400	37.67	37.50	37.00
Marla	EXE	5	1800	37.44	37.44	37.00
Marla	EXE	5	2200	37.67	37.72	37 39

HORSE	ТХТ	DAY	TIME	M/C(°C)	RECTAL(°C)	I/B(°C)
Marla	EXE	6	0600	37.67	37.56	37.11
Marla	EXE	6	1400	37.44	37.06	37.00
Marla	EXE	6	1800	37.17	37.56	36.89
Marla	EXE	6	2200	38.06	38.06	37.61
Marla	EXE	7	0600	37.83	37.61	37.00
Marla	EXE	7	1400	37.56	37.28	36.89
Marla	EXE	7	1800	37.44	37.44	37.00
Marla	EXE	7	2200	37.44	37.72	37.11
Marla	EXE	8	0600	37.17	37.72	37.22
Scoot	EXE	1	1800	38.44	38.50	38.39
Scoot	EXE	1	2200	38.06	38.78	38.00
Scoot	EXE	2	0600	37.56	38.61	38.00
Scoot	EXE	2	1400	37.00	36.39	36.11
Scoot	EXE	2	1800	38.06	38.50	38.22
Scoot	EXE	2	2200	36.94	37.78	37.50
Scoot	EXE	3	0600	36.44	37.56	
Scoot	EXE	3	1400	37.06	37.28	
Scoot	EXE	3	1800	36.17	36.78	36.28
Scoot	EXE	3	2200		38.44	37.61
Scoot	EXE	4	0600	36.78	37.56	37.39
Scoot	EXE	4	1400	36.89	37.83	37.11
Scoot	EXE	4	1800	37.44	38.33	37.89
Scoot	EXE	4	2200	37.44	37.94	37.39
Scoot	EXE	5	0600	37.44	37.94	37.22
Scoot	EXE	5	1400	37.67	38.17	37.50
Scoot	EXE	5	1800	37.67	38.17	37.61
Scoot	EXE	5	2200	37.44	38.11	37.78
Scoot	EXE	6	0600	37.28	37.89	37.22
Scoot	EXE	6	1400	37.44	37.67	37.22
Scoot	EXE	6	1800	37.56	37.94	37.50
Slimer	EXE	1	2200	37.67	37.89	37.61
Slimer	EXE	2	0600	37.44	37.83	37.39
Slimer	EXE	2	1400	37.44	37.39	37.22
Slimer	EXE	2	1800	37.28	37.72	37.50
Slimer	EXE	2	2200	37.44	37.72	37.61
Slimer	EXE	3	0600	37.06	37.61	37.39
Slimer	EXE	3	1400	37.44	37.28	37.22
Slimer	EXE	3	1800	37.94	38.00	37.78
Slimer	EXE	3	2200	37.17	37.61	37.39
Slimer	EXE	4	0600	37.06	37.44	37.22
Slimer	EXE	4	1400	37.28	37.33	37.11
Slimer	EXE	4	1800	37.28	37.44	37.22
Slimer	EXE	4	2200	37.17	37.11	37.50
Slimer	EXE	5	0600	37.56	37.78	37.22

