THE INFLUENCE OF TROPICAL ADAPTATION AND BREEDTYPE ON ADRENAL AND TESTICULAR FUNCTION IN BEEF BULLS

A Dissertation

by

JEFFREY WILLIAM KOCH

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

DOCTOR OF PHILOSOPHY

May 2004

Major Subject: Physiology of Reproduction

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Approved as to style and content by:

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ABSTRACT

The Influence of Tropical Adaptation and Breedtype on Adrenal

and Testicular Function in Beef Bulls. (May 2004)

Jeffrey William Koch, B.S., Texas A&M University;

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Co-Chairs of Advisory Committee: Dr. Ronald D. Randel
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Bulls of various breedtypes including Angus (*Bos taurus*), Bonsmara (*Sanga X Bos taurus*), Brahman (*Bos indicus*), Romosinuano (*Criollo*), Tuli (*Sanga*) and Wagyu (Japanese *Bos taurus*) were utilized to evaluate the influence of tropical adaptation on adrenal and testicular function. The objectives were to determine if tropical adaptation influenced: a) response to management stressors, b) organ and gland weights, adrenal and testis StAR and P450 content and total adrenal, medullary and cortical areas, c) basal and hCG-induced testosterone and d) testis and epididymal sperm concentrations.

Blood samples were obtained within 5 min before and after transportation and during restraint every 15 min for 6 h to evaluate cortical response. Angus, Brahman and Romosinuano bulls were slaughtered following sexual maturity.

Cortical responses to transportation and restraint were not influenced by tropical adaptation. Response to these stressors could be categorized into high responders (Angus, Brahman), intermediate responders (Romosinuano, Tuli) and low responders (Wagyu, Bonsmara). Tropically-adapted breedtypes were not categorized into a single

group; therefore, cortical responses to management stressors were influenced by breedtype, but not by tropical adaptation.

Most organ and gland weights (actual weight and weight corrected for BW) and the steroid precursors, StAR and P450, were not influenced by tropical adaptation, but were by breedtype. Paired adrenal gland weight, total adrenal area, medullary and cortical areas were influenced by tropical adaptation. Tropically-adapted breedtypes had lighter glands and smaller areas than the temperate *Bos taurus* breedtypes.

All breedtypes except Wagyu had similar basal concentrations of plasma testosterone prior to hCG administration; therefore, basal testosterone was not influenced by tropical adaptation, but only by breedtype. Wagyu had greater basal concentrations of testosterone than other breedtypes. Testosterone concentrations following hCG administration was similar between adaptation groups and breedtypes.

As expected, testis and epididymal sperm concentrations were influenced by tropical adaptation. Tropically-adapted breedtypes had greater testicular and epididymal sperm concentrations than the temperate *Bos taurus* breedtypes during the summer months.

In summary, adrenal weight and area and testicular and epididymal sperm concentrations were influenced by tropical adaptation. Cortical response to management stressors, basal testosterone and StAR and P450 content were influenced by breedtype, not tropical adaptation.

DEDICATION

This dissertation is dedicated to my beautiful wife, Shari. Without her encouragement, support, and assistance, this dissertation likely would not have been completed.

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CHAPTER I

INTRODUCTION

Beef cattle operations represent a large portion of the agricultural economy in the United States. Most of the beef cattle operations utilize *Bos taurus* cattle which include both British (such as Angus) and Continental breeds (such as the Simmental). However, Brahman or *Bos indicus* cattle are utilized in the majority of the Gulf Coast region (Cartwright, 1980). The British breeds and Continental breeds are examples of *Bos taurus* breeds of cattle that were developed in the temperate zones of the Northern Hemisphere. The Brahman breed of cattle are members of the *Bos indicus* genus and are tropically-adapted. *Bos taurus* cattle tend to be earlier maturing, faster growing and may yield a more tender carcass compared to *Bos indicus* cattle (Turner, 1980; Solomon et al., 1986). *Bos indicus* cattle tend to be tick and disease resistant and are genetically adapted to utilize poor quality forages (Turner, 1980). Brahman cattle are important in beef crossbreeding systems to increase heterosis and adaptation to hot climates and for their improved utilization of low quality forage (Koger, 1980).

Brahman influenced cattle often have decreased acceptability by cattle feeders and thus are discounted by cattle buyers. Consequently, the scientific evaluation of alternative tropically adapted breeds may be warranted (Thrift, 1997). Currently, there are several tropically-adapted breeds that should be evaluated for potential utilization in crossbreeding. The Romosinuano is a tropically-adapted *Bos taurus* breed of beef cattle

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This dissertation follows the style and format of The Journal of Animal Science.

developed from cattle of Spanish origin in the Sinú Valley of Colombia (Rouse, 1977). The Romosinuano is one of seven Colombian Criollo breeds adapted to tropical and subtropical conditions (Felius, 1995). In Colombia, the Romosinuano breed is recognized for its fertility, heat tolerance, and ability to be utilized in cross-breeding systems with *Bos indicus* cattle (Elzo et al., 1998). The Bonsmara and Tuli are tropically-adapted Sanga cattle breeds that originated in Southern Africa. The Bonsmara is a composite breed which was developed in the Transvaal by Dr. Jan Bonsma and contains 3/16 Hereford, 3/16 Shorthorn and 5/8 Africander, which is considered to be a Sanga breed (Felius, 1995). The Bonsmara are considered to be docile and a highly fertile beef breed (Payne and Hodges, 1997). The Tuli is a Sanga breed that evolved from the mixing of *Bos indicus* and *Bos taurus* germplasm in Africa (Schoeman, 1989). Tuli cattle are considered to have good conformation, to be highly fertile and females are productive until at least 15 years of age (Payne and Hodges, 1997). An additional breed that may be of economic importance in some crossbreeding systems is the Wagyu. The Wagyu is a temperate *Bos taurus* breed of cattle that originated in Japan. Wagyu cattle are referred to as Japanese black and are known for their extremely well-marbled carcasses. The meat from Wagyu cattle is referred to as Kobe beef and is considered to be a delicacy (Felius, 1995).

Stress occurs during common management practices in beef cattle production. The response of a breed to management stressors such as transportation and restraint may determine if that breed will be advantageous in crossbreeding systems. Reproduction is also important in considering the inclusion of a breed of cattle in

crossbreeding systems. Tropically-adapted *Bos taurus* cattle breeds such as the Romosinuano, Sanga influenced or Sanga breeds such as the Bonsmara and Tuli and temperate *Bos taurus* cattle such as the Wagyu may offer unique alternatives in beef cattle crossbreeding systems; however, stress and reproductive parameters must be evaluated to develop a better understanding of how these breeds can be used. The experiments were designed to test the following hypotheses: 1) Tropical adaptation influences adrenal function in bulls and 2) Tropical adaptation influences testicular function in bulls.

Objectives

This research was designed to evaluate stress and reproductive parameters in temperate *Bos taurus* (Angus and Wagyu), tropically-adapted *Bos taurus* (Romosinuano) and tropically-adapted Sanga (Bonsmara and Tuli) and *Bos indicus* (Brahman) bulls. More specifically these experiments were designed to:

1) Determine the influence of breed on response to the common management stressors transportation and restraint.

2) Determine if organ weights, adrenal Steroidogenic acute regulatory (StAR) protein and P450 side-chain cleavage enzyme and total adrenal, medullary and cortical areas of tropically-adapted *Bos Taurus* bulls are more similar to temperate *Bos taurus* bulls or tropically-adapted *Bos indicus* bulls.

3) Determine if differences exist in basal and induced concentrations of testosterone among tropically-adapted *Bos taurus*, Sanga, temperate *Bos taurus* and tropically-adapted *Bos indicus* bulls.

4) Determine if the weights of reproductive organs, testis and epididymal sperm concentrations and content of testis StAR protein and P450 scc enzyme of tropically-adapted *Bos taurus* bulls are more similar to temperate *Bos taurus* bulls or tropically-adapted *Bos indicus* bulls.

CHAPTER II

REVIEW OF THE LITERATURE

The stress and reproductive axes (Figures 1 and 2; respectively) are both important in beef cattle production systems. The ability of animals to reproduce offspring is essential and necessary for beef cattle producers to stay in business. The stress axis maintains homeostasis in the body; however, it can adversely affect reproduction as well as growth in cattle. Different biotypes of cattle may respond differently to management stressors and/or may differ in various reproductive parameters.

The stress and reproductive axes both arise from the same embryonic origin. These axes both have similarities in the hormonal pathways from the hypothalamus to the anterior pituitary to the steroid-producing organ. Steroidogenic pathways are also similar in the adrenal and the testis. The following sections discuss various aspects of the hypothalamic-pituitary-adrenal axis and hypothalamic-pituitary-testis axis as well stimulators of these axes.

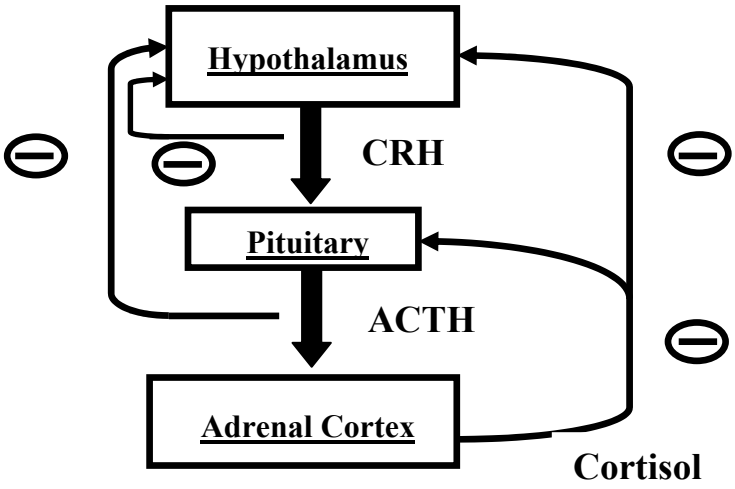

Figure 1. Hypothalamic-pituitary-adrenal axis.

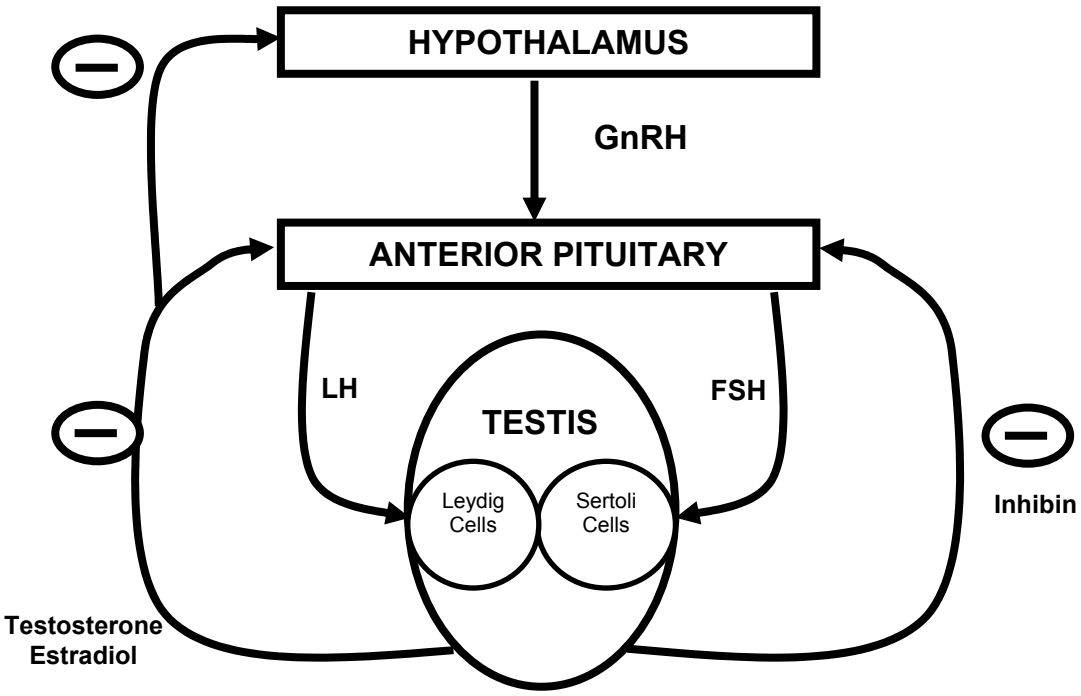

Figure 2. Hypothalamic-pituitary-gonadal axis.

Hypothalamic-Pituitary-Adrenal Axis

The hypothalamic-pituitary-adrenocortical (HPA) axis is a major component of the neuroendocrine response to stressful events (Selye, 1973). This relationship between stress and adrenocortical activation was one of the first recognized in the study of the endocrinology of stress (Selye, 1939). Stress results in activation of both the HPA axis and the sympatho-adrenal medullary (SA) system. Stress response results in production and secretion of glucocorticoids from the HPA axis and the catecholamines (epinephrine and norepinephrine) from the adrenal medulla. In the HPA axis, corticotropin-releasing hormone (CRH) as well as other secretagogues such as vasopressin from the hypothalamus stimulate the secretion of adrenocorticotropic hormone (ACTH) from the corticotropes of the anterior pituitary (Vale et al., 1981). ACTH then enters the systemic circulation and acts at the adrenal gland to stimulate the synthesis and secretion of glucocorticoids (Smith, 1930) and possibly aldosterone (McCarty, 1983) and adrenal androgens (Axelrod and Goldzieher, 1967; Thomas, 1968)).

As shown in Figure 1, the HPA axis is regulated by multiple negative feedback loops. The first feedback loop consists of an ultrashort CRH-mediated loop in which CRH released from the hypothalamus acts back at the hypothalamus to inhibit CRH secretion (Calogero et al., 1988b). The second feedback loop is a short hypothalamic proopiomelancortin (POMC) gene derived peptide loop which includes both ACTH and ß-endorphin inhibiting CRH secretion (Calogero et al., 1988b). The last feedback loop is a long glucocorticoid-mediated feedback loop where the glucocorticoids act at the

hypothalamus to inhibit CRH and inactivate the POMC gene, and also at the anterior pituitary to inhibit ACTH secretion (Keller-Wood and Dallman, 1984).

Corticotropin-Releasing Hormone (CRH)

It has long been recognized that factors produced in hypothalamic neurons regulate the secretion of ACTH from the anterior pituitary (Guillemin and Rosenberg, 1955; Saffran et al., 1955). However, the chemical structure and description of the 41 - amino acid peptide forming CRH was reported just over 20 years ago (Vale et al., 1981). CRH is synthesized in, and secreted primarily from, parvocellular neurons of the paraventricular nucleus of the hypothalamus (Bloom et al., 1982; Olschowka et al., 1982).

