SERUM LUTEINIZING HORMONE AND TESTOSTERONE IN BULLS EXPOSED TO ELEVATED AMBIENT TEMPERATURE

By

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Bachelor of Science

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Bowling Green, Kentucky

1977

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
May, 1980

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IN BULLS EXPOSED TO ELEVATED

AMBIENT TEMPERATURE

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ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Drs. E. J. Turman and R. P. Wettemann for their guidance and advisement during the course of the study and the preparation of this manuscript. Thanks are also extended to Dr. R. L. Hintz for his counsel. Acknowledgment is due to Dr. David Meyerhoeffer and others on the Fort Reno staff for their assistance with collection of data.

The author wishes to thank his colleagues, Tom Hoagland and Colin Muncy for their friendship and help with the collection of data. Additional thanks are extended to other graduate students, Carolynn Evans, Charles Hibberd, Dan Netemeyer, Steve Peterson, Roy Ball, Jocelyn Kapp and others for their friendship and fellowship. Special thanks are extended to John Drew and other laboratory personnel for making the lab an enjoyable place to work.

The author is deeply appreciative of his parents, Dr. and Mrs. John D. Minton, and brother, John. Their unending love and support throughout my life and the course of this study were always present.

Finally, this manuscript is dedicated to my grandfather, the late James Clarence Redick. His life touched and influenced my life and the lives of those who knew him.

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CHAPTER I

INTRODUCTION

Environment has an important role in the reproductive process of farm animals. Thermal stress is an environmental factor implicated in reducing reproductive performance in cattle. Since reproductive performance greatly influences the efficiency of beef production, an understanding of the mechanisms responsible for reduced performance of cattle exposed to increased ambient temperatures is essential for profitable production.

Extensive data is available which evaluates the detrimental effects of heat stress on semen quality and testicular morphology of bulls. However, information on the endocrine changes that are associated with altered pituitary and/or testis function as a result of increased ambient temperature is limited.

Testosterone is the major androgen in pubertal bulls and luteinizing hormone (LH) is the principal gonadotropin controlling testosterone synthesis and secretion. Therefore, changes in peripheral concentrations, secretion patterns or metabolism of these hormones could be used as an index of altered endocrine function in animals exposed to elevated ambient temperature.

The objective of this study was to evaluate the effect of exposing bulls to elevated ambient temperatures for 15 days on blood concentrations and release patterns of LH and testosterone. Since basal concen-

trations of hormones may not indicate secretory activity of the glands, the ability of the hypophyseal-testicular axis to respond to exogenous gonadotropin releasing hormone was determined. In addition, various parameters of semen quality as well as changes in respiratory rates and rectal temperatures were monitored as indices of thermal stress.

CHAPTER II

REVIEW OF LITERATURE

The Effect of Elevated Temperature on Fertility and Spermatogenesis

Fertility

The effects of increasing ambient temperature on subsequent fertility in farm animals is not well characterized. However, the effects of heat stress have been studied more extensively in swine and sheep than in cattle.

Fertility, as measured by fertilization rate and subsequent embryo survival, was reduced in ewes bred to rams which had been exposed to 32°C for 4 days at two to three weeks before mating (Howarth, 1969). Similarly, the percentage of pregnant gilts following insemination with semen from boars exposed to elevated ambient temperatures for 72 hours was reduced 20 to 40 percent when compared with controls (Christenson et al., 1972). This reduction in fertility as well as a reduction in embryo survival and litter size, was apparent by 15 days after boars had been exposed to increased ambient temperature. These data are in agreement with the decrease in conception rate and embryo survival in gilts artificially inseminated with semen from boars exposed to increased ambient temperatures for 6 weeks (Wettemann et al., 1976).

Fertility of dairy bulls is reduced during late July, August and

September (Johnston and Branton, 1953). The correlation coefficient between maximum temperature (weekly averages) and fertility (percent of 60-90 day non-returns to first services) was -.46, suggesting that decreased fertility was associated with increased environmental temperatures.

Although heat reduces male fertility, libido does not appear to be adversely affected in rams (Howarth, 1969), boars (Wettemann et al., 1976) or bulls (Zaremba, 1975).

Germ Cells and the Germinal Epithelium

Application of heat (43°C) directly to the scrotum of rats for 15 minutes selectively damages specific types of germinal epithelial cells (Chowdhury and Steinburger, 1964). Pachytene spermatocytes in stages V and VI were not affected, but primary spermatocytes from stage XI (leptotene) to dividing spermatocytes at stage XIV, as well as spermatids in step 1 and early step 2 of spermiogenesis were susceptible to heat. However, neither resting spermatocytes, spermatogonia or Sertoli cells were damaged by the heat treatment (Chowdhury and Steinburger, 1964). Similarly, Blackshaw et al. (1973) observed that heat treatment of rat testes caused rapid damage to pachytene primary spermatocytes.

The influence of local heating of ram testes is similar to the results for rats (Waites and Ortavant, 1968). When the testes of rams were locally heated at 40.0 to 40.5°C for 140-150 minutes, late stage 7 and early stage 8 pachytene spermatocytes were severely affected.

Exposure of boars to whole body heat for 90 days reduces total testicular sperm numbers, as well as sperm reserves in the epididymides, indicating a reduction in spermatogenesis (Wettemann et al., 1976). A

reduction in spermatids in testicular tissue of heat stressed boars coincided with reduced sperm numbers in testicular homogenates (Wettemann and Desjardins, 1979). Whole body heating (40°C) for as little as 12 hours resulted in vacuolation of spermatids in bulls (Skinner and Louw, 1966). Cross sections of seminiferous tubules of bulls exposed to ambient temperatures exceeding 29°C for five weeks revealed degeneration of the seminiferous epithelium and reduced spermatogenesis (Casady et al., 1953).

Thus it appears that whole body exposure of boars (Wettemann et al., 1976; Wettemann and Desjardins, 1979) and bulls (Casady et al., 1953; Skinner and Louw, 1966) to elevated ambient temperatures produces similar degenerative effects on the testes as does scrotal heating in the rat (Chowdhury and Steinburger, 1964).

Characteristics of Ejaculated Semen

Elevated ambient temperatures produce adverse effects on the quality of ejaculated semen in rams (Howarth, 1969), boars (Christenson et al., 1972; Wettemann et al., 1976) and bulls (Casady et al., 1953; Skinner and Louw, 1966; Rhynes and Ewing, 1973; Meyerhoeffer et al., 1976) and has been coincident with reduced fertility (Howarth, 1969; Christenson et al., 1972; Wettemann et al., 1976). Thus, it appears that reduced semen quality associated with heat stress plays a significant role in reducing reproductive performance.

Exposure to elevated ambient temperature fails to alter the volume of ejacula in bulls (Casady et al., 1953; Duarte Irala, 1973; Zaremba, 1975; Meyerhoeffer et al., 1976), boars (McNitt and First, 1970; Wettemann et al., 1976) or rams (Howarth, 1969). Skinner and Louw (1966)

suggested that heat stress does not alter pH and fructose content in ejacula of bulls. In contrast, the pH of seminal plasma was altered in bulls (Duarte Irala, 1973; Zaremba, 1975) and fructose content increased in the semen of rams (Moule and Waites, 1963) and boars (McNitt and First, 1970) exposed to whole body heat.

Scrotal insulation of Hereford bulls produced a decrease in the percentage of live sperm which was paralleled by an increase in the numbers of morphologically abnormal sperm (primarily tailless heads) (Austin et al., 1961). The alterations in sperm cell quality became apparent by the second and third weeks following scrotal insulation. Johnston et al. (1963) observed a similar increase in morphological abnormalities of sperm cells when they compared Bos indicus crossbred with Bos taurus bulls following heat stress. The Bos indicus crossbred bulls were affected less by heat stress than were the Bos taurus bulls. Skinner and Louw (1966) reported a similar advantage for heat tolerance in Bos indicus bulls.

Exposure of bulls to whole body heat produced an increase in the percentage of sperm cells with aged acrosomes by the third week of treatment (Meyerhoeffer et al., 1976). Similarly, Wettemann et al. (1976) observed an increase in normal and abnormal cells with aged acrosomes from heat stressed boars by the second week of treatment.

Exposure of animals to increased ambient temperatures causes reduced motility of sperm cells in bulls (Casady et al., 1953; Austin et al., 1961; Johnston et al., 1963; Skinner and Louw, 1966; Rhynes and Ewing, 1973; Meyerhoeffer et al., 1976), boars (McNitt and First, 1970; Christenson et al., 1972; Wettemann et al., 1976) and rams (Moule and Waites, 1963; Howarth, 1969). In all three species, the effects of

elevated temperature on semen quality do not become apparent until approximately two to three weeks following the onset of treatment. Reduced sperm cell concentrations and reduced percentages of motile cells, as well as increased percentages of abnormal cells were not apparent in the ejacula of heat stressed rams until the third week following treatment (Howarth, 1969). A similar delay from the onset of treatment until the decrease in sperm motility was observed in heat treated bulls (Meyerhoeffer et al., 1976). The delay from the onset of heat stress until the reduction in semen quality suggests that epididymidal spermatozoa may be more resistant to heat than testicular spermatozoa (Howarth, 1969; Zaremba, 1975). Ross and Entwistle (1979) observed that scrotal insulation did not affect passage of sperm in the epididymides of bulls. They observed an epididymal transport time of 13.5 days, which is within the range (6-14 days) previously reported for normal bulls (Orgebin, 1961).

Fifty five to sixty five days were required before semen quality returned to normal after termination of heat stress of boars (Christenson et al., 1972). A shorter recovery period was observed in rams (Moule and Waites, 1963). Differences in the length of the interval from the onset of heat stress until semen quality is reduced and the time required for return to normal semen quality after heat stress may not only reflect differences between species, but may also reflect differences in the application or duration of the treatment.

Scrotal insulation caused a reduction in sperm cell concentration in ejacula of bulls by the fourth week following treatment (Austin et al., 1961). Whole body exposure of bulls (Rhynes and Ewing, 1973) and rams (Howarth, 1969) to heat stress also causes a decrease in the con-

centration of sperm cells in ejacula. Similarly, decreased sperm output and reduced sperm cell reserves occur in heat stressed boars and confirm the observation that heat treatment reduces spermatogenesis (Wettemann et al., 1976).