HORSE	TXT	DAY	TIME	M/C(°C)	RECTAL(°C)	I/B(°C)
Slimer	EXE	5	1400	37.06	37.22	37.00
Slimer	EXE	5	1800	37.06	37.33	37.11
Slimer	EXE	5	2200	37.44	37.89	37.61
Slimer	EXE	6	0600	37.89	37.89	37.61
Slimer	EXE	6	1400	36.94	37.39	37.00
Slimer	EXE	6	1800	37.44	37.44	37.22
Slimer	EXE	6	2200	37.67	37.78	37.50
Slimer	EXE	7	0600	37.11	37.83	37.11
Sun	EXE	1	1800	38.44	38.78	38.11
Sun	EXE	1	2200	39.06	38.33	38.39
Sun	EXE	2	0600	38.44	38.00	37.89
Sun	EXE	2	1400	38.56	38.28	38.11
Sun	EXE	2	1800	38.56	38.39	38.22
Sun	EXE	2	2200	38.44	38.28	38.22
Sun	EXE	3	0600	37.89	37.89	38.00
Sun	EXE	3	1400	38.44	38.28	38.11
Sun	EXE	3	1800	38.94	38.83	38.61
Sun	EXE	3	2200	38.44	38.56	38.50
Sun	EXE	4	0600	38.50	38.83	38.00
Sun	EXE	4	1400	38.78	38.67	38.22
Sun	EXE	4	1800	39.06	38.78	38.61
Sun	EXE	4	2200	38.56	38.78	38.00
Sun	EXE	5	0600	38.17	38.17	
Sun	EXE	5	1400	37.56	37.72	37.89
Sun	EXE	5	1800	38.06	38.33	37.78
Sun	EXE	5	2200	37.94	37.72	37.89
Sun	EXE	6	0600	37.56	37.72	37.22
Sun	EXE	6	1400	37.56	37.50	37.22
Sun	EXE	6	1800	37.56	37.94	37.50
Sun	EXE	6	2200	37.94	38.11	37.61
Sun	EXE	7	0600	37.56	37.72	37.22
Sun	EXE	7	1400	37.56	37.44	37.11
Miss Lectric	EXE	1	1800	38.44	38.39	
Miss Lectric	EXE	1	2200	38.56	38.50	
Miss Lectric	EXE	2	0600	38.44	38.28	
Miss Lectric	EXE	2	1400	38.44	38.28	
Miss Lectric	EXE	2	1800	38.28	38.33	
Miss Lectric	EXE	2	2200		38.39	
Miss Lectric	EXE	3	0600	38.33	37.72	
Miss Lectric	EXE	3	1400	37.06	37.67	
Miss Lectric	EXE	3	1800	37.56	37.83	
Miss Lectric	EXE	3	2200	36.56	37.11	
Miss Lectric	EXE	4	0600	36.44	36.56	
Miss Lectric	EXE	4	1400	37.28	37.39	

HORSE	ТХТ	DAY	TIME	M/C(°C)	RECTAL(°C)	I/B(°C)
Miss Lectric	EXE	4	1800	37.56	37.50	
Miss Lectric	EXE	4	2200	37.06	37.39	
Miss Lectric	EXE	5	0600	37.83	37.17	
Miss Lectric	EXE	5	1400	37.67	37.33	
Miss Lectric	EXE	5	1800	37.67	37.50	
Miss Lectric	EXE	5	2200	37.28	37.56	
Miss Lectric	EXE	6	0600	36.67	37.50	
Miss Lectric	EXE	6	1400	37.56	37.44	
Miss Lectric	EXE	6	1800	37.28	37.50	
Girly	Cont N	1	1400	37.67	37.83	
Girly	Cont N	1	1800	37.28	37.89	
Girly	Cont N	1	2200	37.06	36.22	
Girly	Cont N	2	0600	36.56	37.33	
Girly	Cont N	2	1400	38.06	38.06	
Girly	Cont N	2	1800	37.17	37.67	
Girly	Cont N	2	2200	36.44	37.72	
Girly	Cont N	3	0600	36.94	37.56	
Girly	Cont N	3	1400	37.28	37.33	
Girly	Cont N	3	1800	37.06	37.11	
Girly	Cont N	3	2200	37.44	37.72	
Girly	Cont N	4	0600	36.44	37.44	
Girly	Cont N	4	1400	37.28	37.67	
Girly	Cont N	4	1800	37.17	37.61	
Girly	Cont N	4	2200	36.94	37.78	
Girly	Cont N	5	0600	36.78	37.61	
Girly	Cont N	5	1400	37.17	37.06	
Girly	Cont N	5	1800	37.06	37.78	
Girly	Cont N	5	2200	36.56	36.89	
Girly	Cont N	6	0600	37.56	37.56	
Girly	Cont N	6	1400	36.56	37.44	
Girly	Cont N	6	1800	37.06	37.33	
Destiny	Cont N	1	1400	38.06	38.06	
Destiny	Cont N	1	1800	38.06	37.94	
Destiny	Cont N	1	2200	38.28	37.00	
Destiny	Cont N	2	0600	37.94	37.78	
Destiny	Cont N	2	1400	38.78	38.33	
Destiny	Cont N	2	1800	37.94	37.83	
Destiny	Cont N	2	2200	37.56	38.06	
Destiny	Cont N	3	0600	37.56	37.83	
Destiny	Cont N	3	1400	37.94	38.06	
Destiny	Cont N	3	1800	37.67	37.72	
Destiny	Cont N	3	2200	38.06	38.00	
Destiny	Cont N	4	0600	37.94	37.94	
Destiny	Cont N	4	1400	37.94	38.00	