The receptor for CRH has been detected in the brain, anterior pituitary, adrenal medulla as well as in sympathetic ganglia of the rat (Cummings et al., 1983) and primate (Valentino, 1988). The CRH receptors concentrated in the corticotropes of the pituitary appear to be sensitive to circulating concentrations of glucocorticoids since receptor numbers decrease shortly after adrenalectomy (Wynn et al., 1985) or during chronic stress (De Souza and Battaglia, 1988). CRH enters the hypothalamic-hypophyseal portal blood system to act at receptors on the corticotropes (Plotsky et al., 1985). These receptors are G-protein specific and when bound to CRH the protein kinase A and protein kinase C pathways are activated (Millan et al., 1987).

CRH secretion is regulated by several stimuli and factors. Stimuli such as pain and blood pressure regulate CRH secretion. Increases in blood pressure result in inhibition of CRH secretion whereas decreases in blood pressure result in increased

CRH secretion (Ganong, 1988). CRH secretion is also regulated by several neurotransmitter systems. Secretion of CRH is stimulated by norepinephrine, epinephrine, acetylcholine and serotonin (Calogero et al., 1988a) as well as cytokines such as Interleukin - 1, 2, 6 and tumor-necrosis factor (Woloski et al., 1985). Calogero et al. (1988a) reported that gamma-aminobutyric acid (GABA), opioids, ACTH and glucocorticoids inhibit the secretion of CRH.

Adrenocorticotropic Hormone (ACTH)

ACTH is synthesized in and secreted from the corticotropes of the anterior pituitary. Proopiomelanocortin (POMC) is the prohormone for ACTH (Mains et al., 1977). Expression of the POMC gene is stimulated by CRH and vasopressin. These authors also report that POMC, in the anterior pituitary, is converted into ACTH, a 39-amino acid fragment, and β-lipotropin, a 92-amino acid fragment. ACTH is not only regulated by CRH, but also by arginine vasopressin, oxytocin, Angiotensin II, vasoactive intestinal polypeptide, and serotonin (Plotsky et al., 1985). Glucocorticoids have been shown to inhibit ACTH secretion in vitro and in vivo (Keller-Wood and Dallman, 1984). CRH passes through the hypothalamo - hypophyseal portal vessels to act upon the corticotropes to stimulate secretion of ACTH. ACTH then enters the systemic circulation and acts at the adrenal gland to stimulate the synthesis and secretion of glucocorticoids and aldosterone and adrenal androgens.

The adrenal gland consists of two regions, the outer cortical region and the inner medullary region. The catecholamines, epinephrine and norepinephrine, are secreted from the chromaffin cells of the adrenal medulla (sympathomedullary system). The

adrenal cortex can be divided into three morphologically distinct zones: the zona glomerulosa, zona fasiculata and the zona reticularis. The primary product of the zona glomerulosa is the mineralocorticoid aldosterone. The primary product of the zona fasiculata and zona reticularis is the glucocorticoid cortisol; however, the zona reticularis is also responsible for secretion of other steroids such as androgens (dehydroepiandrosterone, DHEA; testosterone) and estrogens (estradiol and estrone; Black, 1993). Glucocorticoids, epinephrine, and norepinephrine act to inhibit glucose uptake, fatty acid storage, and protein synthesis at storage sites and stimulate the release of energy substrates, including glucose, amino acids, and free fatty acids, from muscle, fat tissue and liver (Munck et al., 1984). This is paralleled by the stimulation of cardiovascular and pulmonary function (Yates et al., 1980) and suppression of anabolic processes such as digestion, growth, reproduction and immune function (Munck et al., 1984).

Administration of CRH and ACTH in Cattle

Studies describing CRH challenges in cattle are somewhat limited. Boran heifers infected with *Trypanosoma congolense* and administered bovine CRH (bCRH) had a less responsive anterior pituitary and adrenal gland than heifers not infected with Trypanosoma congolense (Abebe et al., 1993). Vesslier et al. (1999) reported that male Holstein calves administered 0.01 μg/kg bCRH did not increase plasma ACTH or cortisol concentrations, but administration of 0.03 μg/kg bCRH resulted in a five-fold increase in ACTH and cortisol and administration of 0.10 μg/kg bCRH resulted in a twenty-fold increase in ACTH and a ten-fold increase in cortisol concentrations. These

same authors suggest that calves seem less sensitive to CRH than other animals since the threshold for a calf's response to bCRH is 0.03 μg/kg compared to thresholds of 0.01 μg/kg for humans (Orth et al., 1983) and pigs (Zhang et al., 1990). Various doses of bCRH and arginine-vasopressin (AVP) administered together to Holstein male calves resulted in a marked increase in ACTH and cortisol concentrations above basal concentrations for at least 150 minutes following bCRH injection (Vesslier et al., 1999). These authors did not utilize a group receiving no AVP to compare the effects of AVP to bCRH on ACTH and cortisol concentrations. However, AVP in addition to bCRH has been shown to have increased the integrated response and peak concentration of ACTH and cortisol in humans (DeBold et al., 1984), sheep (Pradier et al., 1986; Keller-Wood, 1998), and pigs (Janssens et al., 1995).

Numerous studies investigating the influence of ACTH on adrenocortical response have been performed. Various doses of ACTH have been utilized to evaluate adrenal cortical response. Holstein-Friesian steer calves administered ACTH (0.01mg ACTH/100 kg body weight) at 6, 8, 10 and 15 months of age had greater concentrations of plasma cortisol at 8, 10, and 15, but not 6 months of age compared to Holstein-Friesian bull calves receiving the same dose of ACTH over the same sampling period (Verkerk and Macmillan, 1997). Barnes et al. (1983) reported no differences in peak plasma cortisol concentrations following ACTH (0.45 IU/kg BW) treatment in steers and bulls; however, concentrations of plasma cortisol remained elevated for a longer period of time in the steers. Brahman heifers (Lay et al., 1996) and cows of various breeds (Friesian, Hereford, Ayrshire) (Alam et al., 1986) treated with varying doses of ACTH

(0.01, 0.03, 0.06, 0.12, 0.25, 0.50, 1.0, or 2.0 mg ACTH) had marked increases in plasma cortisol concentrations with cows receiving the lowest dose (0.01 mg ACTH) doubling pre-treatment cortisol concentrations at 0.5 and 3 h following injection. These authors as well as others (Gwazdauskas et al., 1980) concluded that the increase in cortisol concentrations following ACTH treatment is directly dependent on the dose of ACTH with increased stimulation of the adrenal gland resulting from larger doses of ACTH.

Breedtype influences cortisol response to ACTH treatments. Koch et al. (2000) reported that the proportional response of concentrations of serum cortisol immediately prior to and 30 min following ACTH treatment was significantly greater for *Bos taurus* steers compared to ½ *Bos taurus* – ½ *Bos indicus* and *Bos indicus* steers but not ¾ *Bos taurus* – ¼ *Bos indicus* steers.

Genetic Differences in the HPA

Differences in the HPA between breeds of cattle (Bruner et al., 1996; Carroll et al., 1996), goats (Engelbrecht et al., 2000), pigs (Desautes et al., 1999), strains of rats (Malendowicz, 1987; Sternberg et al., 1992; Moncek et al., 2001) and ethnic races (Yanovski et al., 1993; Yanovski et al., 1995; Yanovski et al., 1996) have been reported. These differences have been shown to be at various levels of the HPA including the hypothalamus and the pituitary gland as well as the adrenal glands.

Genetic differences concerning the hypothalamus have primarily been demonstrated in various strains of rats. Lewis rats have been reported to have a markedly smaller CRH response to stress than that of the Fischer (F344) rats (Sternberg

et al., 1992). These authors suggest this may be due to a decreased responsiveness of the CRF neurons to afferent stressful stimuli; however, Windle et al. (1998) suggest that differences in CRH release between the Fischer rat strain and the Lewis rat strain may be compounded by differences in pituitary sensitivity. Following a restraint stress, CRH mRNA was significantly increased in the CFY strain of rats over Sprague-Dawley or Wistar strains (Harbuz et al., 1994).

Differences in the HPA are not only associated at the level of the hypothalamus, but also at the level of the anterior pituitary. At the level of the pituitary the Lewis strain of rats had significantly lower POMC mRNA concentrations following restraint stress compared to the Fischer rat strain (Moncek et al., 2001). These authors also reported significantly lower concentrations of ACTH in plasma of Lewis rats following restraint than that in Fischer rats. This is similar to the findings of Dhabhar et al. (1997) reporting that the Fischer strain of rats had significantly higher ACTH following an acute stress than Sprague-Dawley rats or Lewis rats. Differences have been reported in anterior pituitary gland weight between 3/4 Angus and 3/4 Brahman steers with 3/4 Angus steers having heavier anterior pituitary glands (Bruner et al., 1996; Carroll et al., 1996). Bruner et al. (1996) also reported that 3/4 Angus steers had higher concentrations of ACTH than did 3/4 Brahman steers.

Genetic differences have been reported for CRH-induced ACTH secretion in various ethnic groups of people. Higher concentrations of ACTH following CRH administration have been reported in African-American women compared to Caucasian women (Yanovski et al., 1993) and African-American men compared to Caucasian men

(Yanovski et al., 1995). Yanovski et al. (1996) also reported higher ACTH concentrations following CRH administration in prepubertal and early pubertal African-American girls compared with prepubertal and early pubertal Caucasian girls. In each of these studies involving ethnic races of people, only ACTH but not cortisol was significantly elevated in African-American people compared to Caucasian people.

Genetic differences at the level of the adrenal gland have been associated with adrenal gland weights, adrenal gland morphology as well as cortisol production in various species. Bruner et al. (1996) reported 3/4 Angus steers had heavier paired adrenal gland weight, greater plasma cortisol concentrations and larger total adrenal area, medullary area and cortical area than 3/4 Brahman steers. Brown Norway rats had heavier adrenal weights compared to Wistar, Fischer 344 rats and F1 hybrid Brown Norway X Fischer 344 rats (Sarrieau et al., 1998). These authors also reported that the Brown Norway rats had the lowest concentration of corticosterone compared to the other three rat strains. Engelbrecht et al. (2000) reported Angora goats had lower cortisol concentrations following ACTH administration compared to Boer goats or Merino sheep. Angora goats also have lower adrenal mitochondrial P450 content compared to Boer goats and Merino sheep (Engelbrecht and Swart, 2000). Basal cortisol concentrations as well as ACTH-induced cortisol concentrations are higher in Chinese Meishan pigs compared to large white pigs (Desautes et al., 1999). Differences were reported in both adrenal weight and volumes of zona fasiculata and zona reticularis between the CFY and Wistar rat strains (Malendowicz, 1987). Wilkinson et al. (1999)

reported Dark Agouti rats had a significantly smaller zona reticularis compared to Sprague-Dawley and Wistar rats.

Genetic differences have been reported at all levels of the HPA axis. These genetic differences indicate that heredity may play an important role in controlling the mechanisms that lead to different physiological responses to stress. Consequently, an animal's genotype or genetic make-up may affect its susceptibility and/or resistance to stressors.

Factors Stimulating the Stress Response

The stress response can be stimulated by many factors called stressors in normal livestock production systems. A stressor is defined as an environmental factor that contributes to, or elicits, stress responses which can threaten or adversely affect the health of the body. Stressors can be physiological (Dobson and Smith, 1995) or psychological (Grandin, 1997). Many, but not all, of these stressors are caused by normal or poor management practices. Normal management practices which elicit a stress response include handling and restraining animals in squeeze chutes, hot iron branding of animals (Lay et al., 1992a), change in housing (Friend et al., 1987) as well as transportation of animals (Jacobson and Cook, 1998). Poor management practices include nutritional deprivation and overcrowding of animals (Ewing et al., 1999). An additional type of stressor, thermal stress, involves extreme temperatures and is a direct result of the environment.

Various stressors will elicit a stress response in livestock. These stressors can primarily be placed into one of three categories, transportation stress, handling and

restraint stress and thermal stress. The following sections will detail each of these categories.

Transportation Stress

A common management technique in beef cattle production systems is transportation of cattle to a new destination. These destinations can include transporting weaned calves to stocker programs, stocker calves to feedlots, fed cattle to slaughter facilities, as well as cows and bulls from ranch to ranch or to sale barns. Transportation is a stressor that has been identified as a physical stressor (Dobson and Smith, 1995) or a psychological stressor (Grandin, 1997) because of the possible fear-factor encountered by cattle that have had a bad experience previously. Jacobson and Cook (1998) suggest that transportation involves both physical and psychological types of stressors. The primary physiological indicators used to assess the response of livestock to transport are changes in plasma hormones (Reid and Mills, 1962), live weight (Shorthose et al., 1972), heart rate (Stephens and Toner, 1974) and factors involved in immune function (Gwazdauskas et al., 1978).

The stress response most commonly reported in transportation stress is that of increased circulating cortisol concentrations. Zavy et al. (1992) reported that weaned, *Bos indicus* influenced (Angus X Brahman, Hereford X Brahman), castrated, male beef calves had higher concentrations of cortisol prior to transportation than weaned *Bos taurus* (Angus X Hereford) steer calves; however, no differences in cortisol concentration were detected following transportation. Calves that were transported for 12 h immediately following weaning had increased cortisol concentrations (for 24 h and

remained constant for 24 h) for a prolonged time (return to initial or pre-transportation concentrations between d 4 and 7) compared to calves that were weaned with no transportation or calves that were weaned two weeks early in order to stabilize and become acclimated prior to transportation (Crookshank et al., 1979). Cortisol concentrations were greater in crossbred Friesian steers and bulls following 1 h of transportation compared with stationary confinement on the trailer for one h with no transport (Kenney and Tarrant, 1987a, b). Mitchell et al., (1988) reported that Brahman/Hereford/Afrikander cross heifers and steers 15 to 18 mo of age had greater concentrations of cortisol following a 180-km transportation stress compared to control animals which were not transported.

In addition to circulating cortisol, weight loss and percentage of shrink is commonly measured. This is usually a result of increased urination and defecation. Zavy et al (1992) reported that weaned steers lost 8.1% of their body weight but this weight loss was not related to genotype. Lay et al., (1996) reported that pregnant Brahman cows that were subjected to transportation had a greater (4%) shrink at 60 d gestation compared to pregnant Brahman cows that were administered ACTH (1 IU/Kg BW; 2% shrink) or sham (2% shrink); however, the cows receiving ACTH had higher concentrations of cortisol than did transported or sham cows. Friend (2000) reported that horses which were transported for 30 h without water had a greater percentage shrinkage than horses provided water during transportation.

Transportation results in changes in the immune system. Kent and Ewbank (1986a) reported significant increases in neutrophil and lymphocyte numbers in two

groups of 1 to 3 wk old calves transported for 6 or 18 h (sampled during and after the trip) compared to non-transported calves of the same age. Similar results were reported with goats (Kannan et al., 2000) and horses (Stull and Rodick, 2000). Angus and Angus X Brahman feeder steers subjected to a 14 h transportation stress also had increased mature neutrophils but decreased mononuclear cells and eosinophils compared to control feeder steers which were not transported (Blecha et al., 1984).