Thus, it appears that exposure of the testes to increased temperatures adversely affects reproductive performance of bulls as well as boars and rams. Perhaps the most dramatic effects of heat stress are the reduction in spermatogenesis as evidenced by reduced sperm numbers and alterations in the histological appearance of the testis and the reduction in semen quality characterized by a decreased percentage of motile sperm and an increase in the percentage of morphologically abnormal sperm.

Endocrine Function in Bulls

General

A functional hypothalmo-hypophyseal-gonadal axis is characterized by pulsitile releases of luteinizing hormone (LH) from the pituitary, followed by similar releases of testosterone from the testes of rams (Purvis et al., 1974; Sanford et al., 1974; Schanbacher and Ford, 1976; Schanbacher and Ford, 1977), boars (Brock and Wettemann, 1976; Welsh and Johnson, 1978; Welsh and Johnson, 1979) and bulls (Katongole et al., 1971; Smith et al., 1973; Mongkonpuna et al., 1974; Haynes et al., 1976; Kiser et al., 1976; Welsh et al., 1978). The temporal relationship of LH and testosterone in males with apparently random release of hormones throughout a 24-hour period, contrasts the more regular cyclic patterns of gonadotropin release and ovarian response that are well documented in nonpregnant females.

In the bull, episodic surges of plasma LH followed by elevations in plasma testosterone apparently occur at irregular intervals throughout a 24-hour period. Further, this pattern of release does not appear to be associated with external stimuli such as feeding, lighting or sleep (Katongole et al., 1971). However, sexual stimulation has been reported to cause increased serum LH within 30 minutes (Katongole et al., 1971). Katongole et al. (1971) observed that if plasma testosterone concentrations were low at the time of the LH release, the increase in LH was followed by an increase in plasma testosterone. In contrast, other investigators observed that, on the average, sexual preparation or ejaculation did not significantly alter LH (Convey et al., 1971; Bindon et al., 1976; Short et al., 1979) or testosterone (Smith et al., 1973) in bulls.

Frequent determination of plasma hormone concentrations reveals that episodic releases of LH and testosterone in bulls appear to occur 3 to 4 times in a 24-hour period (i.e. every 6 to 8 hours). The average number of increases in LH range from about 3 LH releases in 24 hours (Welsh et al., 1978) to 5 to 10 LH surges in 24 hours (Katongole et al., 1971). Schanbacher (1978) failed to observe the episodic release of LH in Hereford bulls and suggested that either pulsitile releases do not occur in some bulls, or the frequency of blood sampling was not adequate to detect them.

Katongole et al. (1971) observed that each increase in plasma LH was followed by an increase in plasma testosterone within 1.5 hours. Peripheral plasma concentrations of LH ranged from 5 to 50 ng/ml and testosterone concentrations ranged from 2 to 20 ng/ml. In contrast to the limited report by Katongole et al. (1971), Smith et al. (1973) found that only 64 percent of LH secretory spikes were followed by an increase

in testosterone of 2 ng/ml or more within one hour in the sera of five dairy bulls. Similarly, Welsh et al. (1978) observed that only 60 percent of all episodic elevations of LH were accompanied by or followed within one hour by an increase in testosterone.

Some inconsistancies exist in values reported for basal concentrations and magnitudes of episodic releases of LH and testosterone. Basal values of 5 ng/ml and 2 to 4 ng/ml and episodic elevations of 20 to 30 ng/ml and 16 to 20 ng/ml for LH and testosterone, respectively, were observed by Katongole et al. (1971). In contrast, lower basal concentrations (2.0 ng/ml and 1.50 ng/ml for LH and testosterone, respectively) were found in 11 Angus bulls (Welsh et al., 1978). Similarly, lower concentrations of LH and testosterone during episodic releases (1.6 ng/ml and 7.0 ng/ml, respectively) were observed by Haynes et al. (1976) and Welsh et al. (1978) (9.0 ng/ml and 11.5 ng/ml, respectively) as compared to those observed by Katongole et al. (1971). Some of the variation observed for basal and maximal concentrations can probably be explained by differences in hormone standards used or different definitions of the parameters measured.

Response to Gonadotropin Releasing Hormone (GnRH)

The response of the bovine pituitary to exogenous GnRH is well documented. Mongkonpunya et al. (1975) observed that LH increased 24-fold within 45 minutes after an injection of bulls with GnRH. Further, testosterone was elevated threefold in the sera of pubertal bulls in response to the GnRH stimulated LH release. Schanbacher and Echternkamp (1978) observed a similar LH and testosterone response in the sera of Hereford bulls after injection of 500 µg of GnRH. Thibier (1976)

found a 25- to 30-fold increase in LH and a 3- to 4-fold increase in testosterone after treatment of young post-pubertal bulls with 250 μg of GnRH.

The temporal relationship between LH and testosterone concentrations in bulls (Katongole et al., 1971; Smith et al., 1973; Mongkonpunya et al., 1974; Haynes et al., 1976; Kiser et al., 1976; Welsh et al., 1978) and the responses to exogenous LH (Smith et al., 1973) and GnRH (Mongkonpunya et al., 1975; Thibier, 1976; Schanbacher and Echternkamp, 1978) strongly suggests that LH is the major gonadotropin regulating the secretion of testosterone by the bovine testis.

Androgen Synthesis In Vitro

A variety of experimental techniques have been used to determine whether the adverse effects of increased ambient temperature on spermatogenesis are accompanied by alterations in endocrine function. Eik-Nes (1966) utilized naturally occurring cryptorchid dogs while several other investigators utilized surgically induced cryptorchid animals (Llaurado and Dominguez, 1963; Inano and Tomaoki, 1968; Schanbacher and Ford, 1977; Schanbacher, 1978; Schanbacher, 1979) to examine the effect of increasing the temperature of the testis to abdominal temperature on androgen synthesis and secretion. These methods have established the endocrine function of the cryptorchid testis and provide valuable insight into the regulation of gonadotropin secretion and testicular endocrine function (Schanbacher, 1978; Schanbacher, 1979). However, they have only indirectly assessed the effects that elevated environmental temperature may have on testicular endocrine function.

Incorporation of acetate-1-^{14}C by slices of rabbit testicular

tissue into testosterone in vitro was greater at 38°C (a temperature resembling that of the scrotum) than at 40°C (abdominal temperature) (Hall, 1965). Further, this effect of temperature was the same when testicular slices were incubated with or without LH. Studies with cryptorchid rats suggest that androgen biosynthesis may be reduced as a result of altered testicular enzyme activity. The conversion of progesterone to 17α-hydroxyprogesterone, 4-androstenedione and testosterone was greater in the scrotal testis when compared with the cryptorchid testis in unilaterally cryptorchid rats (Llaurado and Dominguez, 1963). Similarly, androgen production from pregnenolone was reduced in testicular homogenates from cryptorchid rats as compared to homogenates of scrotal testes (Inano and Tamaoki, 1968). Using in vitro techniques, Chap et al. (1977) found that testosterone is primarily synthesized via the 4-ene-pathway (progesterone→hydroxyprogesterone→androstenedione→testosterone) and that the last step (androstenedione→testosterone) occurs at a reduced rate in mice subjected to whole body heat (33-35°C, 25-40 percent RH for five weeks) as compared with controls (20-22°C, 30-50 percent RH). The activity of 4-ene-17β-hydroxysteroid dehydrogenase, the enzyme catalyzing the conversion of androstenedione to testosterone, was decreased sharply as a result of heat acclimation in mice.

Effects of Local or Whole Body Heat Stress on Peripheral LH and Testosterone Concentrations

Rams made cryptorchid at 6 weeks of age had increased serum concentrations of LH and FSH by 9 months of age (Schanbacher and Ford, 1977). However, serum testosterone concentrations were not altered by locating the testes in the abdominal cavity and the temporal relation-

ship between LH and testosterone was similar to that observed in intact rams (Schanbacher and Ford, 1977). Secretory spikes of LH in cryptorchid bulls occurred more frequently and were greater in magnitude than those in intact bulls (Schanbacher, 1979). Increases in LH could not be associated with increased testosterone in cryptorchid bulls (Schanbacher, 1979).

The application of whole body heat stress more closely resembles natural environmental heat stress that an animal may be exposed to as a result of increased solar heating than does artificially or naturally occurring cryptorchidism. However, the effect of whole body heat stress on testicular endocrine function in bulls is not well documented.

When bulls were exposed to 35.5°C and 50 percent RH for seven weeks, plasma testosterone decreased to 43 percent of the pretreatment concentration during the first two weeks of treatment, but returned to near pretreatment concentration in subsequent weeks (Rhynes and Ewing, 1973). These authors employed an infrequent sampling schedule which, in view of the episodic pattern of testosterone release reported for bulls (Katongole et al., 1971; Smith et al., 1973; Mongkonpunya et al., 1974; Haynes et al., 1976; Kiser et al., 1976; Welsh et al., 1978; Schanbacher, 1979), may have been inadequate to detect treatment differences. Although peripheral plasma concentrations of testosterone may have been altered, the disappearance of testosterone-1-2-^{3}H indicated that the testosterone half-life, metabolic clearance rate and production rate were not altered in bulls after seven weeks of exposure to 35.5°C (Rhynes and Ewing, 1973).

Elevated ambient temperature alters testicular endocrine function of boars (Wettemann and Desjardins, 1979). With frequent blood sampling, serum testosterone was reduced after 7 days, but not after 14 days of

heat stress (Wettemann and Desjardins, 1979). In vitro incubation of 1-^{14}C acetate with testicular slices from heat stressed boars indicated a reduced ability to form androstenedione, testosterone and dihydrotestosterone. In addition, the pattern of androgen biosynthesis was similar when testicular tissue from stressed boars was incubated in the presence of LH and FSH (Wettemann and Desjardins, 1979).

It appears that whole body exposure to heat stress alters testicular androgen biosynthesis in some species. However, the androgen content of the peripheral circulation may not reflect alterations in testicular synthesis. Alterations in blood dilution or metabolism of androgens may occur following heat stress which could hinder the detection of altered testicular synthesis in peripheral blood.