HORSE	ТХТ	DAY	TIME	M/C(°C)	RECTAL(°C)	I/B(°C)
Destiny	Cont N	4	1800	37.94	37.94	
Destiny	Cont N	4	2200	38.06	37.94	
Destiny	Cont N	5	0600	37.83	38.17	
Destiny	Cont N	5	1400	37.94	38.39	
Destiny	Cont N	5	1800	37.67	37.39	
Destiny	Cont N	5	2200	38.17	38.33	
Destiny	Cont N	6	0600	38.11	38.28	
Destiny	Cont N	6	1400	37.56	38.00	
Destiny	Cont N	6	1800	38.06	38.33	
Oakla	Cont N	1	1800	38.06	37.72	
Oakla	Cont N	1	2200	37.56	36.44	
Oakla	Cont N	2	0600	37.56	37.94	
Oakla	Cont N	2	1400	39.06	38.00	
Oakla	Cont N	2	1800	37.56	37.61	
Oakla	Cont N	2	2200	37.28	37.67	
Oakla	Cont N	3	0600	37.44	37.67	
Oakla	Cont N	3	1400	38.00	37.61	
Oakla	Cont N	3	1800	38.06	37.56	
Oakla	Cont N	3	2200	37.94	37.83	
Oakla	Cont N	4	0600	37.56	37.72	
Oakla	Cont N	4	1400	37.94	37.94	
Oakla	Cont N	4	1800	37.67	37.89	
Oakla	Cont N	4	2200	37.56	37.61	
Oakla	Cont N	5	0600	37.61	37.72	
Oakla	Cont N	5	1400	37.56	37.56	
Oakla	Cont N	5	1800	37.72	37.72	
Oakla	Cont N	5	2200	37.28	37.22	
Oakla	Cont N	6	0600	37.50	37.83	
Oakla	Cont N	6	1400	37.28	37.44	
Oakla	Cont N	6	1800	37.33	37.56	
Rocky	Cont N	1	1400	38.06	37.94	
Rocky	Cont N	1	1800	37.67	37.44	
Rocky	Cont N	1	2200	38.17	37.94	
Rocky	Cont N	2	0600	37.06	37.33	
Rocky	Cont N	2	1400	38.28	37.94	
Rocky	Cont N	2	1800	37.06	37.06	
Rocky	Cont N	2	2200	37.28	37.67	
Rocky	Cont N	3	0600	37.17	37.44	
Rocky	Cont N	3	1400	37.56	37.50	
Rocky	Cont N	3	1800	37.67	37.72	
Rocky	Cont N	3	2200	37.94	37.72	
Rocky	Cont N	4	0600	37.28	37.72	
Rocky	Cont N	4	1400	37.94	37.78	
Rocky	Cont N	4	1800	38.06	37.72	