Heart rate usually increases following transportation. Suckling calves (2-4 mo) transported for one h had an average increase in heart rate from 80 beats per min (prior to transportation) to 110-115 beats per min following transportation (Stephens and Toner, 1974). Friesian bulls that were loaded onto a trailer five times (held on trailer for at least 15 min) over a 2 wk period had a lower heart rate during loading but not following a 2 h transportation experience than bulls not acclimated to the trailer (Cook and Jacobson, 1998). These same authors reported greater circulating concentrations of lipid (g/liter), lactate (mmol/liter) and glucose (mmol/liter) but not total protein (g/liter) in transported animals compared to the control animals.

Restraint and Handling Stress

Restraining cattle is necessary to perform management practices such as implanting, dehorning, branding, castrating, and deworming. Restraint is also utilized in operations where artificial insemination and pregnancy determination are performed. Restraint has been classified as a psychological stressor (Dobson and Smith, 1995; Grandin, 1997). Grandin (1997) suggests that procedures such as restraint in a squeeze chute do not usually cause significant pain, but fear may be a major psychological

stressor in cattle that are raised in extensively managed beef operations. During restraint, animals are often separated and isolated from other animals.

Zavy et al. (1992) reported that Brahman-cross (Angus X Brahman, Hereford X Brahman) steers had higher cortisol concentrations when restrained in a squeeze chute for one h than English-cross (Angus X Hereford) steers. Crossbred heifers and steers (15 to 18 mo Brahman/Hereford/Afrikander) that were handled and restrained for 15 min had increased concentrations of ACTH, cortisol and triiodothyronine (T_3) as well as increased lipid, lactate, and glucose concentrations compared to animals that had previously been fitted with an indwelling jugular catheter and were not handled or restrained (Mitchell et al., 1988). Crookshank et al., (1979) reported that weaned crossbred calves became used to handling over a 16 d time period as the calves became less excitable over the sampling period. In addition, the calves had lower concentrations of cortisol. Holstein heifer calves (seven to sixteen weeks old) had increased cortisol concentrations 30 min following restraint in a squeeze chute and a 2-3 min application of either an unheated or heated electrical dehorner to the horn stumps compared to cortisol concentrations from blood samples collected from the animals in their individual pens prior to restraint and stimulus (Boandl et al., 1989).

Holstein and Jersey cows that were freeze-branded or hot-iron branded had greater heart rates and plasma cortisol concentrations from 5.5 min to 25.5 min after branding compared to cows subjected to a brand maintained at room temperature (Lay et al., 1992a). The cows subjected to freeze-branding had similar concentrations of epinephrine compared to hot-iron branded cows but greater than that of the sham-

branded cows 1 min postbranding and greater concentrations of norepinephrine compared to hot-iron branded or sham-branded cows 0.5 min after branding. Crossbred calves (1/2 Simmental, ¼ Hereford, ¼ Brahman; Lay et al., 1992b) and Angus calves (Lay et al., 1992c) acclimated to restraint and subjected to freeze-branding, hot-iron branding or sham-branding all had increased concentrations of plasma cortisol compared to plasma cortisol concentrations prior to applying the stimulus and hot-iron branded calves (both Angus and crossbred) had greater plasma concentrations of epinephrine at 0.5 min after branding than freeze-branded or sham-branded calves.

Friesian and crossbred beef calves (1-3 mo of age) that were surgically castrated had greater concentrations of salivary cortisol after castration compared to salivary cortisol concentrations in calves that were castrated via application of rubber bands or intact controls (Fell et al., 1986). Similar findings were reported for plasma cortisol concentrations in Angus, Hereford, and Brahman bulls (approximately 20-21 mo old) castrated surgically or by latex rubber band (Chase et al., 1995). These authors also reported that white blood cell counts were higher in animals castrated (surgically or banding) than in intact control animals.

Thermal Stress

Climate can influence the efficiency of cattle production systems. Breeds of cattle differ in their degree of heat or cold tolerance and thus some cattle suffer at extreme environmental temperatures. It has long been recognized that breeds of cattle that are considered to be heat tolerant have greater weight gains under hot conditions than breeds that are not heat tolerant (Ragsdale et al., 1957). Cattle living in naturally

hot climates, or those subjected to artificially hot environments, must become adapted or acclimated and must undergo homeostatic adjustments to maintain thermal balance (Rhynes and Ewing, 1973). Rectal temperature as well as respiration are often associated with thermal stress responses.

Thermal stress affects several bodily functions. Nonlactating Holstein cows exposed to a constant environmental temperature of 43°C for 4.5 h had increased rectal temperature and concentrations of epinephrine and norepinephrine, but decreased concentrations of glucocorticoids compared to a 4.5 h exposure at 40°C (Alvarez and Johnson, 1972). These same authors also reported similar findings (increased rectal temperature and concentrations of epinephrine and norepinephrine, but decreased glucocorticoid concentrations) in nonlactating Holstein cows subjected to 35°C for 24 d compared to nonlactating Holstein cows kept at 18°C for the same period of time.

Heifers representing heat-tolerant breeds (Brahman, Santa Gertrudis, Criollo and Brown Swiss X Zebu) and dairy breeds (Holstein, Guernsey, Ayrshire and Jersey) exposed for 6 h to 40.5°C had greater rectal temperature and respiratory rate during nighttime and daytime readings compared to heifers of the same breeds kept at 25°C for 6 h (Alba and Sampaio, 1988). Rhynes and Ewing (1973a) found that Hereford bulls (approximately 2 years of age) had decreased concentrations of plasma cortisol but similar plasma corticosterone concentrations during a 7 wk exposure to 35.5°C compared to 7 wk at 21°C. The reports of Christison and Johnson (1972) conflict with some of the previous results. These authors reported that mature non-lactating Jersey cows exposed to 35°C for 4 h had a significant increase in plasma cortisol in the first 20

min of exposure. Plasma cortisol continued to rise for two hours and plateaued between 2 and 4 h following the onset of heat exposure. Christison and Johnson (1972) also reported that skin temperature sharply rose in the first 20 min and then continued to rise very slightly during the remainder of the exposure period.

Cattle that are cold tolerant are stressed during periods of high temperatures. Similar findings, reported by Rhynes and Ewing (1973a) reported that rectal temperature and respiration rate were stable and constant in Hereford bulls for seven weeks at 21°C but rapidly rose and remained significantly elevated for seven weeks at 35.5°C. It is important to note that the majority of the studies evaluating heat stress in cattle were performed in climatic laboratories. The results from these studies using climatic laboratories may be extrapolated to indicate likely responses in natural environments. Murray (1982) reported that Hereford and Santa Gertrudis heifers had greater rectal temperature and respiratory rate during an exposure period of 4 h at 40°C in a climatic laboratory compared to a natural setting or a natural setting including enforced movement of the cattle. These differences were likely due to differences in temperature (40°C in climate laboratory and approximately 10°C lower in the natural or field setting). However, Murray (1982) did report that the Hereford and Santa Gertrudis heifer each with the lowest rectal temperature and respiratory rate in the climatic laboratory exhibited the highest rectal temperature and respiratory rate in the field as well as in the field following enforced movement.

Temperature stress is not always a result of breeds that are not adapted to extreme heat conditions. Brahman calves placed at 4°C immediately following birth for

two h had greater concentrations of plasma glucose, lactate, blood urea nitrogen, triglyceride, triiodothyronine, thyroxine and cortisol compared to crossbred (1/2 Simmental X ¼ Brahman X ¼ Hereford) calves placed at 4°C or 31°C for 2 h following birth or Brahman calves placed at 31°C for 2 h following birth (Godfrey et al., 1991). Berardinelli et al. (1992) reported that Brahman (from Texas) and Hereford (from Montana and Nebraska) bulls located in Montana had greater serum cortisol concentrations than the same breeds of bulls (from the respective origin) located in Nebraska or Texas. Thermal stress therefore affects various hormone concentrations and bodily functions and is an environmental stressor that can be reduced by having tropically and /or subtropically adapted cattle.

Tropical Adaptation

The ability to survive and continue efficient production in tropical and subtropical regions is referred to as tropical adaptation. Cattle coming from temperate climates fail to adapt and consequently become unproductive in subtropical and tropical areas (Rhoad, 1935; Bonsma, 1949; Bonsma, 1951). Several factors including sweat glands, hair follicles, sweating rate and respiration rate must be considered in cattle that are tropically adapted.

The number of sweat glands, shape of the sweat glands and hair follicle characteristics have been shown to be different in tropically-adapted cattle and cattle from temperate climates. Nay and Hayman (1956) reported *that Bos indicus X Bos taurus* crossbred cattle have larger and more sweat glands per unit area of the skin than pure-bred *Bos taurus* cattle. The activity of the sweat gland is dependent upon the

shape. It has been reported that tropically-adapted *Bos indicus* cattle have baggy shaped sweat glands, whereas *Bos taurus* breeds have tubular or coiled sweat glands and crossbred cattle have club shaped sweat glands (Yeates et al., 1975). Indigenous tropical cattle had a lower sweat gland length to diameter ratio than European cattle (Jenkinson and Nay, 1972; Jenkinson and Nay, 1973). These authors also suggest that the smaller sweat glands of tropically-adapted cattle may also be indicative of greater activity. Hair follicles appear to play a role in tropical adaptation of cattle. Jenkinson and Nay (1972 and 1973) reported that tropically - adapted cattle have shallower hair follicle depths as well as thicker hair follicles than cattle from temperate regions.

Respiration rates and sweating rates are important in thermoregulation of tropically-adapted cattle. Finch (1986) demonstrated that indigenous tropically-adapted cattle were able to lower resistance to internal heat transfer and regulate body temperature during high levels of heat stress better than *Bos taurus* from temperate regions. Tropically-adapted *Bos indicus* cattle have been reported to have greater sweating rates compared to temperate *Bos taurus* cattle in which the sweating rates tend to reach a plateau after the first increase (Finch, 1986). Additionally, resistance of the hair coat to environmental heat is greater in temperate *Bos taurus* cattle, thus resulting in greater accumulation of heat at the skin (Finch, 1986). Carvalho et al. (1995) reported that *Bos indicus* cattle as well as Simmental cattle native to tropical conditions had lower respiration rates than Simmental cattle imported into tropical regions.

The studies in this section demonstrate the importance of the number and shape of the sweat glands, the hair follicle depth and dynamics, as well as sweating and

respiration rates in tropical adaptation of cattle. All of these factors play a role in dissipating heat to help regulate body temperature in tropical and subtropical climates. Tropical adaptation is not only important in reducing heat stress but also has an effect on several production related parameters.

Hypothalamic-Pituitary-Gonadal Axis

Function of the reproductive system depends on the hypothalamic-pituitary-gonadal (HPG) axis (Senger, 1997) (Figure 2). This section will primarily focus on the HPG axis of the male. The hypothalamus synthesizes and secretes the 10-amino acid neuropeptide gonadotropin releasing hormone (GnRH) (Matsuo et al., 1971). GnRH then acts upon the gonadotropes of the anterior pituitary to regulate the synthesis and secretion of the gonadotropins; luteinizing hormone (LH) (Mongkonpunya et al., 1974) which is a 245-amino acid glycoprotein and follicle-stimulating hormone (FSH) (Amann, 1983) which is a 207-amino acid glycoprotein. LH and FSH have two subunits an α-subunit (96-amino acids; Liu et al., 1972a; Sairam et al., 1972a; Shome and Parlow, 1974a), which is identical for all glycoprotein hormones, and each has a specific β-subunit (149-amino acids for LH and 111-amino acids for FSH) which is the active form (Liu et al., 1972b; Sairam et al., 1972b; Shome and Parlow, 1974b). LH secretion in bulls occurs in a pulsatile fashion with three to eight pulses randomly occurring in a 24 h period (Amann, 1983). The gonadotropins must enter the systemic circulation to act on the gonads to regulate steroidogenesis and spermatogenesis. LH binds to the plasma membrane receptors at the Leydig cells to activate the cAMP-protein kinase second messenger system which results in the production of steroids, especially

androgens such as testosterone (Hafez, 2000). Testosterone synthesis increases approximately 30 min after increased concentrations of LH and consequently is also released in a pulsatile fashion (Amann, 1983). However, continuous exposure of Leydig cells to high concentrations of LH causes them to become refractory and thus less testosterone is produced (Bergfeld et al., 1996). Testosterone produced from Leydig cells is transported into the Sertoli cells where it is then converted into estradiol (Hafez, 2000). FSH acts upon the Sertoli cells located in the seminiferous epithelium to influence Sertoli cell functions which include nourishment of maturing spermatids (Hochereau-de Reviers et al., 1987). The Sertoli cells produce and secrete various compounds including androgen binding protein (ABP), a protein that transports testosterone; sulfated glycoproteins (SGP) 1 and 2, which are thought to be associated with fertility acquisition (SGP1), and aid cellular and fluid movement through the testicular tubular network; transferrin, which transports iron and is thought to be necessary for the process of spermatogenesis and inhibin, which is a hormone that is involved in suppressing FSH at the level of the anterior pituitary via a negative feedback mechanism (Hochereau-de Reviers et al., 1987). Testosterone and estradiol act through a negative feedback mechanism at the level of the hypothalamus and the anterior pituitary to inhibit or suppress GnRH, LH and FSH synthesis and secretion (Gooren, 1989). The physiological functions of androgens (testosterone) consist of differentiation and development of the male urogenital system, the accessory sex organs, the external genitalia and secondary sex characteristics (Hafez, 2000). Testosterone is also involved

in spermatogenesis and has anabolic actions on skeletal and cardiac muscle fibers (Hafez, 2000).

Testosterone production has been reported to be stimulated via exogenous sources such as human chorionic gonadotropin (hCG) (Sundby et al., 1975) and GnRH (Mongkonpunya et al., 1975). GnRH acts directly to stimulate LH secretion which then acts on the cells of Leydig to stimulate testosterone production. Human chorionic gonadotropin (hCG) exhibits LH-like activity and acts directly on the cells of Leydig to stimulate testosterone production. The common dose of hCG that is administered to stimulate production of testosterone is 750 to1000 IU, with intravenous administration being faster acting and more effective than intramuscular administration (Sundby 1981). GnRH has been administered intramuscularly at a dose of 200 μg (Godfrey et al., 1990). Both hCG- and GnRH- induced testosterone production allow for consistent methods to induce testosterone production in male animals.