CHAPTER III

MATERIALS AND METHODS

General

Eight Angus bulls, maintained at the Southwestern Livestock and Forage Research Center, El Reno, Oklahoma, were involved in this study. Treatment and sampling of the bulls occurred during January, 1978. The bulls were housed in environmental chambers for approximately three weeks prior to and during the experimental period.

Design

The bulls used in this experiment ranged from 21.5 to 23 months of age and weighed 445 ± 25 kg at the beginning of treatment. For three weeks prior to the onset of heat treatment, bulls were maintained at 22 ± 1°C in one of two environmentally controlled chambers. Each 3m x 12m chamber had four stanchions with rubber mats on the floor. During the pretreatment period, bulls were haltered and exercised daily to accustom them to handling.

The bulls were fed a nutritionally complete, 65 percent concentrate diet and water was supplied ad libitum. Feed intake was controlled so that the bulls gained about .58 kg/day. Respiratory rates and rectal temperatures were recorded daily following the morning feeding (0900 hours).

Prior to the onset of treatment, bulls were maintained with

flourescent and incandescent lighting (600 lux) from 0700 hours to 1900 hours and only incandescent lighting (40 lux) from 1900 hours to 0700 hours. After the onset of treatment, flourescent and incandescent lighting (600 lux) was maintained in the chambers from 0700 hours to 1900 hours and lights were turned off from 1900 hours to 0700 hours. An exception to this procedure was on days when blood samples were collected, when the lights were on from approximately 0600 hours to 2400 hours.

At the start of treatment, the temperature was increased to 34 ± 1°C in one chamber, while the other chamber remained at 22 ± 1°C. Relative humidity was not controlled, but ranged from 38 to 68 percent in the control chamber and 26 to 34 percent in the hot chamber.

Jugular vein cannulae were inserted approximately 15 hours before each blood sampling period. Bulls were removed from the chambers and restrained in a headgate and squeeze chute for cannulation. The hair was clipped from the lower neck and the area was thoroughly washed with a 5 percent solution of Roccal-D. A 12-gauge thin wall needle, 5 cm in length, was attached to a 50 ml syringe and inserted into the jugular vein (either right or left side). Upon penetration, the syringe was removed from the needle and 120 cm of polyvinyl tubing (Medical Vinyl Tubing; Bolab, v/10) was inserted through the needle until about 40 cm were in the vein. A 2 cm collar of silastic tubing (1.98 mm, ID; 3.17 mm, OD) was placed around the cannula at the point of insertion and secured on the cannula by spray adhesive (Hollister Silicone Medical Adhesive; Dow Corning Type-B). The collar was then sutured to the skin. A leather pouch about 9 x 9 cm was sutured to the skin at the top of the neck and the exposed cannula was placed in it between collection of samples. Adhesive tape was placed around the neck to secure the cannula

from the point where it was exteriorized to the leather pouch. The tubing was flushed with a 3 percent sterile sodium citrate solution. A blunted 17-guage needle and plug were inserted into the free end of the cannula to permit closure of the cannula and to facilitate blood sampling. Following cannulation, the bulls were returned to the environmental chambers. Blood was withdrawn via the cannula using 10 ml syringes. Initially a 2.5 ml sample was taken and discarded. Then a 10 ml sample was withdrawn and retained. After sampling, the cannula was flushed with approximately 2 ml of sterile 3 percent sodium citrate.

On days -2, 6 and 15 of treatment, blood samples were collected at 30 minute intervals from 0600 hours to 1800 hours. Following collection of the 1800 hour sample, 200 μg of synthetic gonadotropin releasing hormone (GnRH; Abbott Laboratories, North Chicago, Illinois) were infused via the cannula, then the cannula was flushed with 25 ml of .9 percent sterile saline. After the infusion of GnRH, blood samples were collected at 15 minute intervals until 1900 hours and then at 30 minute intervals until 2400 hours.

Blood samples were transferred into glass tubes and allowed to clot at $20^{o}C$ for 30 minutes, then stored at $4^{o}C$ for approximately 15 hours. Samples were then centrifuged at 1000 x g for 30 minutes, the serum decanted and stored in plastic vials at $-10^{o}C$ until assayed for luteinizing hormone and testosterone.

Semen Analyses

Bulls were electroejaculated prior to and following heat treatment. At the time of ejaculation, sperm concentrations were determined spectrophotometerically (Willett and Buckner, 1951; Bratton et al., 1956)

and ejacula volumes were measured. Estimates were also made of the percentage motile sperm, rate of motility and percentage abnormal sperm in the ejacula.

Duplicate slides were prepared and stained for evaluation of percentage dead sperm (Hancock, 1952) and acrosomal morphology (Wells et al., 1971). Briefly, 100-cell-counts were performed on each slide. Cells were determined to be dead if complete or partial uptake of dye occurred. Sperm cell types and acrosome characteristics were classified as normal non-aged, normal aged, abnormal non-aged or abnormal aged.

Luteinizing Hormone

Serum luteinizing hormone (LH) concentrations were determined by radioimmunoassay utilizing rabbit anti-sera to bovine LH (B225, generously supplied by Dr. G. D. Niswender, Department of Physiology and Biophysics, Colorado State University, Fort Collins). The procedure used for the assay and radioiodination was similar to that described by Niswender et al. (1969) except that ^{125}I instead of ^{131}I was used. Validation of this assay in our laboratory has previously been reported (Hallford et al., 1979).

A modification to the procedure described by Hallford et al. (1979), purification of ^{125}I-LH on the day of use, increased the binding of ^{125}I-LH to the anti-sera. The general procedure used has been previously described (Rayford et al., 1974). Bovine LH-^{125}I (approximately .3 µg and 20 µc in .5 to 1.0 ml of PBS-Gel; Appendix Table VII) was layered onto an anion exchange column (AG 1 x 10 chloride form, Bio-Rad Laboratories, Richmond, California) then eluted with PBS. Eight, .5 ml fractions were collected. The largest portion of the radioactivity was

eluted in fractions 2, 3 and 4. These fractions were then diluted in phosphate buffered saline plus .1 percent Knox gelatin (PGS-Gel, Appendix Table VII) and used as the radio-ligand in the assay system.

Bovine serum was used as the standard in the assay. The concentration of LH in two large pools of steer serum with low and high LH concentrations was determined in 3 assays at 7 and 12 dilutions, respectively, from a standard curve utilizing bovine LH (NIH-LH-B9) at concentrations ranging from .1 to 6.4 ng. The concentrations of LH in the two pools of steer serum were 7.5 ± .2 ng/ml (mean ± SE, n = 58) and 16.3 ± .5 ng/ml (mean ± SE, n = 38). Standard curves for steer sera and NIH-LH-B9 were parallel within a range of .1 ng to 3.3 ng.

Luteinizing hormone in two samples of bovine sera was quantified in duplicate in each assay. The samples contained 1.4 ± .1 ng/ml (mean ± SE, n = 20) and 6.2 ± .2 ng/ml (mean ± SE, n = 20) of LH. The between-assay coefficient of variation was 16 percent and the within-assay coefficient of variation was 14 percent.

Testosterone

Serum testosterone concentrations were quantified by a radioimmunoassay similar to that described by Smith and Hafs (1973) utilizing rabbit anti-sera to testosterone-3-oxime bovine serum albumin (#666, generously supplied by Dr. G. D. Niswender, Department of Physiology and Biophysics, Colorado State University, Fort Collins). Validation of this assay for porcine serum in our laboratory has been reported (Wettemann and Desjardins, 1979). To validate the assay for bovine serum, samples were assayed before and after testosterone was isolated by chromatography on LH-20 columns (Mongkonpunya et al., 1975). Testosterone in samples before chromatography (5.9 ± .6 ng/ml, mean ± SE, n = 11) did not differ

from the testosterone concentration determined after chromatographic isolation (5.6 ± .6 ng/ml, mean ± SE, n = 11, r = .95). When steer serum and steer serum plus 5 or 10 ng/ml of testosterone were included in each assay, values of .4 ± .1 ng/ml, 5.6 ± .2 ng/ml and 12.0 ± .5 ng/ml (mean ± SE, n = 10 observations at each concentration), respectively, were obtained. The between assay coefficient of variation was 11 percent and the within assay coefficient of variation was 4 percent.

Statistical Analyses

Data obtained on semen quality parameters, respiratory rates, rectal temperatures and bull weights can be described by:

$$Y_{ij} = \mu + T_i + e_{ij}\ (\text{Bull/Trt}_{ij})$$

where Y_{ij} is a semen quality parameter, respiratory rate, rectal temperature or body weight, T is treatment and e is bull nested within treatment. The components μ and T_i were treated as fixed effects of all records of treatment i. Random error effect e_{ij} was specific to each observation. Estimated and significant differences between treatments were obtained by the method of least squares.

Luteinizing hormone and testosterone secretory spikes were defined as increases in serum concentrations greater than one standard deviation above the overall mean for that bull at that bleeding period. A secretory spike began when the serum hormone concentration first exceeded one standard deviation greater than the mean and ended when the concentration became less than one standard deviation greater than the mean. Peak height was defined simply as the highest concentration of hormone

within a secretory spike. Mean concentrations, peak heights, areas under 12-hour profiles and the number of LH and testosterone peaks were analyzed by analysis of variance using a split-plot design (Gill and Hafs, 1971). Data obtained from hormone parameters can be described by:

$$Y_{ijk} = \mu + T_i + P_j + B/T_{ki} + TP_{ij} + e_{ijk}$$

where Y_{ijk} is either mean hormone concentration, area under 12-hour profiles, number of peaks/12 hours or average peak height and where T is treatment, P is period, B/T is bull within treatment and TP is the treatment by period interaction. The components μ, T_i, P_j, B/T_{ki} and TP_{ij} were treated as fixed effects of all records of treatment i, period j and bull k within treatment i. Random error effect e_{ijk} was specific to each observation.

Polynomial equations were fit to the LH and testosterone data obtained following GnRH to illustrate the response. Time after GnRH was used as the independent variable and LH or testosterone concentration as the dependent variable. Regression coefficients for time through the fourth (LH) or second (testosterone) order were calculated. The highest order equation with statistical significance ($P < .05$) was used.