HORSE	ТХТ	DAY	TIME	M/C(°C)	RECTAL(°C)	I/B(°C)
Rocky	Cont N	4	2200	37.56	36.56	
Rocky	Cont N	5	0600	37.94		
Rocky	Cont N	5	1400	37.56	37.28	
Rocky	Cont N	5	1800	38.06	37.28	
Rocky	Cont N	5	2200	37.44	37.72	
Rocky	Cont N	6	0600	37.83	37.50	
Rocky	Cont N	6	1400	37.56	37.44	
Rocky	Cont N	6	1800	37.94	37.72	
Trinket	Cont N	1	1800	37.67	37.83	
Trinket	Cont N	1	2200	37.28	37.00	
Trinket	Cont N	2	0600	36.67	37.28	
Trinket	Cont N	2	1400	38.44	37.94	
Trinket	Cont N	2	1800	36.94	37.67	
Trinket	Cont N	2	2200	36.44	37.67	
Trinket	Cont N	3	0600	36.94	37.67	
Trinket	Cont N	3	1400	37.06	36.28	
Trinket	Cont N	3	1800	37.17	37.17	
Trinket	Cont N	3	2200	37.44	37.67	
Trinket	Cont N	4	0600	36.56	37.78	
Trinket	Cont N	4	1400	37.94	37.78	
Trinket	Cont N	4	1800	37.44	37.72	
Trinket	Cont N	4	2200	37.44	37.56	
Trinket	Cont N	5	0600	37.72	37.56	
Trinket	Cont N	5	1400	37.17	37.44	
Trinket	Cont N	5	1800	37.17	37.50	
Trinket	Cont N	5	2200	37.06	37.83	
Trinket	Cont N	6	0600	37.00	37.17	
Trinket	Cont N	6	1400	37.28	37.28	
Trinket	Cont N	6	1800	37.44	37.28	

APPENDIX 15. TEMPERATURE RECORDS FOR EXERCISED HORSES

HORSE	ТХТ	DAY	TIME	M/C(°C)	RECTAL(°C)	I/B(°C)
Slimer	EXE	1	0	37.44	37.39	37.89
Slimer	EXE	1	10		38.28	
Slimer	EXE	1	20		39.17	
Slimer	EXE	1	30		39.50	
Slimer	EXE	1	45	39.56	39.83	39.00
Slimer	EXE	1	60	39.06	39.22	38.00
Slimer	EXE	1	75	38.56	38.28	37.61
Slimer	EXE	1	105	37.56	37.72	37.50
Slimer	EXE	1	165	37.28	37.44	37.39
Slimer	EXE	2	0	37.44	37.28	37.78
Slimer	EXE	2	10	37.67	38.00	
Slimer	EXE	2	20	38.28	38.78	
Slimer	EXE	2	30	38.56	39.28	
Slimer	EXE	2	45	39.94	40.22	39.50
Slimer	EXE	2	60	39.56	40.11	38.89
Slimer	EXE	2	75	38.94	39.56	38.22
Slimer	EXE	2	105	38.06	38.44	38.00
Slimer	EXE	2	165	37.94	38.00	37.61
Slimer	EXE	3	0	37.28	37.33	37.00
Slimer	EXE	3	10	37.56	37.72	
Slimer	EXE	3	20	38.06	38.28	
Slimer	EXE	3	30	38.94	38.94	
Slimer	EXE	3	45	39.94	39.83	38.22
Slimer	EXE	3	60	39.28	39.28	39.00
Slimer	EXE	3	75	38.56	39.00	39.28
Slimer	EXE	3	105	37.17	37.83	38.22
Slimer	EXE	3	165	37.56	37.56	37.39
Slimer	EXE	4	0	37.06	37.22	37.11
Slimer	EXE	4	10	37.28	37.94	
Slimer	EXE	4	20	38.17	38.28	
Slimer	EXE	4	30	38.78	38.83	
Slimer	EXE	4	45	39.56	39.56	39.11
Slimer	EXE	4	60	39.17	39.44	38.50
Slimer	EXE	4	75	38.44	38.89	38.11
Slimer	EXE	4	105	37.56	37.89	37.50
Slimer	EXE	4	165	37.06	37.33	37.00
Slimer	EXE	5	0	36.94	37.39	37.00
Slimer	EXE	5	10	37.17	37.00	
Slimer	EXE	5	20	37.44	37.94	
Slimer	EXE	5	30	37.94	38.56	
Slimer	EXE	5	45	38.94	39.22	38.61
Slimer	EXE	5	60	39.17	39.06	38.61
Slimer	EXE	5	75	38.50	38.44	37.78
Slimer	EXE	5	105	37.56	37.72	37.39