Spermatogenesis

Spermatogenesis is the process whereby spermatozoa are formed from spermatogonia and occurs in the seminiferous tubule of the seminiferous epithelium (Berndtson, 1977). Spermatogenesis can be subdivided into spermatocytogenesis, meiosis and spermiogenesis which each consist of specific cellular divisions (Berndtson, 1977). The process of spermatocytogenesis initiates at the basal membrane of the seminiferous tubule with A1 spermatogonia (Berndtson and Desjardins, 1976). A pool of stem cells mitotically divide to provide a continual source of A1 spermatogonia (Abdel-Raouf, 1961). The A1 spermatogonia undergoes a mitotic cell division resulting

in A2 spermatogonia and continues until A4 spermatogonia are present (Knudsen, 1958). A4 spermatogonia divide into intermediate (I) spermatogonia and then the I spermatogonia divide into B spermatogonia (Knudsen, 1958). The last division of spermatocytogenesis and the last occurring in the basal compartment is the division of the B spermatogonia into primary spermatocytes (Knudsen, 1958). All of these divisions are a result of mitosis.

The first division in the adluminal compartment is a meiotic (I) division of primary spermatocytes into secondary spermatocytes. Spermiogenesis then begins with a rapid meiotic (II) division of secondary spermatocytes into spherical spermatids (Amann, 1962). The spermatids must undergo four phases to mature into spermatozoa. These phases also occur during the process of spermiogenesis and include the Golgi phase, cap phase, acrosomal phase and the maturation phase (Senger, 1997). During the Golgi phase, the first steps in development of the acrosome and axoneme occur (Senger, 1997). The cap phase results in the formation of a "cap" over the anterior portion of the nucleus. The tail begins to protrude towards the lumen during the cap phase. The spermatid nucleus begins to elongate and the acrosome eventually covers the majority of the anterior nucleus during the acrosomal phase with the spermatids embedding themselves deeply into the Sertoli cells and their tails projecting into the lumen of the seminiferous tubule (Senger, 1997). Within the acrosome are hydrolytic enzymes such as acrosin, hyaluronidase, zona lysin, esterases and acid hydrolases that are required for fertilization to occur (Hafez, 1987). The last phase is the maturation phase. During the maturation phase, the final events including mitochondria assembled around the

flagellum to form the middle piece and dense outer fibers of the flagellum and the fibrous sheath are produced to complete the assembly process (Hafez, 1987). Amann (1988) suggests that maturation is not final until motility has been acquired by the spermatozoa. Following maturation of the spermatozoa, the process of spermiation, or release of spermatozoa into the lumen of the seminiferous tubule, occurs. Breakage of the cytoplasmic bridges occur during the release of the spermatozoa into the lumen of the seminiferous tubule. The complete process of spermatogenesis takes approximately 61 days in the bull (Berndston and Desjardins, 1976).

Spermatozoa that are released into the lumen of the seminiferous tubule are collected in the rete testis and then pass through the vas efferens to get to the caput epididymus (Foote, 1962). The spermatozoa then pass through the corpus epididymus to the cauda epididymus (Foote, 1962). The epididymus functions to transport, mature, concentrate and store spermatozoa. Spermatozoa leave the cauda epididymus through the vas deferens which connects to the urethra that serves as an exit out of the body for the spermatozoa (Foote, 1962).

Daily Sperm Production and Extra-Gonadal Sperm Reserves

The technique for determining testis and epididymal (caput, corpus, and cauda) sperm concentrations in the bull was first described by Amann and Almquist (1962) and these authors described the calculation for determining daily sperm production in bulls. Daily sperm production and extra-gonadal sperm reserves are affected by breed, age and plane of nutrition in bulls. Seven yr old Angus bulls had greater spermatozoal reserves in the caput, corpus and cauda epididymus compared to Hereford bulls of the same age

but less epididymal spermatozoal reserves than six yr old Charolais bulls (Weisgold and Almquist, 1979). These same authors reported that three yr old Charolais bulls had less epididymal spermatozoal reserves than did six yr old Charolais bulls.

The level of nutrition, particularly energy, has been shown to affect epididymal spermatozoal reserves. Angus and Hereford yearling bulls receiving a medium energy diet had greater epididymal sperm reserves than Angus and Hereford yearling bulls receiving a high energy diet (Coulter and Bailey, 1988). Similar findings were reported for 15 mo old Angus and Hereford bulls (Coulter et al., 1987) as well as for two yr old Angus and Hereford bulls (Coulter and Kozub, 1984).

Stress and Reproduction

Activation of the HPA axis during stress can affect reproductive function at all three levels of the hypothalamic-pituitary-testicular axis as well as at the end-organs (target tissues) for the sex steroids (Rabin et al., 1990). At the level of the hypothalamus, endogenous and exogenous CRH and glucocorticoids have been shown to inhibit the secretion of GnRH in the rat (Rivier and Vale, 1984)). Exogenous CRH or glucocorticoid administration has also been shown to inhibit GnRH secretion in the human (Rivier and Rivest, 1991). Rabin et al. (1990) suggests that opioid peptides administered exogenously or produced during stress or after administration of CRH, also suppress GnRH secretion. However, this can be reversed in the rat by the prior administration of naloxone (Rivier and Vale, 1984). The inhibition of GnRH secretion by CRH and glucocorticoids results in a decrease in LH and FSH secretion from the

anterior pituitary and ultimately a decrease in testosterone production by the cells of Leydig and an alteration in the spermatogenic process.

There is considerable evidence *in vivo* and *in vitro* that the glucocorticoids can act directly upon the pituitary gonadotropes of domestic animals to reduce the secretion of gonadotropins, especially LH (Moberg, 1987). It has been reported that only prolonged exposure of the pituitary to glucocorticoids seems to have an effect on the basal secretion of LH (Padmanabhan et al., 1983 and Suter and Schwartz, 1985). Moberg (1991) suggests the primary action of the glucocorticoids on the gonadotropins appears to be blockage of the ability of GnRH to stimulate secretion of LH. Although this mechanism is not understood, there have been several proposed mechanisms. The first mechanism was proposed by Kamel and Kubajak (1987) and they suggest that the glucocorticoids inhibit LH secretion by interfering with the hydrolysis and turnover of phospholipids by blocking the enzymatic activity of phospholipase A_2, thus depressing arachidonic acid release. A second mechanism proposed by Moberg (1991), suggests that glucocorticoids may alter gonadotropin function by modifying the feedback of the gonadal steroids onto the gonadotrophs. Administration of ACTH to adrenalectomized rams has been shown to prevent exogenous GnRH from stimulating the secretion of LH (Fuquay and Moberg, 1983). However, Matteri et al. (1986) reported that treating ovine pituitaries *in vitro* with synthetic ACTH altered gonadotroph function in a biphasic manner both stimulating basal secretion of the gonadotropins and at the same time diminishing the amount of gonadotropins released in response to a subsequent GnRH challenge.

There are several components from the HPA that have an effect at the gonadal level. CRH has been shown to inhibit testosterone synthesis in the rat Leydig cell (Leers-Sucheta et al., 1999) yet stimulates steroidogenic acute regulatory protein (StAR) and steroid synthesis in the murine Leydig cell (Huang et al., 1997). Acute treatment with ACTH inhibits testosterone secretion by the bovine (Johnson et al., 1982) and ovine (Juniewicz et al., 1987) testis, but stimulates testosterone secretion from the testes of boars and rabbits (Liptrap, 1993). In bulls, there is an inverse relationship between plasma concentrations of glucocorticoids and the amount of testosterone secreted in response to exogenous LH (Welsh et al., 1979). It appears that most of the influence of the adrenal axis in regulating the gonads is through altered gonadotropin secretion.

The Affects of High Ambient Temperature on Spermatogenesis

It has long been known that increased scrotal temperature, either due to high ambient air temperature or scrotal insulation, affects spermatogenesis. Spermatogenesis in both beef and dairy bulls appears to be affected. Casady et al. (1953) reported sterility lasting at least 100 d in one Guernsey bull and temporarily reduced semen quality in another Guernsey bull following 17 d of exposure to 100ºF ambient temperature in a heat chamber. Hereford bulls exposed to scrotal insulation for 24 or 72 h were reported to have a decrease of approximately 65% in number of live sperm and a 60% decrease in normal sperm in the second and third wk following insulation compared to non-insulated control bulls (Austin et al., 1961). Similar results were reported in Shorthorn bulls exposed to 10 or 20 h of scrotal insulation (Ross and Entwistle, 1979). A decrease in motile sperm and an increase in abnormal cells was reported in yearling Angus bulls

exposed to 35ºC for 8 h or 31ºC for 16 h (Meyerhoeffer et al., 1985); however, these differences were not as large as those reported by others (Austin et al., 1961; Ross and Entwistle, 1979) but tended to have a longer recovery period. Skinner and Louw (1966) reported that spematozoal characteristics (% live sperm, % abnormal sperm) in Afrikander (Sanga) bulls were not as severely affected as that of Friesland (*Bos taurus*) bulls after various exposure times to 40ºC; however, optimum spermatogenesis was impaired in both breeds. Increased ambient air temperature has been shown to influence secretion of LH and testosterone and thus possibly impair spermatogenesis. Minton et al. (1981) reported a decrease in average LH concentrations 6 days following initiation of a 15 d exposure to 34ºC. Concentrations of plasma testosterone in bulls exposed to 35.5ºC for seven wk were reported to fall to 43% of that of control bulls during the first two wk of heat exposure (Rhynes and Ewing, 1973b).

CHAPTER III

RESPONSE TO MANAGEMENT STRESSORS AND ADRENAL CORTEX CONTENT OF STEROIDOGENIC ACUTE REGULATORY PROTEIN DIFFERS IN TEMPERATE AND TROPICALLY-ADAPTED *Bos taurus* AND *Bos indicus* BULLS

Introduction

In livestock production systems, the stress response can be stimulated by many factors called stressors. A stressor is defined as an environmental factor that contributes to a stressful circumstance or elicits stress responses which can threaten or adversely affect the health of the body. Stressors can be physiological (Dobson and Smith, 1995) or psychological (Grandin, 1997). Many, but not all, of these stressors are caused by normal or poor management practices. Normal management practices which elicit a stress response include handling and restraining animals in squeeze chutes, hot iron branding of animals (Lay et al., 1992a), as well as transporting of animals (Jacobson and Cook, 1998). Breed of cattle can also affect the level of response to a stressor. Zavy et al. (1992) reported that British x Brahman steers had greater plasma cortisol concentrations following restraint and transportation than did British cross steers. Not only have differences been detected in cortisol concentrations among breeds, differences in organs of the hypothalamo-pituitary-adrenal axis have been detected. Bruner et al. (1996) reported that paired adrenal weight in ¾ Angus (*Bos taurus*) was 15 % higher than in ¾ Brahman (*Bos indicus*) steers and that morphometric analysis of adrenal gland cross-sections indicated that total, cortical, and medullary areas were greater for ¾

Angus than for ¾ Brahman steers. These same authors also report that the anterior pituitary gland weighed more in ¾ Angus relative to ¾ Brahman steers which is consistent with the findings of Carroll et al. (1996). Producers could select breeds or types of animals which might be better suited to withstand the stressors inherent in their production systems provided that comparative information was developed. A better understanding of response to stressors by tropically-adapted *Bos taurus* and tropically-adapted Sanga cattle as well as the temperate Wagyu cattle is necessary to determine how they can best fit into beef crossbreeding systems. Therefore, the objectives of this study were to 1) determine the influence of breedtype on response to common management stressors which include transportation and restraint and 2) determine if organ weights, adrenal steroidogenic acute regulatory (StAR) protein and P450 side-chain cleavage enzyme and total adrenal, medullary and cortical areas of tropically-adapted *Bos taurus* bulls are more similar to that of temperate *Bos taurus* bulls or to tropically-adapted *Bos indicus* bulls.

Materials and Methods

Experiment 1

Animals

Bulls of six different breeds, Bonsmara (n = 8; Sanga type), Romosinuano (n = 10; *Bos taurus*), Tuli (n = 10; Sanga), Brahman (n = 8; *Bos indicus*), Angus (n = 7; *Bos tuarus*) and Wagyu (n = 10; *Bos taurus*), were utilized for this experiment. Bulls arrived at the Texas Agricultural Experiment Station in Overton, Texas following weaning. Upon arrival, bulls were pastured together and managed similarly during a 5 mo

acclimation period. There were several important cooperators that generously supplied bulls for this study. The cooperators were Dr. Chad Chase at the Subtropical Agricultural Research Station (Angus and Romosinuano bulls; Brooksville, FL.), the Texas Agricultural Experiment Station (Brahman bulls; Overton, TX.), Mr. George Chapman (Bonsmara bulls; Amarillo, TX.), and Mr. Kent Briggs (Tuli and Wagyu bulls; Rice, TX.).

After reaching sexual maturity (defined as two consecutive semen samples collected via electroejaculation at 2 week intervals containing $\geq$ 500 million sperm per ejaculate as well as $\geq$ 50 % motility of viable spermatozoa), bulls were weighed, body condition scored as described by Godfrey et al. (1988; 1-9 scale; 1 = emaciated and 9 = obese) and subjected to a transportation stress and a serial blood collection. Bulls representing each breed were utilized during all sampling periods. Body condition scores were assessed by the same two people. During each subjected stressor, bulls from each breed were represented. A heparinized blood sample was collected via tail vessel puncture immediately prior to transporting. The transportation stress consisted of a pre-determined trip which was 30 km long and lasted 30 min (average speed was 60 km/h). The transportation stress was done in replicates using three bulls which were loaded on a 7.3-m trailer so that each bull had his own compartment (2.0 m by 2.4 m). The transportation stress was applied at approximately the same time each day (1600 h $\pm$ 45 min). Following the transportation stress, bulls were weighed and an additional heparinized blood sample was collected via tail vessel puncture. Bulls were then fitted with an indwelling jugular catheter (o.d. = 1.7 mm, i.d. = 1.1 mm; Teflon, TFE, Cole-

Parmer, Chicago, IL) as described by Lay et al. (1996). Bulls were randomized for the trailer compartment and the fitting of the indwelling catheter by the order in which they went through squeeze chute. Bulls were confined together and allowed to rest overnight before the serial blood samples were collected. The serial blood collection took place the next day to ensure that cortisol concentrations from the restraint stressor were not elevated as a result of the transportation stress.

Heparinized blood samples were collected every 15 min for 6 h to determine concentrations of cortisol in the systemic circulation. The blood samples were placed on ice and centrifuged (20 min at 2700 rpm) within 30 min of collection. Plasma was harvested and stored at -20°C until radioimmunoassays could be performed. Area under the curve was calculated for cortisol using the trapezoidal rule.