LH data obtained from these equations can be explained by:

$$Y_{ij} = \mu + B_i + T_j + T_j^2 + T_j^3 + T_j^4 + e_{ij}$$

and that for testosterone by:

$$Y_{ij} = \mu + B_i + T_j + T_j^2 + e_{ij}$$

where Y_{ij} was the LH or testosterone response to GnRH and where B is bull and T is time. The components μ, B_i and T_j were treated as fixed

effects of all records of bull i and time j. Random error e_{ij} was specific to each observation.

Response curves between treatment and within period by hormone described by this model were determined to be different if either the intercepts or one or more of the beta values were significantly different ($P < .05$).

Partial correlation coefficients were calculated to determine the temporal relationship between LH and testosterone. Observed LH and testosterone concentrations were adjusted for bull, treatment and period effects and correlations were obtained between LH concentrations and testosterone concentrations in sera collected at 0, .5, 1.0, 1.5 and 2.0 hours later.

CHAPTER IV

RESULTS AND DISCUSSION

Rectal Temperatures and Respiratory Rates

Rectal temperatures and respiratory rates were analyzed on a daily basis throughout the experimental period (Figure 1). Rectal temperatures were similar between heat stressed and control bulls prior to exposure to elevated ambient temperature and remained similar through day 5 of treatment. Rectal temperatures were elevated ($P < .05$) in heat stressed bulls ($39.1 \pm .1^{o}C$) as compared to controls ($38.5 \pm .1^{o}C$) on day 6 of treatment. Similarly, stressed bulls had increased ($P < .05$) rectal temperatures on days 9 and 10 of treatment. Rectal temperatures were similar ($P > .10$) between the two treatment groups on days 7, 8, 11 and 13 of treatment, but tended ($P < .10$) to be increased in bulls exposed to elevated ambient temperature on days 12 and 15. Respiratory rates were similar for heat stressed and control bulls through day 5 of treatment, but were elevated in stressed bulls on day 6 (58.2 ± 2.9 vs 47.0 ± 2.9 breaths/min.). Respiratory rates were similar ($P > .10$) between the two treatment groups on day 7, but were elevated ($P < .05$) in stressed bulls on days 8, 9 and 10. Respiratory rates remained similar ($P > .10$) between heat stressed and control bulls on days 11-15 of treatment.

Large variations in respiratory rates were observed between bulls and within bulls on different days and may partially account for the

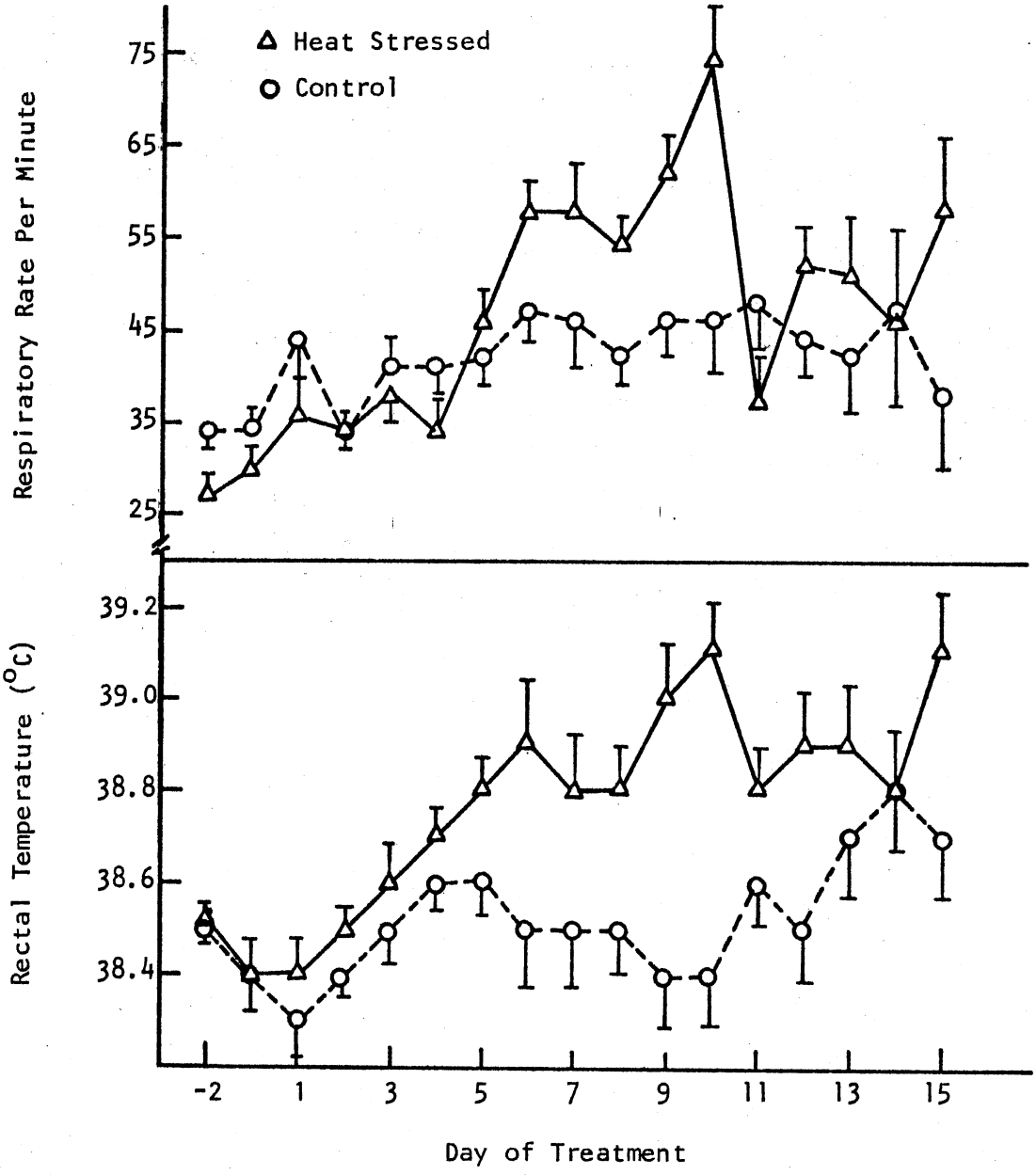

Figure 1. Respiratory Rates and Rectal Temperatures of Control and Heat Stressed Bulls.

absence of a treatment effect on each day of exposure to elevated temperature, particularly days 7 and 11-15. Part of this variation is likely due to inaccuracy of estimation of respiratory rate. In addition, the limited number of animals per treatment (n=4) probably was not adequate to detect a greater number of significant treatment effects even though the bulls in the elevated temperature chamber were obviously uncomfortable.

The increases in both rectal temperatures and respiratory rates observed in the present experiment are in agreement with the results of others who observed increased respiratory rates (Duarte Irala, 1973; Meyerhoeffer et al., 1976) and rectal temperatures (Duarte Irala, 1973) when bulls were exposed to elevated ambient temperatures. These results suggest that exposure of bulls to 34°C is sufficient to elevate body temperature and thus increased thermal stress.

Semen Quality

Estimates of semen quality in this experiment were conducted to characterize the influence of the temperatures used on testicular and epididymidal function. The intent was not to exhaustively measure the detrimental effects of exposure of bulls to elevated temperature on semen quality.

The characteristics of ejacula collected from bulls four days prior to and two days following treatment are summarized in Table I. Characteristics of the fresh ejacula as well as the determination of live/dead and acrosomal characteristics (Table II) from stained slides were similar for both treatment groups prior to exposure to elevated ambient temperature.

TABLE I

CHARACTERISTICS OF EJACULA FROM HEAT STRESSED AND CONTROL BULLS FOUR DAYS PRIOR TO (Day -4) AND TWO DAYS FOLLOWING (DAY +2) TREATMENT

Criteria	Day of Collection or Evaluation			
	-4		+2	
	Control	Heat Stressed	Control	Heat Stressed
Volume (ml)	5.9 ± .9[a]	5.0 ± .9	6.6 ± 2.1	7.0 ± 2.1
Rate of Movement[b]	3.4 ± .3	3.5 ± .3	4.4 ± .2[c]	3.0 ± .2[d]
Concentration ($X\ 10^6$)/ml	965 ± 206	798 ± 206	1000 ± 147	970 ± 147
Motile Sperm (%)	63.8 ± 4.0	68.8 ± 4.0	83.8 ± 4.2[c]	65.0 ± 4.2[d]
Abnormal Sperm (%)	35.5 ± 4.3	30.2 ± 4.3	26.5 ± 7.8	32.8 ± 7.8

[a]Mean ± SE of 4 bulls.

[b]Scale of 1-5.

[c,d]Means in the same row on the same day of treatment with different superscripts are significantly different ($P < .05$).

TABLE II

LIVE/DEAD AND ACROSOMAL CHARACTERISTICS OF SPERM FROM HEAT STRESSED AND CONTROL BULLS FOUR DAYS PRIOR TO (DAY -4) AND TWO DAYS FOLLOWING (DAY +2) TREATMENT

Criteria	Day of Treatment			
	-4		+2	
	Control	Heat Stressed	Control	Heat Stressed
Live Sperm (%)	66.8 ± 4.3[a]	71.0 ± 4.3	74.0 ± 3.7[b]	57.8 ± 3.7[c]
Morphologically Normal Sperm (%)	64.0 ± 9.7	69.0 ± 9.7	69.0 ± 7.3	60.8 ± 7.3
Sperm With Aged Acrosomes (%)	2.2 ± 1.0	2.5 ± 1.0	9.2 ± 2.9	10.8 ± 2.9

[a]Mean ± SE of 4 bulls.

[b,c]Means in the same row on the same day of treatment with different superscripts are significantly different ($P < .05$).

The percentage of motile sperm in ejacula from heat stressed bulls was reduced ($P < .05$) when compared with controls (65.0 ± 4.2 and 83.8 ± 4.2%, respectively) at two days following the end of heat treatment. Similarly, the rate of cell movement declined in the ejacula of stressed bulls ($P < .05$) after treatment. Semen volume, sperm concentration and the percentage of abnormal sperm were similar between the two treatment groups following exposure to elevated ambient temperature.

Evaluation of stained slides of ejacula revealed a decrease ($P < .05$) in the percentage of live sperm in ejacula from heat stressed bulls after treatment. In contrast, the percentage of morphologically abnormal sperm or sperm with aged acrosomes was similar for both groups of bulls following treatment.