HORSE	ТХТ	DAY	TIME	M/C(°C)	RECTAL(°C)	I/B(°C)
Slimer	EXE	5	165	37.44	37.44	37.00
Sun	EXE	1	0	37.00	36.39	37.50
Sun	EXE	1	10	38.17	38.17	
Sun	EXE	1	20	38.56	38.72	
Sun	EXE	1	30	39.44	39.56	
Sun	EXE	1	45	39.67	39.72	39.00
Sun	EXE	1	60	39.06	39.39	37.89
Sun	EXE	1	75	38.67	38.28	38.11
Sun	EXE	1	105	38.44	38.33	38.22
Sun	EXE	1	165	38.06	38.50	38.22
Sun	EXE	2	0	37.56	37.00	29.11
Sun	EXE	2	10	38.28	38.06	
Sun	EXE	2	20	38.78	38.83	
Sun	EXE	2	30	39.06	39.06	
Sun	EXE	2	45	38.78	39.06	38.00
Sun	EXE	2	60	38.17	38.78	38.39
Sun	EXE	2	75	37.56	37.06	38.11
Sun	EXE	2	105	36.89	36.94	37.00
Sun	EXE	2	165	36.17	36.78	35.61
Sun	EXE	3	0	37.44	38.28	37.44
Sun	EXE	3	10	38.28	38.39	38.28
Sun	EXE	3	20	39.28	39.06	39.28
Sun	EXE	3	30	39.28	39.61	39.56
Sun	EXE	3	45	39.56	39.94	38.78
Sun	EXE	3	60	38.78	39.56	38.22
Sun	EXE	3	75	38.22	39.00	37.94
Sun	EXE	3	105	37.94	38.22	37.44
Sun	EXE	3	165	37.44	38.33	37.89
Sun	EXE	4	0	37.56	37.67	37.50
Sun	EXE	4	10	38.17	38.17	
Sun	EXE	4	20	38.56	38.61	
Sun	EXE	4	30	39.06	39.33	
Sun	EXE	4	45	39.78	39.44	39.50
Sun	EXE	4	60	38.94	39.33	38.78
Sun	EXE	4	75	38.44	38.89	38.22
Sun	EXE	4	105	37.44	38.22	37.78
Sun	EXE	4	165	37.67	38.17	37.61
Sun	EXE	5	0	37.44	37.67	37.89
Sun	EXE	5	10	38.94	38.44	
Sun	EXE	5	20	39.94	39.22	
Sun	EXE	5	30	39.78	39.83	
Sun	EXE	5	45	40.17	40.56	39.11
Sun	EXE	5	60	39.56	39.83	39.00
Sun	EXE	5	75	38.78	39.44	38.50

HORSE	ТХТ	DAY	TIME	M/C(°C)	RECTAL(°C)	I/B(°C)
Sun	EXE	5	105	38.17	38.78	37.78
Sun	EXE	5	165	37.56	37.94	37.94
Marla	EXE	1	0	37.67	37.17	33.78
Marla	EXE	1	10		38.78	
Marla	EXE	1	20		39.28	
Marla	EXE	1	30	39.17	40.11	
Marla	EXE	1	45	39.67	40.28	36.28
Marla	EXE	1	60	39.67	39.83	30.11
Marla	EXE	1	75	39.17	39.17	38.00
Marla	EXE	1	105	37.94	37.33	37.39
Marla	EXE	1	165	37.56	37.50	37.50
Marla	EXE	2	0	37.67	37.28	37.89
Marla	EXE	2	10	38.00	38.72	
Marla	EXE	2	20	38.61	39.17	
Marla	EXE	2	30	39.06	39.72	
Marla	EXE	2	45	39.28	40.06	38.89
Marla	EXE	2	60	39.44	39.78	37.89
Marla	EXE	2	75	38.78	38.72	37.61
Marla	EXE	2	105	38.06	38.11	37.39
Marla	EXE	2	165	37.67	37.94	37.22
Marla	EXE	3	0	37.67	37.50	37.11
Marla	EXE	3	10	37.67	38.06	
Marla	EXE	3	20	38.28	38.67	
Marla	EXE	3	30	38.78	39.22	
Marla	EXE	3	45	39.56	40.06	38.22
Marla	EXE	3	60	39.67	39.56	39.00
Marla	EXE	3	75	38.94	38.50	39.28
Marla	EXE	3	105	38.06	37.89	37.50
Marla	EXE	3	165	37.56	37.67	37.00
Marla	EXE	4	0	37.28	37.39	37.11
Marla	EXE	4	10	37.67	38.00	
Marla	EXE	4	20	38.44	38.83	
Marla	EXE	4	30	38.78	39.11	
Marla	EXE	4	45	39.17	39.83	39.00
Marla	EXE	4	60	39.28	39.67	38.50
Marla	EXE	4	75	39.06	39.17	37.89
Marla	EXE	4	105	37.67	38.89	37.11
Marla	EXE	4	165	37.17	37.56	36.89
Marla	EXE	5	0	37.56	37.28	36.89
Marla	EXE	5	10	37.56	37.61	
Marla	EXE	5	20	38.06	38.11	
Marla	EXE	5	30	38.28	38.83	
Marla	EXE	5	45	38.94	39.67	38.61
Marla	EXE	5	60	39.39	39.17	38.39