Hormone Radioimmunoassay Procedure

Plasma concentrations of cortisol were determined by radioimmunoassay as described by Willard et al. (1995, See Appendix 1 for complete description). Antiserum (rabbit anti-cortisol) was purchased from Pantex, Inc. (Santa Monica, CA) and tritiated hydrocorticosterone was purchased from New England Nuclear, Inc. (Boston, MA). The cross-reactivity of the antiserum was approximately 60%, 48%, 0.01%, 0.01 % and 0.01% with corticosterone, deoxycorticosterone, progesterone, androstenedione and estradiol, respectively. The cortisol was purchased from Steraloids (Wilton, NH) and standards were made which had concentrations ranging from 3.9 pg/ml to 16,000 pg/ml,. The intraasssay coefficient of variation was 5.4 % and the interassay coefficient of variation was 7.2%.

Statistical Analysis, Experiment # 1

Differences in percent shrink, pre-transportation cortisol concentrations, post-transportation cortisol concentrations, absolute change in cortisol concentrations from pre-transportation to post-transportation, cortisol area under the curve and average, lowest and highest cortisol concentrations during the restraint period were analyzed using the GLM procedure for analysis of variance (SAS, 1992). The plasma concentrations of cortisol during the first 2 h, second 2 h and third 2 h of restraint were analyzed using the repeated measures procedure of SAS (1992). Mean separation was accomplished using the PDIFF option of SAS (1992).

Experiment # 2

Animals

Angus (n = 10), Brahman (n = 8) and Romosinuano (n = 10) bulls were fed an 8.85:1.0:0.15 corn:soybean meal:limestone ration for a minimum of 69 d (range was 69 – 111 d) following the serial blood collection. The range in time on feed existed due date availability to slaughter at the slaughter facility. These bulls were penned together and given Coastal bermuda grass hay and water ad libitum. Following the feeding period, the bulls were transported to the Texas A&M University Rosenthal Meat Science and Technology Center where they were processed for food utilization. Every breed was represented at each slaughter date. The dates of slaughter, number of head slaughtered and the average daily temperature at slaughter are included in Table 1.

Table 1. Slaughter dates, number of head slaughtered and the average daily temperature at slaughter

Slaughter date	Number of head slaughtered	Average temperature of each slaughter date (°F)
May 30, 2000	8	82.5
June 13, 2000	6	80
July 13, 2000	6	88.5
August 8, 2000	5	86.5
September 5, 2000	3	92.5

Tissue Collection

Upon slaughter, whole pituitary glands and adrenal glands were collected and stored on ice until weights could be obtained. Weights (± 0.1 g) were also obtained for the liver, spleen, heart and lungs (trachea included). Adrenal glands were trimmed free of adherent tissue, weighed (± 0.01 g) and a cross-sectional slice of the right adrenal gland was fixed in 4 % paraformaldehyde for histology. The cortical component was dissected from the medullary component and a piece weighing approximately 0.5 g was snap frozen in liquid nitrogen and stored at -80°C until analysis for StAR protein and P450 side – chain cleavage enzyme. Morphometrical analysis of the histology section

was used to determine total, cortical and medullary areas of the adrenal gland. Pituitary glands were cleaned of adherent tissue, weighed (± 0.01 g) and the anterior pituitary were dissected from the whole pituitary and weighed (± 0.01 g).

Western Blot Analysis

A 200 mg section of adrenal cortical tissue was homogenized in 0.25 M sucrose buffer which contained 0.1mM EDTA and 10mM Tris (See Appendix D for complete description). Sample buffer (25mM Tris/Cl, pH 6.8, 1% SDS, 5% β-mercaptoethanol, 1mM EDTA, 4% glycerol and 0.01% bromophenol blue) was used to solubilize protein pellets and subsequently loaded onto a 12.5% SDS-PAGE mini-gel. The samples were then electophoresed at 200 V for 45 min using standard SDS-PAGE buffer. Transfer of proteins from the SDS-PAGE mini-gel to a polyvinylidene diflouride membrane (PVDF; Bio-Rad Hercules, CA) occurred electrophoretically at 100 V for 2 h. The PVDF membrane containing the proteins was exposed to an antibody specific for StAR. This polyclonal StAR antibody used for Western blot analysis was generated in rabbits against a recombinantly produced StAR human protein which lacks the first 62 amino acids. This N-62 antibody was generously supplied by Dr. Walter L. Miller (UC San Francisco). Preincubation of the membrane in a blocking buffer [phosphate buffered saline (PBS) buffer with 4% Carnation non-fat dry milk and 0.5% Tween-20] occurred for 1 h at room temperature followed by a 1 h incubation in fresh buffer containing the primary antibody. PBS buffer containing 0.5% Tween-20 was then used to wash the membrane three times for 10 min per wash. Following the washes, the membrane was placed in fresh blocking buffer containing the second antibody (donkey-anti-rabbit

immunoglobin G) conjugated with horseradish peroxidase (Amersham, Arlington Height, IL). The membrane then underwent an additional three washes with PBS buffer containing 0.5% Tween-20 for 10 min per wash. Chemiluminesence via the Renaissance Kit (Dupont-New England Nuclear) was used to detect the specific signal emitted by the antibody. A BioImage Visage 2000 analytical system was used to quantitate the bands and express the bands as integrated optical density (IOD) units. After completion of the analysis, the membranes were stripped of the StAR antibody using a buffer containing: 2% SDS, 62mM Tris and 100mM β-mercaptoethanol. The membranes were then reprobed using an antibody specific for P450 scc (Chemicon, Temecula, CA) following an identical procedure used to probe the blots for the StAR protein.

Adrenal Morphometric Analysis

Entire cross sections of the right adrenal gland fixed in 4% paraformaldehyde were sectioned at 5 micron intervals and stained with hematoxilin and eosin (H & E; 3 sections per slide). The H&E sectioned images were visualized using an Olympus SZH Zoom Stereo Microscope and acquired by a Sony 960MD 3-CCD color camera attached to the microscope. A PowerMac 8100 computer system using a LG3-8-bit frame grabber (Scion Corp.) which was under the control of the NIH Image Program was used for image acquisition from the Sony color camera. NIH Image (Scion Image Software, Scion Corp.) was used to determine morphometric measurements for total adrenal area, medullary and adrenal artery areas. The total, medullary and adrenal artery areas were measured three times for each of the three H&E sections per slide. An average was

calculated for the nine measurements for each of the total medullary and adrenal artery areas. Adrenal artery area was subtracted from the total adrenal area and medullary area. The adrenal cortical area was calculated as follows: Adrenal Cortical Area = Total Area – (Medullary Area + Adrenal Artery Area).

Statistical Analysis, Experiment # 2

Differences in weights of whole pituitary glands, anterior pituitary glands, adrenal glands, differences in total, cortical and medullary areas of adrenal glands and differences in StAR protein and P450 side – chain cleavage enzyme content were determined using the GLM procedure for analysis of variance (SAS, 1992). Mean separation was accomplished using the PDIFF option of SAS (1992).

Results, Experiment # 1

Transportation Stress

A significant ($P<0.001$) breed affect was detected for average body condition score (BCS) prior to transportation. Brahman and Angus bulls had higher ($P<0.05$) average BCS than Bonsmara, Romosinuano, Tuli, and Wagyu bulls (Table 2).

Table 2. Body condition score (LS mean ± SE) for sexually mature Angus, Bonsmara, Brahman, Romosinuano, Tuli, and Wagyu bulls

Breed	Average body condition score
Brahman	6.3 ± 0.1^{a}
Angus	6.1 ± 0.1^{a}
Tuli	5.8 ± 0.1^{b}
Bonsmara	5.7 ± 0.1^{b}
Wagyu	5.7 ± 0.1^{b}
Romosinuano	5.5 ± 0.1^{b}

Different superscripts within a column differ $P<0.05$

Percent shrink of bodyweight following transportation was significantly ($P<0.05$) influenced by breed. Brahman and Angus bulls had greater ($P<0.05$) shrink than Bonsmara or Wagyu bulls with the Romosinuano and Tuli bulls being intermediate (Table 3).

Table 3. Transportation shrinkage (LS mean ± SE) of sexually mature Angus, Bonsmara, Brahman, Romosinuano, Tuli, and Wagyu bulls

Breed	Shrink (%)
Brahman	1.24 ± 0.19^{a}
Angus	1.20 ± 0.20^{a}
Tuli	$0.84 \pm 0.17^{a,b}$
Romosinuano	$0.82 \pm 0.17^{a,b}$
Bonsmara	0.63 ± 0.19^{b}
Wagyu	0.53 ± 0.17^{b}

Different superscripts within a column differ $P<0.05$

Plasma cortisol concentrations prior to transportation were significantly ($P<0.05$) influenced by breed. Angus and Brahman bulls had greater ($P<0.05$) plasma concentrations of cortisol prior to transportation than Wagyu and Bonsmara bulls with Romosinuano and Tuli bulls being intermediate (Table 4).

There was a significant ($P<0.0002$) breed influence on plasma cortisol concentrations following transportation. Romosinuano, Brahman, Angus and Tuli bulls had greater ($P<0.02$) concentrations of plasma cortisol following transportation than Wagyu and Bonsmara bulls (Table 4).

Absolute change in plasma concentrations of cortisol from pre-transportation to post-transportation was significantly ($P<0.0007$) influenced by breed. Bonsmara was the only breed that did not show an increase of pre-transportation to post-transportation cortisol concentrations. Wagyu bulls had a significantly ($P<0.05$) lower absolute change in concentrations of plasma cortisol from pre-transportation to post-transportation than

the Romosinuano and Brahman bulls which had the greatest increase in cortisol concentrations (Table 4). Tuli and Angus bulls had similar absolute change in concentrations of plasma cortisol from pre-transportation to post-transportation to Wagyu bulls but were greater ($P<0.05$) than the Bonsmara bulls. A non-significant ($P<0.08$) elevation in plasma cortisol concentration was detected in Wagyu compared to Bonsmara bulls.

Table 4. Cortisol concentrations (ng/ml; LS mean ± SE) prior to and following transportation and the absolute change in post transportation and pre transportation cortisol concentration for sexually mature Angus, Bonsmara, Brahman, Romosinuano, Tuli, and Wagyu bulls

Breed	Cortisol concentration prior to transportation (ng/ml)	Cortisol concentration following transportation (ng/ml)	Change in cortisol concentration for post and pre transportation (ng/ml)
Angus	14.72 ± 2.79^{a}	24.52 ± 3.14^{x}	$9.80\pm2.96^{a,b}$
Bonsmara	3.62 ± 2.61^{c}	2.28 ± 2.94^{y}	-1.33 ± 2.77^{c}
Brahman	$12.19\pm2.61^{a,b}$	25.17 ± 2.94^{x}	12.98 ± 2.77^{a}
Romosinuano	9.69 ± 2.34^{b}	25.20 ± 2.63^{x}	15.51 ± 2.47^{a}
Tuli	$7.42\pm2.34^{b,c}$	18.44 ± 2.63^{x}	$11.02\pm2.47^{a,b}$
Wagyu	4.67 ± 2.34^{c}	9.41 ± 2.63^{y}	$4.74\pm2.47^{b,c}$

Different superscripts within a column differ ^{a,b,c} $P<0.05$, ^{x,y} $P<0.02$

Cannulation

Placement of a jugular cannula can be a stressor and there was a difference ($P<0.0002$) in response to this stressor due to breed. Wagyu and Bonsmara bulls were less responsive ($P<0.0002$) to this stressor than all other breeds examined (Table 5).

Table 5. Cortisol concentration (ng/ml; LS mean ± SE) following cannulation for sexually mature Angus, Bonsmara, Brahman, Romosinuano, Tuli, and Wagyu bulls

Breed	Cortisol concentration following cannulation (ng/ml)
Romosinuano	21.11±2.29[a]
Tuli	20.84±2.29[a]
Angus	19.83±2.73[a]
Brahman	19.71±2.56[a]
Wagyu	4.95±2.29[b]
Bonsmara	4.12±2.56[b]

Different superscripts within a column differ $P<0.0002$

Restraint

There were no breed differences over the entire 6 h sampling period. However, a significant ($P<0.03$) breed influence over time was detected during the first h of restraint (Figure 3). There were no differences over time during hour 2 - 6 of restraint. However, there was a significant ($P<0.05$) time * breed interaction for the first and second h of restraint. No ($P<0.08$) difference was detected for a time * breed interaction for the third h of restraint.

There was no significant breed affect detected for average or lowest plasma cortisol concentrations in the bulls over the 6 h restraint period. A significant ($P<0.05$) breed influence was detected for maximum concentration of plasma cortisol in the bulls during the 6 h restraint period. Brahman and Romosinuano bulls had greater ($P<0.05$) maximum concentrations of plasma cortisol during the 6 h restraint period than Bonsmara and Wagyu bulls with Angus and Tuli bulls being intermediate (Table 6).

Table 6. The range (maximum, average, and lowest) of cortisol concentrations (ng/ml; LS mean ± SE) during the 6 h of restraint for sexually mature Angus, Bonsmara, Brahman, Romosinuano, Tuli, and Wagyu bulls

Breed	Maximum cortisol concentration (ng/ml)	Average cortisol concentration (ng/ml)	Lowest cortisol concentration (ng/ml)
Brahman	24.83±3.64[a]	9.67±1.65	3.12±0.65
Romosinuano	22.30±3.25[a]	7.56±1.47	1.45±0.59
Angus	21.63±3.89[a,b]	8.97±1.76	1.70±0.70
Tuli	20.80±3.25[a,b]	7.27±1.47	1.40±0.59
Bonsmara	12.89±3.64[b]	5.00±1.65	1.17±0.65
Wagyu	12.11±3.25[b]	5.16±1.47	0.90±0.59