The failure to observe a reduction in ejacula volumes after heat stress agrees with results observed by others (Casady et al., 1953; Duarte Irala, 1973; Zaremba, 1975; Meyerhoeffer et al., 1976). Scrotal insulation for 24 or 72 hours (Austin et al., 1961) and long term whole body heating (Rhynes and Ewing, 1973) reduced sperm cell concentration in ejacula of bulls. However, this effect was not observed until the fourth week of treatment (Austin et al., 1961). Similarly, two to three weeks were required for the appearance of increased numbers of morphologically abnormal sperm in ejacula after scrotal insulation of bulls (Austin et al., 1961). Thus, failure to observe alterations in sperm concentrations or percentages of abnormal cells in ejacula from the heat stressed bulls in this study is not surprising since semen was evaluated at 2 days after 15 days of exposure to elevated temperature.

The reductions in the percentage of motile sperm and rate of movement of cells in ejacula of stressed bulls were apparent 17 days after

the onset of exposure to elevated temperature. Similar reductions in the percentage of motile sperm at 2 to 3 weeks after heat stress have been observed (Casady et al., 1953; Austin et al., 1961; Johnston et al., 1963; Skinner and Louw, 1966; Rhynes and Ewing, 1973; Meyerhoeffer et al., 1976).

These results suggest that exposure of bulls to 34°C for 15 days adversely affects some criteria of semen quality in ejacula taken two days following treatment. The failure to observe reductions in all parameters of semen quality in this period of time supports the observations of Howarth (1969) and Zaremba (1975) that epididymal spermatozoa are apparently more resistant to heat. Thus, some time must be allowed for epididymal passage and the appearance of sperm with morphological abnormalities and reduced sperm cell concentration in ejacula following heat stress.

Luteinizing Hormone

Average serum luteinizing hormone (LH) concentrations on day -2 of treatment were similar for control (2.9 ± .1 ng/ml) and heat stressed bulls (2.8 ± .1 ng/ml) (Figure 2 and Table III). Similarly, the area under 12-hour LH profiles (Table III), the number of secretory spikes per 12 hours and the average height of secretory spikes (Table IV) were not different between treatment groups prior to exposure to elevated ambient temperature. LH concentrations observed in this experiment are in agreement with values previously reported for mature bulls (Katongole et al., 1971; Mongkonpunya et al., 1974; Haynes et al., 1976; Sitarz et al., 1977; Welsh et al., 1978; Schanbacher, 1979). On the average, 1.5 to 2.0 LH secretory spikes in 12 hours were observed in the present

experiment. Similarly, Welsh et al. (1978) reported 3 LH secretory spikes in 24 hours, but Katongole et al. (1971) observed a range of 5-10 LH secretory spikes in 24 hours. Maximum LH concentrations averaged 10 ng/ml prior to treatment which is similar to 9 ng/ml observed by Welsh et al. (1978). In contrast, other reports of maximum LH concentrations range from 1.6 ng/ml (Haynes et al., 1976) to 20 to 30 ng/ml (Katongole et al., 1971). Differences between the frequency and magnitude of LH secretory spikes in various experiments are likely due to differences in sampling frequency, identification of secretory spikes and/or assay standards used.

Following 6 days of elevated ambient temperature, average LH concentrations were reduced ($P < .05$) in stressed bulls compared to controls. A similar reduction ($P < .05$) was observed in the area under 12-hour LH profiles of stressed bulls (Table III). Although no difference was observed in the frequency of LH secretory spikes on day 6, the average height of these spikes was reduced ($P < .05$) in heat stressed bulls as compared with controls ($6.4 \pm .8$ and $9.0 \pm .8$ ng/ml, respectively).

By day 15 of treatment, average LH concentrations the area under 12-hour LH profiles and average height of LH secretory spikes tended to be reduced in stressed bulls as compared with controls (Tables III and IV) but the magnitude of the differences were not as great as observed on day 6. For all of the above LH criteria, decreases were observed in control bulls and increases in heat stressed bulls during the interval from day 6 to day 15 of treatment. This observation may account for the smaller differences between the LH parameters observed for the two treatments on day 15.

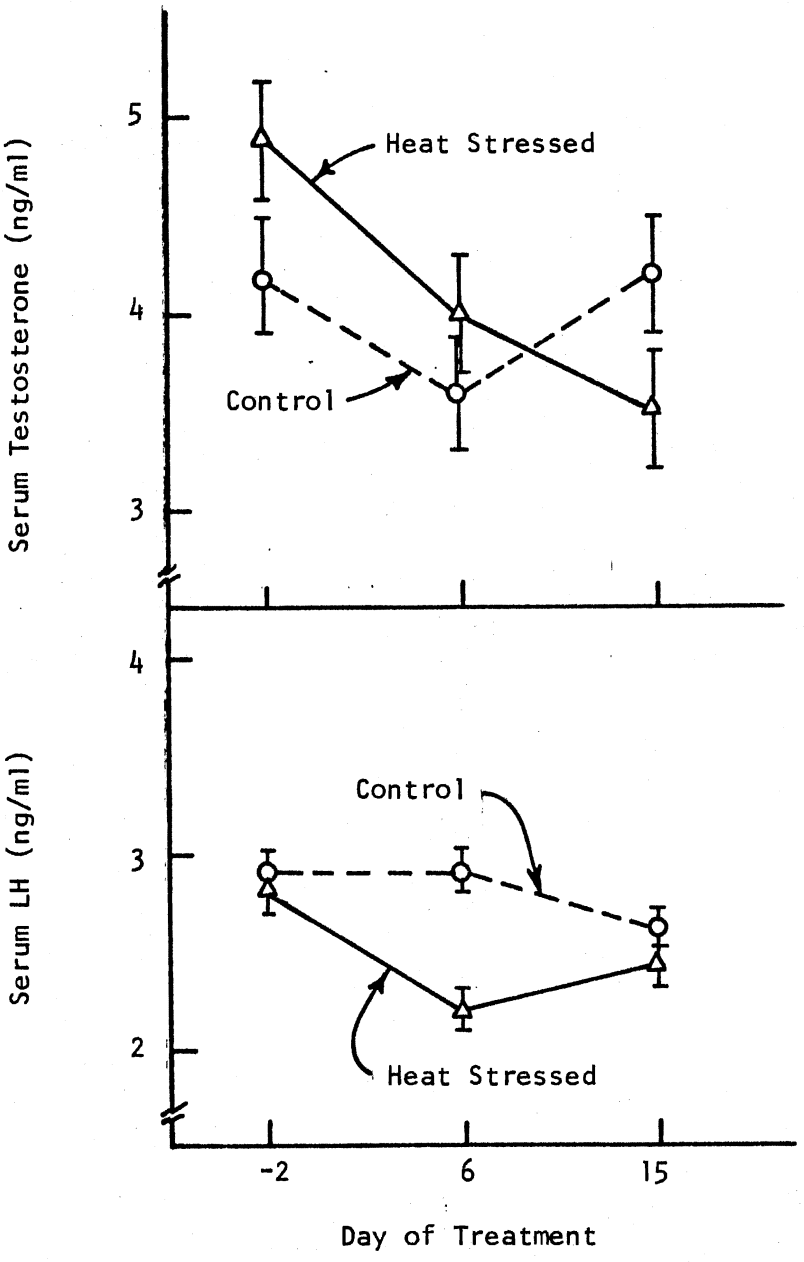

Figure 2. Average Serum LH and Testosterone in Control and Heat Stressed Bulls on Days -2, 6 and 15 of Treatment.

TABLE III

SERUM LH CONCENTRATION IN CONTROL AND HEAT STRESSED BULLS

Criteria	Day of Treatment		
	-2	6	15
Concentration (ng/ml)			
Control	2.9 ± .1[a]	2.9 ± .1[b]	2.6 ± .1[d]
Heat Stressed	2.8 ± .1	2.2 ± .1[c]	2.4 ± .1[e]
Area Under 12 hr. Curve (ng·hr/ml)			
Control	3.5 ± 1.5	33.8 ± 1.5[b]	31.8 ± 1.5[f]
Heat Stressed	3.4 ± 1.5	27.1 ± 1.5[c]	28.7 ± 1.5[g]

[a]Mean ± SE

[b,c]Criteria in the same column with different superscripts are significantly different ($P < .05$).

[d,e]Criteria in the same column with different superscripts are different ($P < .10$).

[f,g]Criteria in the same column with different superscripts are different ($P < .18$).

TABLE IV

SERUM LH SECRETORY SPIKES IN CONTROL AND HEAT STRESSED BULLS

Criteria	Day of Treatment		
	-2	6	15
Secretory Spikes[a] Per 12 hr. (No.)			
Control	1.5 ± .3[b]	3.0 ± .3	2.2 ± .3
Heat Stressed	2.0 ± .3	2.8 ± .3	2.5 ± .3
Ave. Height of Secretory Spikes (ng/ml)			
Control	9.7 ± .8	9.0 ± .8[c]	8.6 ± .8[e]
Heat Stressed	10.0 ± .8	6.4 ± .8[d]	6.8 ± .8[f]

[a]Increase in serum LH greater than one SD above the mean.

[b]Mean ± SE.

[c,d]Criteria in the same column with different superscripts are significantly different ($P < .05$).

[e,f]Criteria in the same column with different superscripts are different ($P < .13$).

These results suggest that exposure of bulls to 34°C for six days reduces peripheral LH concentrations. The slight reductions in serum LH concentrations, areas and secretory spike heights for control bulls on day 15 as compared to day 6 is not easily explained, but may have been an effect of confinement. The slight increase in serum LH concentrations in heat stressed bulls by day 15 suggests that stressed bulls may have acclimated to the hot environment.

In contrast to the observations in the present experiment, Wettemann and Desjardins (1979) reported that exposure of boars to 34.5°C failed to alter serum LH concentrations when boars were sampled three times daily. However, frequent sampling would probably be necessary to assess the influence of heat stress on plasma LH concentrations.