HORSE	ТХТ	DAY	TIME	M/C(°C)	RECTAL(°C)	I/B(°C)
Marla	EXE	5	75	38.72	38.56	37.61
Marla	EXE	5	105	37.56	38.39	37.00
Marla	EXE	5	165	37.44	37.56	36.78
Sun	EXE	1	0	38.56	38.28	38.11
Sun	EXE	1	10	40.06	38.83	
Sun	EXE	1	20	40.56	39.33	
Sun	EXE	1	30	41.44	40.06	
Sun	EXE	1	45	41.28	40.61	38.89
Sun	EXE	1	60	40.11	40.06	39.72
Sun	EXE	1	75	39.56	39.61	40.00
Sun	EXE	1	105	39.06	38.78	38.39
Sun	EXE	1	165	38.56	38.44	38.22
Sun	EXE	2	0	38.56	38.50	38.39
Sun	EXE	2	10	40.17	38.78	
Sun	EXE	2	20	40.17	39.11	
Sun	EXE	2	30	40.22	39.22	
Sun	EXE	2	45	41.06	39.61	39.72
Sun	EXE	2	60	40.17	39.78	39.28
Sun	EXE	2	75	39.56	39.44	39.00
Sun	EXE	2	105	39.06	39.00	38.78
Sun	EXE	2	165	38.94	38.83	38.61
Sun	EXE	3	0	38.78	38.67	
Sun	EXE	3	10	40.06	38.94	
Sun	EXE	3	20	40.06	39.11	
Sun	EXE	3	30	40.78	39.61	
Sun	EXE	3	45	41.17	39.78	
Sun	EXE	3	60	39.06	39.94	
Sun	EXE	3	75	39.44	39.39	
Sun	EXE	3	105	39.28	38.94	
Sun	EXE	3	165	39.06	38.78	
Sun	EXE	4	0	37.56	37.72	38.78
Sun	EXE	4	10	39.56	38.22	
Sun	EXE	4	20	39.94	38.78	
Sun	EXE	4	30	40.44	38.56	
Sun	EXE	4	45	40.78	38.94	39.00
Sun	EXE	4	60	39.28	39.11	39.11
Sun	EXE	4	75	39.06	38.89	39.50
Sun	EXE	4	105	38.17	38.44	38.78
Sun	EXE	4	165	38.06	38.33	38.61
Sun	EXE	5	0	37.56	37.50	33.50
Sun	EXE	5	10	38.94	37.72	
Sun	EXE	5	20	39.94	38.17	
Sun	EXE	5	30	39.94	38.72	
Sun	EXE	5	45	40.44	39.06	39.00
APPENDIX 15. CONTINUED