Different superscripts within a column differ $P<0.05$

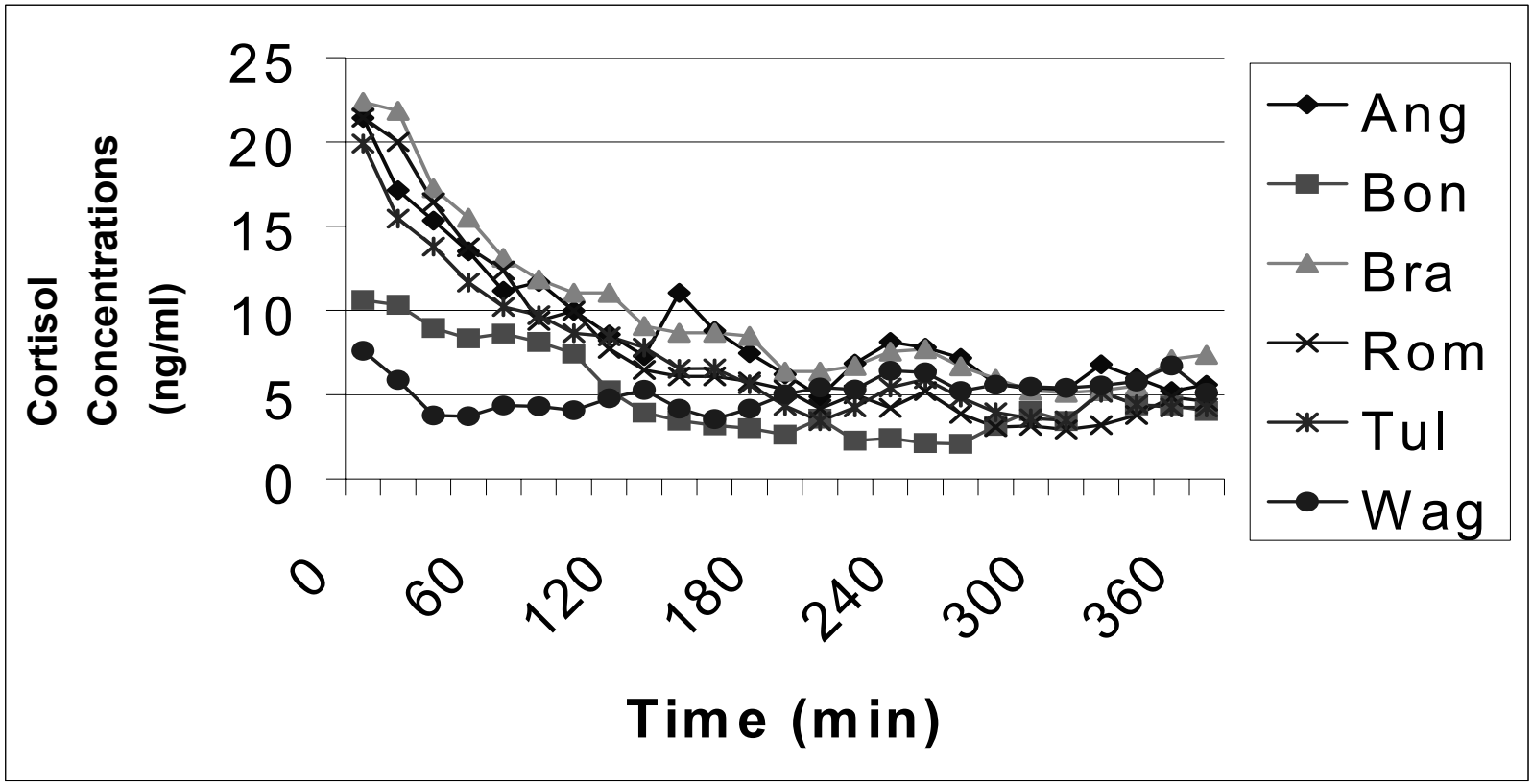

Figure 3. Average concentrations of plasma cortisol in Angus, Bonsmara, Brahman, Romosinuano, Tuli, and Wagyu bulls during the 6 h restraint period. Time*breed interaction ($P<0.05$). Plasma samples taken every 15 min during the 6 h restraint stress to determine cortisol concentrations. Time 0 was the initial sample taken.

There was a significant ($P<0.06$) breed affect for area under the curve over the 6 h restraint period (Fig. 3). Brahman and Angus bulls had greater ($P<0.05$) area under the curve over the 6 h restraint period than Bonsmara and Wagyu bulls with Romosinuano and Tuli bulls being intermediate (Table 7).

Table 7. Area under the curve (LS mean ± SE) for cortisol concentrations during the 6 h restraint stress for sexually mature Angus, Bonsmara, Brahman, Romosinuano, Tuli, and Wagyu bulls

Breed	Total area under the curve (arbitrary units)
Brahman	3483.4±549.0[a]
Angus	3339.8±586.9[a]
Romosinuano	2543.0±491.0[a,b]
Tuli	2302.3±491.0[a,b]
Bonsmara	1758.5±549.0[b]
Wagyu	1488.4±491.0[b]

Different superscripts within a column differ $P<0.05$

There was a significant breed influence on average ($P<0.03$) as well as maximum ($P<0.02$) plasma cortisol concentrations during the first h of restraint. Brahman, Romosinuano, Angus and Tuli bulls had greater ($P<0.05$) average concentrations of plasma cortisol during the first h of restraint than Wagyu bulls with the Bonsmara bulls being intermediate to all breeds except Brahman bulls (Table 8). Brahman, Romosinuano, Angus and Tuli bulls had greater ($P<0.05$) maximum concentrations of plasma cortisol during the first h of restraint than Wagyu bulls with Bonsmara bulls

being intermediate to Tuli and Wagyu bulls (Table 8). Wagyu bulls tended (P<0.09) to have the lowest plasma cortisol concentration during the first h of restraint compared to Angus, Romosinuano and Brahman bulls (Table 8). There was no breed influence detected for average, highest or lowest plasma cortisol concentrations for the second h, third h, fourth h, fifth h or sixth h of restraint.

Table 8. The range (maximum, average, and lowest) cortisol concentrations (ng/ml; LS mean ± SE) during the first h of restraint for sexually mature Angus, Bonsmara, Brahman, Romosinuano, Tuli, and Wagyu bulls

Breed	Highest cortisol concentration (ng/ml)	Average cortisol concentration (ng/ml)	Lowest cortisol concentration (ng/ml)
Brahman	24.21±3.73[a]	18.02±3.16[a]	11.66±2.83
Romosinuano	22.30±3.34[a]	16.79±2.83[a,b]	11.61±2.54
Angus	22.05±3.99[a]	15.70±3.38[a,b]	10.97±3.03
Tuli	20.68±3.34[a,b]	14.21±2.83[a,b]	9.38±2.54
Bonsmara	12.56±3.73[b,c]	9.37±3.16[b,c]	6.54±2.83
Wagyu	9.05±3.34[c]	5.07±2.83[c]	2.20±2.54

Different superscripts within a column differ P<0.05

Results, Experiment # 2

Slaughter Weight

Slaughter weight and hot carcass weight were significantly (P<0.0002) influenced by breed. Brahman bulls were heavier (P<0.0004) at slaughter and had a heavier (P<0.0004) hot carcass weight than Angus and Romosinuano bulls (Table 9).

Dressing percentage was significantly (P<0.004) influenced by breed. Brahman and Angus bulls had greater (P<0.05) dressing percentages than Romosinuano bulls (Table 9).

Table 9. Slaughter weight, hot carcass weight, and dressing percent (LS mean ± SE) of Angus, Brahman, and Romosinuano bulls at 69-111 d following sexual maturity

Breed	Slaughter weight (kg)	Hot carcass weight (kg)	Dressing percent (%)
Brahman	527.39±19.04[a]	318.08±13.25[a]	60.25±0.63[x]
Angus	415.10±17.03[b]	244.06±11.85[b]	58.65±0.57[x]
Romosinuano	385.27±17.03[b]	220.55±11.85[b]	57.02±0.57[y]

Different superscripts within a column differ [a,b] P<0.0004; [x,y] P<0.05

Endocrine Organ Weights

Whole pituitary gland weights and whole pituitary gland weight on a body weight (BW) basis (mg/kg BW) differed significantly (P<0.03) between breeds. Romosinuano and Angus bulls had heavier (P<0.05) whole pituitary glands than Brahman bulls (Table 10). On a BW basis, Romosinuano had a heavier (P<0.02) whole pituitary gland than Angus which were heavier (P<0.02) than Brahman bulls (Table 10).

Table 10. Whole pituitary gland weights and whole pituitary gland weight on a BW basis (mg/kg BW; LS mean ± SE) for Angus, Brahman, and Romosinuano bulls 69-111 d following sexual maturity

Breed	Pituitary weight (g)	Pituitary weight (mg/kg BW)
Romosinuano	2.05±0.09[a]	5.33±0.18[x]
Angus	1.94±0.09[a]	4.68±0.18[y]
Brahman	1.65±0.12[b]	3.16±0.20[z]

Different superscripts within a column differ [a,b] $P<0.05$; [x,y,z] $P<0.02$

There were no breed differences for anterior pituitary gland weights (Table 11); however, anterior pituitary gland weight (BW basis; mg/kg BW) was significantly ($P<0.0002$) influenced by breed. Romosinuano bulls had a heavier ($P<0.02$) anterior pituitary gland on a BW basis than Angus bulls which were heavier ($P<0.02$) than Brahman bulls (Table 11).

Table 11. Anterior pituitary gland weights and anterior pituitary gland weight on a BW basis (mg/kg BW; LS mean ± SE) for Angus, Brahman, and Romosinuano bulls 69-111 d following sexual maturity

Breed	Anterior pituitary weight (g)	Anterior pituitary weight (mg/kg BW)
Romosinuano	1.56±0.09	4.05±0.17[a]
Angus	1.43±0.09	3.45±0.17[b]
Brahman	1.33±0.10	2.53±0.19[c]

Different superscripts within a column differ [a,b] $P<0.02$

Paired adrenal gland weights and paired adrenal gland weight on a BW basis were significantly (P<0.02) influenced by breed. Angus had heavier (P<0.02) paired adrenal gland weights than Romosinuano and Brahman bulls which were similar (Table 12). However, on a BW basis, Angus and Romosinuano had heavier (P<0.0002) paired adrenal gland weights than Brahman bulls (Table 12).

Table 12. Paired adrenal gland weights and paired adrenal gland weight on a BW basis (mg/kg BW; LS mean ± SE) for Angus, Brahman, and Romosinuano bulls 69-111 d following sexual maturity

Breed	Paired adrenal weight (g)	Paired adrenal weight (mg/kg BW)
Angus	17.35 ± 0.67^{a}	42.10 ± 1.54^{x}
Romosinuano	14.86 ± 0.67^{b}	38.79 ± 1.54^{x}
Brahman	14.82 ± 0.75^{b}	28.25 ± 1.72^{y}

Different superscripts within a column differ ^{a,b} P<0.02, ^{x,y} P<0.0002

There were no breed differences in liver weights (Table 13); however, liverweight on a BW basis was significantly (P<0.0005) influenced by breed. Romosinuano and Angus bulls had heavier (P<0.005) liver weights on a BW basis than Brahman bulls (Table 13).

Table 13. Liver weights and liver weight on a BW basis (g/kg BW; LS mean ± SE) for Angus, Brahman, and Romosinuano bulls 69-111 d following sexual maturity

Breed	Liver weight (kg)	Liver weight (g/kg BW)
Romosinuano	5.4±0.3	13.9±0.5[a]
Angus	5.3±0.3	12.8±0.5[a]
Brahman	5.6±0.3	10.6±0.5[b]

Different superscripts within a column differ P<0.005

Actual spleen weights as well as spleen weight per unit BW basis were significantly (P<0.01) influenced by breed. Brahman and Romosinuano had heavier (P<0.003) spleen weights than Angus bulls (Table 14). Romosinuano had heavier (P<0.006) spleen weights on a BW basis than Angus and Brahman bulls (Table 14).

Table 14. Spleen weights and spleen weight on a BW basis (g/kg BW; LS mean ± SE) for Angus, Brahman, and Romosinuano bulls 69-111 d following sexual maturity

Breed	Spleen weight (kg)	Spleen weight (g/kg BW)
Angus	0.78±0.04[b]	1.90±0.08[y]
Brahman	0.98±0.04[a]	1.89±0.09[y]
Romosinuano	0.89±0.04[a]	2.28±0.08[x]

Different superscripts within a column differ [a,b] P< 0.003; [x,y] P<0.006

A significant (P<0.03) breed influence was detected for heart weight, but not for heart weight on a BW basis (Table 15). Brahman and Angus bulls had heavier (P<0.03) actual heart weights than Romosinuano bulls (Table 15).

Table 15. Heart weights and heart weight on a BW basis (g/kg BW; LS mean ± SE) for Angus, Brahman, and Romosinuano bulls 69-111 d following sexual maturity

Breed	Heart weight (kg)	Heart weight (g/kg BW)
Angus	1.7±0.1[a]	6.4±1.5
Brahman	1.7±0.1[a]	3.2±1.7
Romosinuano	1.4±0.1[b]	3.7±1.5

Different superscripts within a column differ [a,b] P<0.03

There was no breed influence detected for lung weight (including the trachea, Table 16); however, lung weight (including the Trachea) on BW basis was significantly (P<0.005) influenced by breed. Romosinuano and Angus had heavier (P<0.04) lung weights on a BW basis than Brahman bulls (Table 16).

Table 16. Lung weights and lung weight on a BW basis (g/kg BW; LS mean ± SE) for Angus, Brahman, and Romosinuano bulls 69-111 d following sexual maturity

Breed	Lung weight (kg)	Lung weight (g/kg BW)
Romosinuano	5.2±0.2	13.57±0.43[a]
Angus	5.2±0.2	12.65±0.43[a]
Brahman	5.9±0.2	11.20±0.48[b]

Different superscripts within a column differ [a,b] P<0.04

Adrenal StAR and P450 Content and Adrenal Morphometrics

Adrenal StAR content was significantly (P<0.03) influenced by breed. Angus had a greater (P<0.007) adrenal StAR content than Brahman with Romosinuano bulls being intermediate (Table 17). Breed differences were not detected for adrenal P450

content (Table 17). A western blot of the adrenal StAR protein and the P450 scc enzyme with three bulls representing each breed is shown in Figure 4.

Table 17. Adrenal StAR protein and P450 scc enzyme content (LS mean ± SE) for Angus, Brahman, and Romosinuano bulls 69-111 d following sexual maturity

Breed	Adrenal StAR content (IOD units)	Adrenal P450 content (IOD units)
Angus	0.223±0.010[a]	0.128±0.01
Romosinuano	0.199±0.010[a,b]	0.139±0.01
Brahman	0.177±0.012[b]	0.141±0.01

Different superscripts within a column differ P<0.007

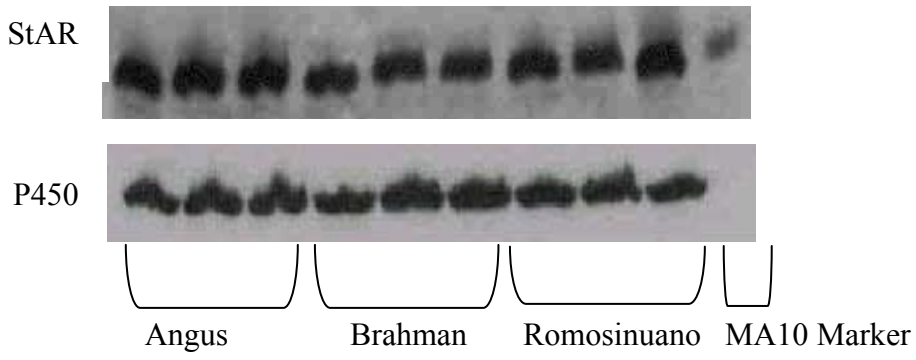

Figure 4. Western blots of adrenal StAR protein and P450 scc for Angus, Brahman, and Romosinuano bulls 69-111 d following sexual maturity.