Elevated LH and FSH concentrations have been observed in artificially cryptorchid bulls (Schanbacher, 1978; Schanbacher, 1979) and rams (Schanbacher and Ford, 1977). Further, episodic surges of LH occur in cryptorchid bulls, but they are more frequent and of a higher magnitude than those observed in intact bulls (Schanbacher, 1979). Although the cryptorchid bull represents an animal whose testes are chronically exposed to increased temperature, a direct comparison to whole body heat treatment may not be valid.

Reduced serum LH in heat stressed bulls by day 6 of treatment may be mediated in several ways. The reduction in average LH concentration as well as reduced area under 12-hour LH profiles suggests that LH release and/or clearance was altered. The fact that the frequency of episodic surges of LH was similar in heat stressed and control bulls suggests that the stimulus for pulsitile release of endogenous GnRH and thus LH was not altered by heat stress. However, a reduction in the

magnitude of secretory spikes of LH suggests that alterations in the synthesis and/or secretion of LH may occur during heat stress. Depressed LH synthesis and pituitary LH stores may account for the observed reduction in LH on day six of treatment, but cannot be confirmed on the basis of the results of this study.

Serum LH concentrations after 200 μg of GnRH are illustrated in Figure 3. Each LH response curve was best fit by a fourth order polynomial equation. Appendix Tables VIII, IX and X list serum LH concentrations after GnRH on days -2, 6 and 15 of treatment, respectively.

The LH response to GnRH was similar in heat stressed and control bulls at all three sampling periods. In general, serum LH increased from 3.4 ng/ml at GnRH treatment to 28.9 ng/ml within 15 minutes after administration of GnRH. The maximum LH response of about 60 ng/ml was observed at 1.5 to 2 hours after infusion. This LH response represents an 18-fold increase in LH concentrations above preinjection values.

The LH response to GnRH challenge suggests that exposure of bulls to 34°C for 15 days did not affect the ability of the pituitary to respond to exogenous GnRH. The LH response observed in this experiment conforms to the observations of others for mature bulls at lower ambient temperatures (Zolman et al., 1973; Thibier, 1976; Schanbacher and Echternkamp, 1978). In addition, plots of mean LH responses to GnRH illustrate that a biphasic response occurs which is similar to that reported by Zolman et al. (1973) and Thibier (1976).

Whether heat stress reduced endogenous gonadotropin synthesis and pituitary stores of LH can not be answered based on these results. A single treatment with 200 μg of GnRH may have been adequate to elicit maximal LH response but probably was not adequate to deplete pituitary

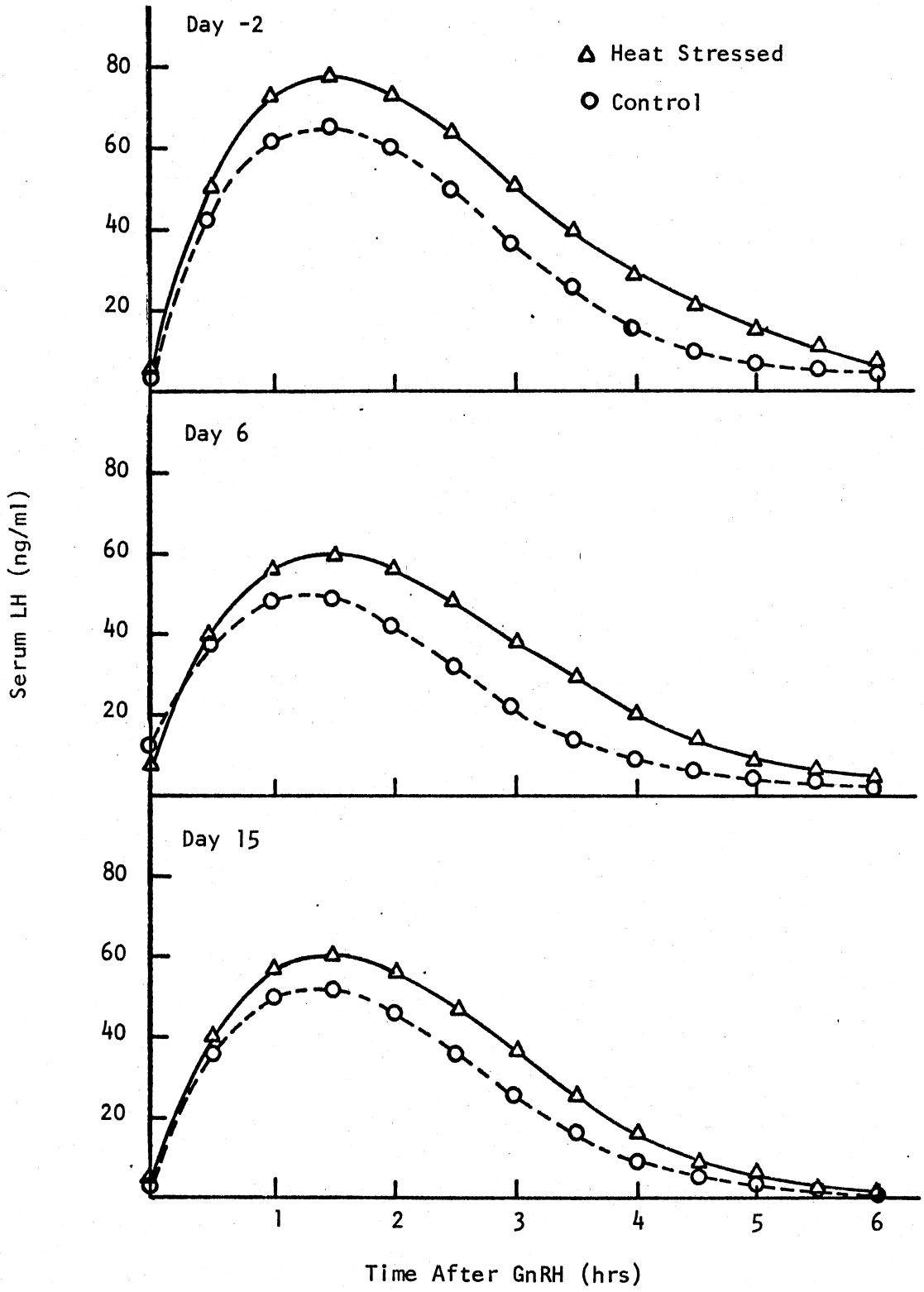

Figure 3. Serum LH Response to GnRH on Days -2, 6 and 15.

stores of LH (Mongkonpunya et al., 1975).

Testosterone

Serum testosterone concentrations in heat stressed bulls prior to treatment tended to be greater than that for control bulls ($P < .10$) (Table V). The concentrations observed in this experiment are in close agreement with those observed by Katongole et al. (1971) and Smith et al. (1973) but are slightly higher than those reported by Mongkonpunya et al. (1975) and Schanbacher and Echternkamp (1978). Reasons for the greater average testosterone concentrations and areas under 12-hour testosterone profiles observed in heat stressed bulls compared to controls prior to treatment are not readily apparent but may be due to variation between bulls in testosterone secretion and/or clearance.

Heat stressed and control bulls had similar serum testosterone concentrations and areas under 12-hour testosterone profiles after 6 days of treatment. In addition, the number of testosterone secretory spikes and the average height of secretory spikes were similar for both treatments after 6 and 15 days (Table VI).

In contrast, average serum testosterone concentrations tended to be reduced ($P < .13$) in stressed bulls ($3.5 \pm .3$ ng/ml) when compared with control bulls ($4.2 \pm .3$ ng/ml) on day 15 of treatment. Similarly, the areas under 12-hour testosterone profiles were slightly reduced ($P < .18$) in heat stressed bulls following 15 days of treatment.

The results of serum testosterone analysis in the present experiment indicate that this hormone may be reduced in the peripheral circulation of bulls by 15 days of exposure to 34°C. The testes of cryptorchid animals have reduced ability to synthesize testosterone *in vitro*

TABLE V

SERUM TESTOSTERONE CONCENTRATION IN CONTROL AND HEAT STRESSED BULLS

Criteria	Day of Treatment		
	-2	6	15
Concentration (ng/ml)			
Control	4.2 ± .3[a,b]	3.6 ± .3	4.2 ± .3[d]
Heat Stressed	4.9 ± .3[c]	4.0 ± .3	3.5 ± .3[e]
Area Under 12 hr. Curve (ng·hr/ml)			
Control	49.8 ± 3.3[b]	43.1 ± 3.3	49.5 ± 3.3[f]
Heat Stressed	58.9 ± 3.3[c]	47.6 ± 3.3	42.8 ± 3.3[g]

[a]Mean ± SE.

[b,c]Criteria in the same column with different superscripts are different ($P < .10$).

[d,e]Criteria in the same column with different superscripts are different ($P < .13$).

[f,g]Criteria in the same column with different superscripts are different ($P < .18$).

TABLE VI

SERUM TESTOSTERONE SECRETORY SPIKES IN CONTROL AND HEAT STRESSED BULLS

Criteria	Day of Treatment		
	-2	6	15
Secretory Spikes[a] Per 12 hr. (No.)			
Control	2.0 ± .4[b]	2.2 ± .4	2.0 ± .4
Heat Stressed	1.8 ± .4	2.0 ± .4	1.8 ± .4
Ave. Height of Secretory Spikes (ng/ml)			
Control	9.2 ± .8	8.6 ± .8	9.4 ± .8
Heat Stressed	10.2 ± .8	8.5 ± .8	9.0 ± .8

[a]Increases in serum testosterone greater than one SD above the mean.

[b]Mean ± SE.

(Llaurado and Dominguez, 1963; Inano and Tamaoki, 1968; Chap et al., 1977). Similarly, testicular slices from heat stressed boars synthesize less androstenedione, testosterone and dihydrotestosterone *in vitro* compared to tissue from control boars (Wettemann and Desjardins, 1979). Chap et al. (1977) suggested that the reduced activity of 4-ene-17β-hydroxysteroid dehydrogenase (the enzyme converting androstenedione to testosterone) may account for the reduced ability to form testosterone in the testes of heat acclimated mice.

In contrast to *in vitro* studies of androgen synthesis, peripheral testosterone concentrations in cryptorchid bulls (Schanbacher, 1979) and rams (Schanbacher and Ford, 1977) are similar to intact animals. These studies suggest that if testosterone synthesis is altered in the abdominal testis, the effect is deminished and not detectable in the peripheral circulation. In addition, altered metabolism or disposition of androgens may accompany dilution in the circulation.