HORSE	ТХТ	DAY	TIME	M/C(°C)	RECTAL(°C)	I/B(°C)
Sun	EXE	5	60	39.56	39.11	39.28
Sun	EXE	5	75	38.28	38.67	38.22
Sun	EXE	5	105	38.06	38.22	37.78
Sun	EXE	5	165	37.56	37.94	37.89
Sun	EXE	6	0	37.56	37.44	37.39
Sun	EXE	6	10	38.56	37.72	
Sun	EXE	6	20	39.17	38.33	
Sun	EXE	6	30	39.78	38.56	
Sun	EXE	6	45	40.06	38.89	38.89
Sun	EXE	6	60	39.44	38.78	38.22
Sun	EXE	6	75	38.56	38.17	37.78
Sun	EXE	6	105	37.67	38.00	37.50
Sun	EXE	6	165	38.06	38.00	37.61
Miss Lectric	EXE	1	0	38.44	38.28	
Miss Lectric	EXE	1	10	38.94	39.00	
Miss Lectric	EXE	1	20	39.44	39.39	
Miss Lectric	EXE	1	30	39.78	39.61	
Miss Lectric	EXE	1	45	40.06	39.22	
Miss Lectric	EXE	1	60	40.06	39.39	
Miss Lectric	EXE	1	75	39.50	39.17	
Miss Lectric	EXE	1	105	38.94	38.33	
Miss Lectric	EXE	1	165	38.28	38.33	
Miss Lectric	EXE	2	0	37.17	37.72	
Miss Lectric	EXE	2	10	37.28	37.44	
Miss Lectric	EXE	2	20	37.67	38.67	
Miss Lectric	EXE	2	30	38.44	39.00	
Miss Lectric	EXE	2	45	39.56	39.50	
Miss Lectric	EXE	2	60	39.78	39.44	
Miss Lectric	EXE	2	75	39.67	39.17	
Miss Lectric	EXE	2	105	38.78	38.56	
Miss Lectric	EXE	2	165	37.56	37.83	
Miss Lectric	EXE	3	0	37.28	37.17	
Miss Lectric	EXE	3	10	37.28	38.17	
Miss Lectric	EXE	3	20	37.28	37.72	
Miss Lectric	EXE	3	30	38.67	37.94	
Miss Lectric	EXE	3	45	38.56	39.17	
Miss Lectric	EXE	3	60	39.17	38.78	
Miss Lectric	EXE	3	75	38.78	38.39	
Miss Lectric	EXE	3	105	38.06	37.94	
Miss Lectric	EXE	3	165	37.56	37.50	
Miss Lectric	EXE	4	0	37.67	37.33	
Miss Lectric	EXE	4	10		38.17	
Miss Lectric	EXE	4	20		38.67	
Miss Lectric	EXE	4	30	38.44	39.00	

APPENDIX 15. CONTINUED

HORSE	ТХТ	DAY	TIME	M/C(°C)	RECTAL(°C)	I/B(°C)
Miss Lectric	EXE	4	45	39.06	39.50	
Miss Lectric	EXE	4	60	39.56	39.28	
Miss Lectric	EXE	4	75	39.17	38.78	
Miss Lectric	EXE	4	105	38.17	37.39	
Miss Lectric	EXE	4	165	37.67	37.50	
Miss Lectric	EXE	5	0	37.56	37.44	
Miss Lectric	EXE	5	10		37.50	
Miss Lectric	EXE	5	20		38.39	
Miss Lectric	EXE	5	30	38.56	38.89	
Miss Lectric	EXE	5	45	39.06	39.33	
Miss Lectric	EXE	5	60	38.94	38.78	
Miss Lectric	EXE	5	75	38.56	38.28	
Miss Lectric	EXE	5	105	37.94	37.72	
Miss Lectric	EXE	5	165	37.28	37.50	

VITA

Lynn Frances Commaille was born in New York, New York and raised in Fairfield County, Connecticut. After graduating from Joel Barlow High School in 2001, she moved to the city of Burlington to pursue a degree at the University of Vermont. Lynn received her bachelor of science in animal science with a concentration in equine studies in May 2005.

Upon graduation, Lynn accepted an internship in the horse management program at The W. H. Miner Agricultural Research Institute in Chazy, New York. She spent a year and a half working with Morgan horses in the areas of training, riding, and reproductive management. In 2006, she was accepted into the master's program at Texas A&M University to study equine reproduction under the guidance of Dr. Martha Vogelsang. During this time, she also worked as a veterinary technician at Van Stavern Small Animal Hospital.

Lynn is currently seeking a position at a veterinary clinic which specializes in equine assisted reproductive techniques as she continues her education prior to attending veterinary school. Contact: Lynn Commaille, 52 Topledge Road, Redding, CT 06896. e-mail: lcommail@neo.tamu.edu.