Total adrenal gland area, adrenal medullary area and adrenal cortical area were significantly ($P<0.05$) influenced by breed. Angus bulls had a greater total adrenal area ($P<0.02$), adrenal medullary area ($P<0.06$) and adrenal cortical area ($P<0.02$) than Brahman and Romosinuano bulls (Table 18).

Table 18. Total adrenal area (LS mean ± SE) for Angus, Brahman, and Romosinuano bulls 69-111 d following sexual maturity

Breed	Total adrenal area (µm)	Medullary adrenal area (µm)	Cortical adrenal area (µm)
Angus	82.00 ± 3.36^{a}	25.29 ± 1.16^{m}	56.32 ± 2.66^{x}
Brahman	68.51 ± 3.75^{b}	21.90 ± 1.29^{n}	46.11 ± 2.97^{y}
Romosinuano	65.51 ± 3.36^{b}	20.49 ± 1.16^{n}	44.49 ± 2.66^{y}

Different superscripts within a column differ ^{a,b} $P<0.002$, ^{m,n} $P<0.06$ and ^{x,y} $P<0.02$.
Note: Adrenal artery area was subtracted from the total adrenal area and medullary area. The adrenal cortical area was calculated as follows: Adrenal Cortical Area = Total Area – (Medullary Area + Adrenal Artery Area).

Discussion

The intent of these experiments was to determine if tropical adaptation influences adrenal function in bulls and to evaluate how temperate *Bos taurus,* tropically-adapted *Bos indicus*, tropically-adapted Sanga and tropically-adapted *Bos taurus* bulls respond to two different types of common management stressors and to determine if organ weights of tropically-adapted *Bos taurus* are more similar to temperate *Bos taurus* or tropically-adapted *Bos indicus* bulls. Consequently, various breeds were utilized to determine if differences exist among different biotypes. Results from experiment # 1 indicate that breed differences do exist in response to different types of management stressors;

however, tropical adaptation was not influenced by these management stressors. The response to two different management stressors was similar for each individual breed; however, not all breeds responded in exactly the same fashion. Animals respond to stressors by stimulating the hypothalamic-pituitary-adrenal axis. In this axis, stress stimulates CRH released from the hypothalamus which acts at the corticotropes of the anterior pituitary to synthesize and secrete ACTH. ACTH then enters systemic circulation and acts at the adrenal gland to release the glucocorticoids, cortisol and corticosterone. Results from experiment # 2 indicate that the weights of organs and adrenal StAR content of tropically-adapted *Bos taurus* bulls tend to be more similar to that of temperate *Bos taurus* than that of tropically-adapted *Bos indicus* bulls; however, paired adrenal weight (actual weight), total adrenal area, medullary and cortical area were similar to that of tropically-adapted *Bos indicus* bulls and thus these parameters were influenced by tropical adaptation.

The reasons why breeds of cattle respond differently are not completely understood, although genetics may potentially be an underlying factor. In the present study, only cortisol, and not ACTH or CRH, was assayed in bull plasma. Consequently, it is unknown as to where in the HPA Axis the differences in cortisol concentrations originate, although central and/or peripheral mechanisms may contribute to this difference. Hypo-or hyperreactivity of the HPA axis may be a result of factors such as negative feedback efficiency, concentrations of ACTH secretagogues in the median eminence, differences in pituitary sensitivity to CRH and/or adrenal sensitivity to ACTH

or factors associated with receptor dynamics as well as availability of steroidogenic enzymes.

Although the HPA axis is similar in all animals, genetic differences have been reported at all levels within the HPA axis. At the level of the hypothalamus, Lewis rats have a significantly smaller CRH response to stress than that of the Fischer rats (Sternberg et al., 1992). Additionally, CRH mRNA significantly increased following a restraint stress in CFY rats compared to Sprague-Dawley and Wistar rats (Harbuz et al., 1994). Bonsmara X Angus steers had lower amplitude of cortisol following CRH administration than Angus, Bonsmara and Brahman steers; however Angus steers had a greater peak plasma cortisol than Bonsmara and Bonsmara X Angus steers, but not Brahman steers (Hollenbeck et al., 2003). Theses authors also reported Angus and Bonsmara X Angus steers displayed a more rapid cortisol peak response to CRH than Brahman or Bonsmara steers and plasma cortisol returned to basal concentrations more slowly in Bonsmara steers than Angus, Brahman or Bonsmara X Angus steers. ACTH concentrations have been reported to be lower in Lewis rats following a restraint stress than in Fischer rats (Moncek et al., 2001) and Sprague-Dawley rats (Dhabhar et al., 1997). ACTH and cortisol concentrations have been reported to be higher in 3/4 Angus steers compared to 3/4 Brahman steers (Bruner et al., 1996). However, in the present study no differences in cortisol concentrations between Angus and Brahman were detected. Selection plays a key role in the genetic differences of the HPA.

Animals that handle stress well and have good temperaments have better growth traits and may have more efficient immune systems. Baker et al. (2003) reported

weaned calves that had a bad temperament and a fast "exit velocity" from the chute lost an average of 11 pounds in the first 50 d after weaning while weaned calves that had a good temperament and a slow exit velocity gained an average of 30 pounds in the 50 d following weaning. This could be important in selecting cattle that can be efficient performers in the stocker and feedlot segments of the beef industry.

Transportation of livestock not only results in stimulation of the HPA axis, but also stimulates shrinkage of live weight. Cattle that are transported typically encounter a loss in body weight (shrink) that may be due to long trips without feed and water, excessive heat as well as increased urination and defecation during transport. Shrinkage of live weight is important in the beef industry as cattle are transported to slaughter facilities. Shrinkage in live weight results in lighter animals at slaughter and can potentially result in reduced profit. Additional body weight shrinkage can occur at the slaughter facility when cattle are co-mingled without feed and water and penned together. Kreikemeier et al. (1998) reported that 36 to 84 h may pass from the time animals arrive at the slaughter facility until they are slaughtered and that many slaughter facilities do not provide feed or water to the cattle unless it is expected to be longer than 36 h before the cattle are slaughtered.

The percent shrink in body weight detected in the bulls in the present study was considerably lower than that reported in other cattle of various ages (Lay et al., 1996; Zavy et al., 1992 and Warriss et al., 1995). A 4% shrink in bodyweight was reported in pregnant Brahman cows which had been transported (Lay et al., 1996). Warriss et al. (1995) reported a 4.6-7.0% shrink in 12-18 mo old Hereford X Friesian steers and

Limousin cross steers which were transported 5, 10, or 15 h. British cross and British X Brahman weaned steers had an 8.1% shrink in body weight (Zavy et al., 1992). It is unknown why the bulls in the present study had lower percent shrink in body weight but it may possibly be a result of a shorter duration of transportation as well as cooler temperatures at the beginning of the experiment. Additionally, these cool temperatures lasted for a longer period of time than normal for the geographic region. The bulls in the present study had access to ad libitum hay and water until immediately prior to transportation. Shrink in bodyweight, following transportation, was not influenced by tropical adaptation.

Transportation is a stressor that typically elicits a stress response. This stress response from transportation, like that of body weight shrinkage, has a commercial component as well. Preharvest stress from transportation and coupled with the additional stress of co-mingling cattle can result in dark cutters in animals that do not handle stress well (Scanga et al., 1998). The preharvest stress depletes muscle glycogen stores and thus reduces the glycogen needed to produce lactic acid that reduces the pH of postmortem muscle which consequently results in an undesirable, dark, firm and dry cut lean surface (McVeigh and Tarrant, 1982).

In the present study, Angus, Brahman, Romosinuano, Tuli and Wagyu bulls all either doubled or almost doubled the concentrations of plasma cortisol from those prior to transportation. A similar doubling response in plasma cortisol concentrations was reported in steers transported for five h (Warriss et al., 1995); however, the plasma cortisol concentrations in these steers following transportation was much lower than that

detected in the bulls in the present study. It was surprising that the Bonsmara bulls in the present study actually had a lower plasma cortisol concentration following transportation compared to the plasma cortisol concentrations prior to transportation. Blecha et al. (1984) reported similar findings in that Angus feeder steers also had decreased cortisol concentrations following 10 h of transportation. These authors suggest that it was likely that this was due to the animals acclimating to the trailer. Warriss et al. (1995) reported that cattle transported 10 or 15 h had an increase in plasma cortisol concentrations but the increases were slight and decreased with longer duration of transportation. Knights and Smith (2003) reported that transportation stress results in an increase followed by a gradual suppression of ACTH secreted by the anterior pituitary accompanied by a decrease in anterior pituitary responsiveness to CRH and arginine vasopressin stimulation. In the present study, bulls were transported for only 30 min. It is possible that the Bonsmara bulls may have acclimated to transportation; however, the likelihood of acclimation occurring during a short trip is not likely.

Cannulation is a procedure that is not commonly performed during management in beef cattle operations; however, it is an important and a necessary technique utilized for research purposes. The technique of cannulation also served as an additional handling procedure. Little research has evaluated the technique of jugular cannulation as a potential stressor to cattle. Angus, Brahman, Romosinuano and Tuli bulls had similar concentrations of plasma cortisol following cannulation to the plasma cortisol concentrations following transportation. Bonsmara bulls doubled the plasma cortisol concentrations from post-transportation to post-cannulation and had cortisol

concentrations similar to that prior to transportation. The Wagyu bulls; however, had a decrease of 50% in plasma cortisol concentrations following cannulation compared to that following transportation. These results clearly indicate that the cannulation procedure may elicit a response similar to that of transportation. It is unclear if the stress response resulted from the cannulation itself or possibly the handling of the area around the neck and head.

Restraint, unlike jugular cannulation, is a management practice that is commonly utilized for multiple reasons in beef production systems. In the present study, differences in average and maximum plasma cortisol concentrations were only detected during the first h of restraint. The maximum plasma cortisol concentration during the six h restraint period was detected in the first h of restraint for all breeds of bulls. The maximum plasma cortisol concentration detected was similar to the concentrations of plasma cortisol following transportation for all breeds of bulls except for the Bonsmara bulls which had nearly a 5-fold higher plasma cortisol concentration during the first h of restraint compared to post-transportation cortisol concentrations. However, the average cortisol concentrations for Bonsmara bulls were similar to that reported by Hollenbeck et al. (2003) in Bonsmara steers in the 2 h duration prior to CRH administration. A possible explanation for the maximum cortisol concentration occurring during the first h of restraint is that the bulls were gathered from pens, placed in a chute alley, extensions were placed on cannulas and cannulas were checked to make sure they worked adequately. The restraint and blood sampling period began after all cannulas for a set of bulls were connected and working properly. Following the first h of restraint, plasma

cortisol concentrations gradually decreased for approximately three h, plateaued for one h and then slightly increased during the last h of restraint. The decreased cortisol concentrations were likely due to the bulls becoming acclimated to the restraint as well as to the blood collection. The slight increase of plasma cortisol concentrations between the fifth and sixth h of restraint may be due to fatigue encountered by the bulls. The bulls also appeared to be uncomfortable at this time since a constant switching from standing to lying down and vice versa occurred with the bulls. The low responses of the Bonsmara and Wagyu bulls to transportation, cannulation and restraint are likely a result of selection for docile cattle which occurred over many years and generations (Bonsma 1951 and Felius, 1995). Hollenbeck et al. (2003) reported that relative to other breedtypes (Angus and Brahman), the Bonsmara and Bonsmara X Angus steers maintained lower plasma cortisol throughout the 2 h prior to and 2 h immediately following CRH administration.

Experiment # 2 was conducted to evaluate if organ weights, adrenal StAR protein and P450 scc enzyme content, total adrenal area, medullary area and cortical area of tropically-adapted *Bos taurus* bulls is more similar to that of temperate *Bos taurus* bulls or tropically-adapted *Bos indicus* bulls. Since sexual maturity occurs later in life in Brahman cattle, Brahman bulls were older and thus had a heavier weight at slaughter as well as a heavier hot carcass weight than Angus and Romosinuano bulls. Brahman bulls also had a greater dressing percentage. This was similar to the trend reported in the study conducted by Chase et al. (2001). They noted that Brahman X Angus bulls had greater hot carcass weights (271 kg) and dressing percentages (55.1 %) than Senepol X

Angus bulls (225 kg, 52.8 %, respectively) or Tuli X Angus bulls (225 kg, 52.7 %, respectively); however, these values were slightly lower than those reported in the present study. Slaughter characteristics including slaughter weight, hot carcass weight and dressing percentage were not influenced by tropical adaptation.

When obtaining organ weights from animals of different breeds, it is important to consider BW and put the actual weights on a BW basis since some animals may be older and heavier at similar physiological endpoints. This allows animals of different weights and sizes to be compared on an equal basis. The whole pituitary gland weight reported for Romosinuano bulls in the present study was similar to those reported in British X Continental bulls and steers of slaughter weight (Doornenbal, 1974). The heavier anterior pituitary gland weight reported for Angus compared to Brahman bulls in the present study was similar to the findings reported in ¾ Angus and ¾ Brahman steers (Carroll et al., 1996; Bruner et al., 1996). A larger anterior pituitary gland may result in the ability to produce and secrete more ACTH as seen in higher concentrations of ACTH in 3/4 Angus compared to 3/4 Brahman steers as reported by Bruner et al. (1996).

Paired adrenal gland weight is influenced by tropical adaptation. Paired adrenal glands were reported to be heavier in ¾ Angus compared to ¾ Brahman steers (Bruner et al., 1996). These are similar to trends reported in the present study with Romosinuano bulls having similar paired adrenal gland weights to that of Brahman bulls. The paired adrenal gland weights reported for bulls in the present study were slightly lower than those reported in ¾ Angus and ¾ Brahman steers (Bruner et al., 1996) but similar to those reported in British X Continental bulls and steers (Doornenbal, 1974). Large

adrenal glands may result in greater sensitivity to ACTH; however, this is beyond the scope of this research and further research is required. Bruner et al. (1996) reported that 3/4 Angus steers had higher concentrations of cortisol compared to 3/4 Brahman steers.

The rate-limiting step in steroidogenesis is the transfer of cholesterol from the outer to the inner mitochondrial membrane by the StAR protein (Stocco and Clark, 1996). Differences in StAR content among breeds may result in differences in the ability of an animal to produce glucocorticoids. No differences in adrenal StAR content were detected in male or female fetal Angus and Brahman calves (Green, 1999). Although differences in adrenal StAR content were detected among breeds of bulls in the present study, no differences were detected in concentrations of plasma cortisol at slaughter or adrenal P450 scc content among the breeds of bulls. Similarly, no differences in adrenal P450 scc were detected between fetal Angus and Brahman calves (Green, 1999). One might expect for P450 scc content to parallel StAR content as it follows next in the process of steroidogenesis. A possible explanation that P450 scc does not parallel StAR protein is the way in which they may be regulated. In the rat ovary, following stimulation by PMSG and hCG, StAR was reported to be acutely regulated while P450 scc was reported to be chronically regulated (Sandhoff and McLean, 1996). Cholesterol concentrations were reported to be higher in tropically-adapted calves compared to temperate calves (O'Kelley and Wallace, 1979). This suggests that tropically-adapted cattle may not need as much StAR to move cholesterol since more cholesterol is readily available.