Exposure of bulls to 35.5°C was observed to dramatically decrease plasma testosterone concentrations (Rhynes and Ewing, 1973), however, an infrequent sampling schedule was utilized in the experiment. With more frequent sampling, serum testosterone was not significantly altered in the present experiment. Reduced serum testosterone concentrations have been observed in heat stressed boars after 7 but not 14 days of treatment (Wettemann and Desjardins, 1979). This work tends to support the findings of the present experiment. However, in neither study was the reduction in testosterone concentrations very great when compared with controls ($P \cong .12$ in both studies). In addition, pulsitile patterns of testosterone release were not affected by heat treatment in this study nor in the study of Wettemann and Desjardins (1979).

Serum testosterone response to GnRH (Figure 4) was similar for heat stressed and control bulls on days -2, 6 or 15 of treatment. Each of the responses was best fit by a quadratic equation.

Serum testosterone concentrations increased from approximately 4 ng/ml prior to GnRH to about 8 ng/ml by 1.5 hours after GnRH. A sustained elevation in serum testosterone (> 8 ng/ml) was observed until about 5 hours after GnRH infusion, after which testosterone concentrations began to decrease. Gonadotropin releasing hormone (500 µg) was observed to increase plasma testosterone in Hereford bulls to 7 ng/ml by about 2 hours following treatment (Schanbacher and Echternkamp, 1978). Thibier (1976) also observed a similar maximum testosterone response to 250 µg of GnRH in yearling bulls.

The testosterone response to GnRH in the present experiment suggests that heat stressed bulls are capable of responding to endogenous LH stimulation with testosterone secretion. Thus bulls exposed to whole body heat stress for short periods appear to be unlike cryptorchid bulls in that their testes retain the ability to respond to GnRH-mediated LH stimulation (Schanbacher, 1979).

The slightly reduced peripheral concentrations of testosterone in heat stressed bulls at day 15 of treatment (Table V) were not reflected by GnRH challenge on that day. Although prolactin was not measured in this experiment, elevated ambient temperature increases prolactin concentration in heifers (Wettemann and Tucker, 1974). Furthermore, prolactin increases the sensitivity of the testes to LH in rats (Bartke and Dalterio, 1976). In addition, LH and prolactin administered in combination increase testosterone concentration in rats more than LH or prolactin administered singularly (Hafiez et al., 1972). Perhaps elevated

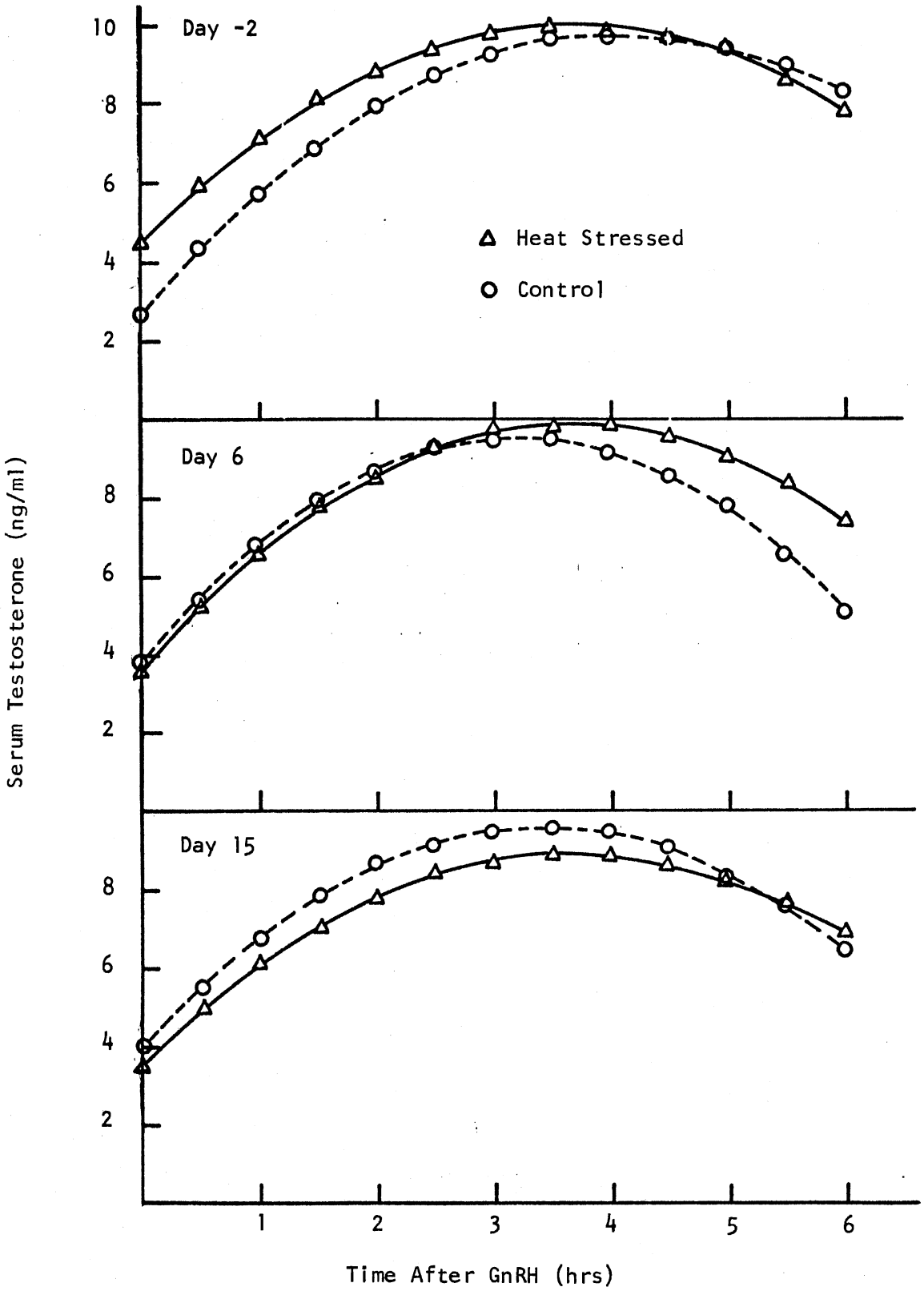

Figure 4. Serum Testosterone Response to GnRH on Days -2, 6 and 15.

prolactin enhanced the GnRH-mediated LH release of testosterone in heat treated bulls. Although this possibility cannot be ruled out, injection of LH and prolactin in bulls resulted in no greater testosterone concentrations than LH treatment alone (Smith et al., 1973).

Taken together, these results suggest that peripheral concentrations of testosterone in bulls may be reduced by elevated ambient temperature after 15 days of exposure. The lack of treatment effect on day 6 and the slight difference on day 15 suggest that altered testicular androgen biosynthesis may not be reflected to the same extent in the peripheral circulation as one might expect from the results of *in vitro* studies. In addition, an alteration in androgen metabolism and/or disposition may occur. Further, the responsiveness of the testis to LH was apparently not altered by elevated temperature as the frequency and magnitude of testosterone secretory spikes and the testosterone response to GnRH-mediated LH release were similar for both treatments.

Temporal Relationship Between LH and Testosterone

Pooled correlations of LH and testosterone in the same sample or samples taken .5, 1, 1.5 or 2 hours later were calculated to establish the temporal association between serum LH and testosterone concentrations. Luteinizing hormone was moderately, but significantly correlated with testosterone in samples taken one hour ($r = .64$; $P < .001$) and 1.5 hours later ($r = .60$; $P < .001$). In contrast, serum LH was not correlated with testosterone in the same sample and was lowly correlated ($P < .001$) with samples taken .5 hour ($r = .39$) and two hours later ($r = .35$). Welsh et al. (1978) observed that the correlation coefficient between LH and testosterone in samples collected one hour apart was .34 in 11 Angus bulls.

The larger correlation coefficients observed in the present experiment may be a reflection of the more frequent sampling interval, enhancing the ability to detect increases in serum LH of short duration.

Over all bulls and days of treatment, 85.4 percent of all testosterone secretory spikes were preceded by an elevation in LH. If episodic releases of testosterone occurring during the first 1.5 hours of the blood sampling period were deleted (those testosterone secretory spikes which we could not have associated with a release of LH), then 95.4 percent of all episodic elevations of testosterone were associated with an elevation in LH.

Reports of temporal association of serum LH and testosterone vary somewhat and may be a function of sampling frequency. Welsh et al. (1978) observed that 69 percent of all testosterone elevations were associated with episodic releases of LH. A limited report by Katongole et al. (1971) suggested that each LH secretory spike was followed by an increase in testosterone within 1.5 hours.

The lack of a complete association of serum LH and subsequent testosterone concentrations is not surprising. The inability to sample continuously limits the ability of the investigator to continuously associate LH and testosterone. Although LH treatment causes increased testosterone (Smith et al., 1973), prolactin (Hafiez et al., 1972) and corticosteroids (Welsh et al., 1979) may modify the response.

CHAPTER V

SUMMARY

The objective of this experiment was to evaluate the effect of elevated ambient temperature on endocrine function in bulls.

Eight Angus bulls, ranging in age from 21.5 to 23 months, were individually stanchioned in temperature controlled chambers at 22 ± 1°C for a 3 week adjustment period. Following the adjustment period, 4 bulls were assigned to either control (22 ± 1°C) or elevated ambient temperature (34 ± 1°C) and exposed continuously for 15 days.

Jugular cannulae were placed in all bulls 15 hours prior to blood sampling on days -2, 6 and 15 of heat treatment. Samples were taken at 30 minute intervals from 0600 hours to 1800 hours. At 1800 hours, 200 µg of GnRH were infused. Blood samples were obtained at 15 minute intervals for the following hour, then at 30 minute intervals until 2400 hours.

Luteinizing hormone (LH) concentrations were similar between both groups of bulls two days prior to the onset of heat treatment. After 6 days of treatment, average serum LH concentrations were reduced ($P < .05$) in stressed bulls (2.9 ± .1 ng/ml vs 2.2 ± .1 ng/ml). In addition, the area under 12 hour LH profiles and the average height of LH secretory spikes were reduced ($P < .05$). By 15 days of treatment, average serum LH concentrations, areas under 12-hour profiles and secretory spike heights tended to be reduced but the magnitude of the reduc-

tion compared to controls ($P \simeq .15$) was less than that observed on day 6. The frequency of LH pulsitile releases was similar in control and heat stressed bulls throughout the experimental period.

Serum testosterone concentrations were similar in control and heat stressed bulls until 15 days of exposure to elevated ambient temperature when concentrations were slightly reduced ($P < .13$) in stressed bulls. Similarly, the areas under 12-hour testosterone profiles tended to be less ($P < .18$) in stressed bulls as compared with controls on day 15. The frequency and magnitude of testosterone secretory spikes were not altered by heat stress.

The LH and testosterone responses to exogenous GnRH were similar for control and heat stressed bulls at all three sampling periods. Generally, LH increased from about 3 ng/ml to 29 ng/ml within 15 minutes after GnRH infusion. Serum LH attained a maximum of 60 ng/ml by 1.5 to 2 hours after injection, then declined gradually to pre-injection concentrations by the end of the sampling period. A two-fold increase in testosterone occurred (> 8 ng/ml) within 1.5 hours after GnRH treatment. The testosterone response to GnRH was sustained much longer than that of LH and began to decline (< 8 ng/ml) by 5 hours after GnRH. Serum testosterone had not returned to basal concentrations by 6 hours after GnRH infusion.

The results of this experiment indicate that exposure of bulls to $34^{\circ}C$ for 15 days reduces serum LH by day 6 of treatment. However, this effect may be transient as serum LH concentrations were similar for both treatments by day 15. Serum LH response to GnRH was similar between control and heat stressed bulls throughout the treatment, suggesting that heat stress did not alter pituitary responsiveness to exogenous

gonadotropin.

Serum testosterone concentrations were not significantly altered by 15 days of heat stress. In addition heat stress does not appear to alter testosterone release in response to GnRH-mediated LH release. If reductions in testicular androgen biosynthesis occur, they may be masked by alterations in androgen metabolism and/or disposition and are not reflected in the peripheral circulation.

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APPENDIX

TABLE VII

STOCK AND WORKING BUFFER SOLUTIONS USED IN IMMUNOASSAY PROCEDURES

1. 0.5 M Sodium Phosphate Buffer, pH 7.5

   A) Weigh 69.0 g $Na_2PO_4 \cdot H_2O$ (monobasic) and dilute to 1000 ml with glass distilled H_2O; store at 5^oC.

   B) Weigh 71.0 g Na_2HPO_4 (dibasic) and dilute to 1000 ml with glass distilled H_2O; store at 5^oC.

   C) To make 0.5 M Sodium Phosphate, mix 1 part monobasic plus 4 parts dibasic. Adjust pH to 7.5.

2. Stock Phosphate Buffered Saline (.01 M, pH 7.0)

   A) 120 ml of 0.5 M Sodium Phosphate, $NaH_2PO_4 \cdot H_2O$ (monobasic).

   B) 240 ml of 0.5 M Sodium Phosphate, $NaHPO_4$ (dibasic).

   C) 143 g Sodium Chloride, NaCL.

   D) 1.75 g Thimerosol (Merthiolate).

   E) Add glass distilled H_2O to a final volume of 3500 ml.

   F) Check pH, adjust to 7.0 if necessary.

3. Phosphate Buffered Saline Working Solution (PBS, .01 M, pH 7.0)

   A) Dilute one part PBS stock with four parts glass distilled water.

4. Phosphate Buffered Saline Plus 0.1% Gelatin (PBS+Gel)

   A) Weigh 1g of Knox Gelatin and dilute to 1000 ml with PBS working solution.

TABLE VIII

SERUM LH AND TESTOSTERONE RESPONSE TO GnRH ON DAY -2 OF TREATMENT

Time After GnRH (Hr)	LH(ng/ml)		Testosterone (ng/ml)	
	Control[a]	Heat Stressed[a]	Control[a]	Heat Stressed[a]
0.00	1.8 ± 0.5[b]	2.6 ± 0.5	3.6 ± 1.5	4.9 ± 1.5
0.25	33.9 ± 5.1	33.2 ± 5.1	3.0 ± 1.4	4.4 ± 1.4
0.50	42.8 ± 7.4	54.4 ± 7.4	3.6 ± 1.0	5.0 ± 1.0
0.75	55.4 ± 7.0	69.4 ± 7.0	5.3 ± 0.9	6.6 ± 0.9
1.00	55.7 ± 7.1	70.6 ± 7.1	5.5 ± 0.4	7.3 ± 0.4
1.50	63.2 ± 8.8	74.6 ± 8.8	8.0 ± 0.9	10.4 ± 0.9
2.00	71.6 ± 7.6	64.9 ± 8.7	8.1 ± 0.9	8.8 ± 0.9
2.50	48.6 ± 9.5	65.7 ± 9.5	9.2 ± 0.7	9.3 ± 0.7
3.00	33.4 ± 6.2	51.8 ± 6.2	8.5 ± 1.0	9.3 ± 1.0
3.50	25.3 ± 5.7	40.6 ± 5.7	10.3 ± 1.6	8.4 ± 1.6
4.00	16.0 ± 4.0	30.7 ± 4.0	10.6 ± 2.6	10.4 ± 2.6
4.50	10.4 ± 2.7	18.4 ± 2.7	8.3 ± 1.2	9.3 ± 1.2
5.00	7.6 ± 1.8	14.5 ± 1.8	9.1 ± 0.8	10.0 ± 0.8
5.50	6.4 ± 1.7	10.3 ± 1.7	8.9 ± 1.6	8.6 ± 1.6
6.00	3.5 ± 1.5	7.6 ± 1.5	8.9 ± 1.2	7.9 ± 1.2

[a]Values in each column are the means for 4 bulls.

[b]Mean ± SE.

TABLE IX

SERUM LH AND TESTOSTERONE RESPONSE TO GnRH ON DAY 6 OF TREATMENT

Time After GnRH (Hr)	LH (ng/ml)		Testosterone (ng/ml)	
	Control[a]	Heat Stressed[a]	Control[a]	Heat Stressed[a]
0.00	7.1 ± 1.6[b]	1.7 ± 1.6	4.1 ± 1.0	4.2 ± 1.0
0.25	30.2 ± 5.1	28.0 ± 5.1	4.5 ± 0.9	4.3 ± 0.9
0.50	42.1 ± 7.6	50.1 ± 7.6	5.7 ± 0.9	4.8 ± 0.9
0.75	46.1 ± 6.5	45.5 ± 6.5	6.1 ± 0.7	5.7 ± 0.7
1.00	43.5 ± 10.1	53.6 ± 10.1	7.2 ± 1.0	7.3 ± 1.0
1.50	47.0 ± 11.2	52.9 ± 11.2	8.4 ± 1.6	9.7 ± 1.6
2.00	41.8 ± 9.6	53.6 ± 9.6	9.7 ± 1.2	8.9 ± 1.2
2.50	36.4 ± 11.5	52.9 ± 10.0	9.0 ± 2.0	10.4 ± 2.0
3.00	25.7 ± 8.3	39.9 ± 7.2	8.9 ± 1.0	9.2 ± 1.1
3.50	13.4 ± 5.2	29.9 ± 5.2	10.3 ± 1.3	8.5 ± 1.3
4.00	9.3 ± 2.4	19.5 ± 2.4	10.0 ± 1.4	9.6 ± 1.4
4.50	6.4 ± 2.2	12.4 ± 2.2	8.1 ± 0.7	10.1 ± 0.7
5.00	4.6 ± 1.4	9.5 ± 1.4	8.3 ± 1.8	9.9 ± 1.8
5.50	3.3 ± 0.8	6.4 ± 0.8	6.1 ± 0.6	8.0 ± 0.6
6.00	2.3 ± 0.6	4.1 ± 0.6	6.0 ± 0.7	7.4 ± 0.7

[a]Values in each column are the means for 4 bulls.

[b]Mean ± SE.

TABLE X

SERUM LH AND TESTOSTERONE RESPONSE TO GnRH ON DAY 15 OF TREATMENT

Time After GnRH (Hr)	LH (ng/ml)		Testosterone (ng/ml)	
	Control[a]	Heat Stressed[a]	Control[a]	Heat Stressed[a]
0.00	3.0 ± 0.9[b]	4.0 ± 0.9	4.0 ± 0.5	3.1 ± 0.5
0.25	24.6 ± 6.0	23.7 ± 6.0	4.8 ± 0.8	3.7 ± 0.8
0.50	40.8 ± 7.3	47.0 ± 7.3	5.3 ± 0.9	5.1 ± 0.9
0.75	45.8 ± 5.9	52.8 ± 5.9	5.9 ± 0.5	5.8 ± 0.5
1.00	46.2 ± 5.1	47.3 ± 5.1	7.2 ± 0.7	6.4 ± 0.7
1.50	51.4 ± 7.6	59.1 ± 7.6	8.4 ± 1.1	8.3 ± 1.1
2.00	51.5 ± 5.9	60.3 ± 5.9	8.2 ± 1.0	7.9 ± 1.0
2.50	40.7 ± 4.7	53.7 ± 4.7	9.8 ± 1.4	8.7 ± 1.4
3.00	22.8 ± 5.8	35.0 ± 5.8	9.4 ± 1.0	6.9 ± 1.0
3.50	15.3 ± 3.6	25.0 ± 3.6	10.4 ± 1.2	10.3 ± 1.2
4.00	11.3 ± 2.1	16.7 ± 2.1	9.4 ± 1.5	8.5 ± 1.5
4.50	7.2 ± 1.5	12.4 ± 1.5	8.2 ± 1.1	7.4 ± 1.1
5.00	5.4 ± 1.3	9.2 ± 1.3	8.2 ± 1.2	8.7 ± 1.2
5.50	3.7 ± 0.8	6.6 ± 0.8	6.9 ± 0.8	7.9 ± 0.8
6.00	3.1 ± 0.6	5.3 ± 0.6	7.4 ± 1.1	7.3 ± 1.1

[a]Values in each column are the means for 4 bulls.

[b]Mean ± SE.

VITA 2

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Master of Science

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