Total adrenal area, medullary area and cortical area for Angus and Brahman bulls were similar to that reported by Bruner et al. (1996) in 3/4 Angus and 3/4 Brahman steers. This is likely a direct result of the size of the adrenal glands. The larger adrenal glands would be expected to have larger total, medullary and cortical areas. The heavier and larger adrenal gland may possibly have the potential to produce and secrete greater amounts of cortisol. Koch et al. (2000) reported that the proportional response of concentrations of serum cortisol immediately prior to and 30 min following administration of ACTH was greater for *Bos taurus* (Angus) steers compared to ½ *Bos taurus* – ½ *Bos indicus* and *Bos indicus* (Brahman) steers but not ¾ *Bos taurus* – ¼ *Bos indicus* steers. Bruner et al. (1996) reported that ¾ Angus had higher mean concentrations of plasma cortisol than ¾ Brahman steers. However, results from experiment # 1 of the present study show no differences in plasma cortisol concentrations between Angus and Brahman bulls.

Various organs, including the liver, spleen, heart and lungs (including the trachea) were weighed to determine if tropically-adapted *Bos taurus* bulls are more similar to temperate *Bos taurus* bulls or to tropically-adapted *Bos indicus*. The actual weights of the heart and liver in the present study are approximately 33% lower than those reported in 13 mo old Simmental bulls which were produced in vitro (McEvoy et al., 1998). Cattle produced in vitro usually have larger organs than cattle produced in vivo. The heavier organ weights (corrected for BW) from Romosinuano bulls may possibly be similar to a heterosis effect where crossbred calves have greater body weights than the purebred counterparts. The Romosinuano bulls may have a similar

phenomenon to that of heterosis since they are tropically-adapted yet they are also *Bos taurus* cattle. It is possible that the larger organs may be advantageous to Romosinuano cattle in handling extreme heat and thermal stress via respiration and heart rate.

Conclusion

It is concluded that various breeds of bulls respond differently to management stressors, but tropical adaptation does not influence these management stressors. However, bulls within a breed had similar responses to both transportation and restraint. Consequently, either transportation or restraint can be utilized to rank cattle as high, intermediate or low responders. This can be important in the selection of animals.

Most organ and gland weights corrected for BW are not influenced by tropical adaptation; however, actual paired adrenal gland weight, total adrenal area, medullary and cortical areas of tropically-adapted *Bos taurus* are more similar to those of tropically-adapted *Bos indicus* bulls and consequently are influenced by tropical adaptation. Adrenal StAR content was not influenced by tropical adaptation. Tropically-adapted *Bos taurus* breeds may offer a unique alternative to beef crossbreeding systems. It appears that heredity may play an important role in controlling the mechanisms that lead to the physiological response to stress. Also, genotype may affect an animals' resistance and/or susceptibility to stressors

CHAPTER IV

BASAL AND INDUCED TESTOSTERONE CONCENTRATIONS, REPRODUCTIVE ORGAN WEIGHTS, TESTIS STEROIDOGENIC ACUTE REGULATORY PROTEIN CONTENT, AND TESTIS AND EPIDIDYMAL SPERM CONCENTRATIONS OF TROPICALLY-ADAPTED AND TEMPERATE *Bos taurus* AND TROPICALLY-ADAPTED *Bos indicus* BULLS

Introduction

Reproduction is critical and essential in beef production systems. Functions of the reproductive system depend on the hypothalamic-pituitary-gonadal (HPG) axis (Senger, 1997). The hypothalamus synthesizes and secretes gonadotropin-releasing hormone (GnRH) which acts at the gonadotrophs of the anterior pituitary to secrete the gonadotropins, luteinizing hormone (LH) (Mongkonpunya et al., 1974) and follicle-stimulating hormone (FSH) (Amann, 1983). The gonadotropins enter the circulation and act on the gonads to regulate steroidogenesis and spermatogenesis. LH acts at the Leydig cells in the testis to produce testosterone (Amann, 1983). In bulls, LH and testosterone are released in a pulsatile fashion with testosterone concentrations increasing approximately 30 min following increased LH concentrations (Amann, 1983).

Testosterone is involved in spermatogenesis as well as the development of accessory sex glands and secondary sex characteristics. Exogenous hormones such as human chorionic gonadotropin (hCG; Sundby et al., 1975) and GnRH (Mongkonpunya et al., 1975) have been shown to induce testosterone secretion in bulls. Inducing testosterone secretion in this manner allows for testosterone production to be measured

in different breeds of bulls. Breed can have an affect on testosterone concentrations. Godfrey et al. (1990) reported lower basal concentrations of testosterone in Brahman bulls compared Hereford bulls.

Determining testicular and epididymal sperm content enables daily spermatozoa production (DSP) to be calculated as well as an understanding of the capacity of spermatozoa storage in the epididymus (Amann and Almquist, 1961). A difference in DSP has been reported in Angus, Hereford and Charolais bulls (Weisgold and Almquist, 1979). Knowledge of differences in DSP among various breeds could be important in selecting the best bull for a particular system. A better understanding of reproductive traits in tropically-adapted *Bos taurus* bulls is necessary to determine how they would best fit into crossbreeding programs. Therefore, the objectives of this study are to 1) determine if differences exist in basal and hCG-induced concentrations of testosterone among tropically-adapted *Bos taurus*, Sanga, temperate *Bos taurus* and tropically-adapted *Bos indicus* bulls and 2) determine if the weights of reproductive organs, testis and epididymal sperm concentrations and content of testis StAR protein and P450 scc enzyme of tropically-adapted *Bos taurus* bulls is more similar to that of temperate *Bos taurus* bulls or tropically-adapted *Bos indicus* bulls.

Materials and Methods

Experiment 1

Animals

Bulls of six different breeds, Bonsmara (n = 8), Romosinuano (n = 10), Tuli (n = 10), Brahman (n = 8), Angus (n = 7) and Wagyu (n = 10), were utilized for this experiment. Bulls arrived at the Texas Agricultural Experiment Station in Overton, Texas following weaning. Upon arrival, bulls were pastured together and managed similarly during a 5 mo acclimation period. There were several important cooperators that generously supplied bulls for this study. The cooperators were Dr. Chad Chase at the Subtropical Agricultural Research Station (Angus and Romosinuano bulls; Brooksville, FL.), the Texas Agricultural Experiment Station (Brahman bulls; Overton, TX.), Mr. George Chapman (Bonsmara bulls; Amarillo, TX.), and Mr. Kent Briggs (Tuli and Wagyu bulls; Rice, TX.).

After reaching sexual maturity (defined as two consecutive semen samples collected via electroejaculation every other week containing at least 500 million sperm per ejaculate as well as at least 50 % motility of viable spermatozoa), bulls were then fitted with an indwelling jugular catheter (o.d. = 1.7 mm, i.d. = 1.1 mm; Teflon, TFE, Cole-Parmer, Chicago, IL) as described by Lay et al. (1996). Bulls were confined together and allowed to rest overnight before the serial blood samples were collected.

Blood samples were collected every 15 min for the first seven h and every 30 min for the next five h. At the end of the first six h, human chorionic gonadotropin (hCG, Chorulon, Intervet, Millsboro, DE) was administered (1000 IU total; 10 ml

consisting of 100 IU/ml) via the indwelling jugular catheter. The blood samples were placed on ice and centrifuged within 30 min of collection. Plasma was harvested and stored at -20°C until radioimmunoassays can be performed. Area under the curve was calculated for testosterone using the trapezoidal rule.

Hormone Radioimmunoassay Procedure

Plasma harvested from blood samples collected during the first six h (prior to hCG administration) and after hCG administration were analyzed via radioimmunoassay to determine basal concentrations of testosterone as described by Godfrey et al. (1990) (see Appendix 1 for complete description). Antiserum (11-BSA # S – 250) was purchased from Dr. G. D. Niswender (Colorado State University, Fort Collins, Colorado) and tritiated testosterone was purchased from New England Nuclear, Inc. (Boston, MA). The cross-reactivity of the antiserum is approximately 0.01 %, 0.01 %, 0.11 % and 1.7 % with pregnenolone, progesterone, estradiol and androstenedione, respectively. The testosterone was purchased from Steraloids (Wilton, NH) and standards were made which had a concentration ranging from 3.9 pg/ml to 16,000 pg/ml. The intraasssay coefficient of variation was 9.1 % and the interassay coefficient of variation was 11.3 %. The extraction efficiency was 84.12 %.

Statistical Analysis, Experiment # 1

The plasma concentrations of testosterone (basal) were analyzed using ANOVA specific for repeated measures procedures of SAS (1992). Average, lowest (prior to hCG administration), highest concentration of testosterone and area under the curve (prior to and following hCG administration) were analyzed using the GLM procedure for

analysis of variance (SAS, 1992). Mean separation was accomplished using the PDIFF option of SAS (1992).

Experiment # 2

Animals

Angus (n = 7), Brahman (n = 8) and Romosinuano (n = 10) bulls were fed an 8.85:1.0:0.15 corn:soybean meal:limestone ration for a minimum of 69 d (range was 69 – 111 d) following the serial blood collection. The range in time on feed existed due date availability to slaughter at the slaughter facility. These bulls were penned together and given Coastal bermuda grass hay and water ad libitum. Following the feeding period, the bulls were transported to the Texas A&M University Rosenthal Meat Science and Technology Center where they were processed for food utilization. Every breed was represented at each slaughter date. The dates of slaughter, number of head slaughtered and the average daily temperature at slaughter are included in Table 1.

Tissue Collection

Upon slaughter, testicles (including the epididymus), seminal vesicles and whole pituitaries were collected and stored on ice until weights could be obtained. The testes and epididymus were cleaned of adherent tissue, the epididymus was dissected from the testis and weights (to nearest 0.01 g) were obtained for both the testis and epididymus. A tissue slice from the right testis was fixed in 4 % paraformaldehyde and used for histology to measure seminiferous tubule diameter. A piece of tissue (approximately 0.2 g) was cut from the testis, snap frozen in liquid nitrogen, and stored at -80°C until analysis for StAR protein and P450 side – chain cleavage enzyme protein content could

be performed. The right testis and right epididymus were frozen at -20° until testis sperm concentration and epididymal sperm concentrations from the caput, corpus, and caudal sections of the epididymus could be determined. Pituitary glands were cleaned of adherent tissue, weighed (to nearest 0.01 g) and the anterior pituitary was dissected from the whole pituitary and weighed (to nearest 0.01 g).

Testis and Epididymal Sperm Concentrations

Concentrations of sperm from the right testis and right epididymus were determined using procedures described by Amann and Almquist (1961). Testis and epididymal tissues were removed from the freezer and thawed at room temperature. A piece of testicular parenchyma weighing 1.5 to 1.7 g was obtained. The tissue was placed in a plastic weigh boat and minced thoroughly using a scalpel followed by scissors. The minced tissue was then diluted in 150 ml of homogenization fluid (See Appendix 3 for complete description) and placed in a Waring blender (Model: #7009; New Hartford, CT) and blended for 2 min. The epididymus was separated into three sections, the caput, corpus and cauda, and homogenized separately in 150, 150 and 200 ml of homogenization fluid, respectively. Each section was minced as previously described and homogenized for 2 min and 15 sec. Following homogenization, the fluid was poured into a 250 - ml Erlenmeyer flask, sealed with parafilm and refrigerated for 24 h at 4°C. Following refrigeration, the flasks were shaken vigorously and a sample was removed using a glass Pasteur pipette and one side of the hemacytometer chamber was filled. The process was repeated for filling the other side of the hemacytometer chamber. The hemacytometer was then placed within a humidified petri dish for 2 to 3

min. An inverted phase microscope (Model # IMT-2 Olympus Optical Co., LTD, Tokyo, Japan) was utilized to count sperm cells at 400X. The spermatids counted from the testis included all homogenization resistant sperm nuclei, which for the bull includes stages VI, VII and VIII of the cycle of the seminiferous epithelium. The spermatids counted from the caput, corpus and cauda epididymus included all sperm present in the hemacytometer grid. All sperm counts (testis and epididymus) were made by the same person. A new sample for both sides of the hemacytometer was utilized if there was greater than 15 % difference noted between counts (each side of the hemacytometer chamber) to insure accuracy of counts. The homogenized sample was diluted 1:6 for caput epididymus and 1:2 for cauda epididymus because the number of spermatids was too great to count. Sperm content of testis (per g and total) and regions of the epididymus were calculated based on tissue weight fluid (dilution if necessary) and spermatid counts. Daily sperm production (DSP) and daily sperm production/gram (DSP/g; efficiency of sperm production) were then calculated by dividing the testis sperm content by 5.32 (Amann, 1970). The number 5.32 represents the number of days for production of spermatozoa represented by the spermatids counted in the testicular homogenate (Amann et al., 1974). To calculate DSP, it was assumed that testicular parenchyma constituted 87.2 % of gross testis weight (Swierstra, 1970).

Testicular Histological Measurements

Sections of the right testis fixed in 4% paraformaldehyde were sectioned at 5 micron intervals and stained with hematoxilin and eosin (H & E; at least 3 sections per slide). The H&E sectioned images were visualized using an Olympus SZH Zoom Stereo

Microscope and acquired by a Sony 960MD 3-CCD color camera attached to the microscope. A PowerMac 8100 computer system using a LG3-8-bit frame grabber (Scion Corp.) which is under the control of the NIH Image Program was used for image acquisition from the Sony color camera. Fifty randomly chosen, circular, seminiferous tubules with lumens were evaluated for each bull. The diameter (micrometers) of the seminiferous tubule and the diameter of the lumen of the seminiferous tubule were measured using NIH Image (Scion Image Software, Scion Corp.). The diameter of each seminiferous tubule and lumen were an average of two perpendicular measurements.

Western Blot Analysis

The western blot analysis was the same as described in Chapter III.

Statistical Analysis, Experiment # 2

Differences in weights of testis, epididymus, seminal vesicles, whole pituitary and anterior pituitary, diameter of seminiferous tubules and lumen, daily sperm production, daily sperm production/gram, sperm concentration in the testis, caput epididymus, corpus epididymus and cauda epididymus and differences in StAR protein and P450 scc enzyme content were determined using the GLM procedure for analysis of variance (SAS, 1992). Mean separation was accomplished using the PDIFF option of SAS (1992).

Results, Experiment # 1

Basal concentrations of testosterone differed ($P<0.04$) between breeds over the 6 h sampling period prior to hCG administration (Figure 5).

There was a significant ($P<0.04$) breed difference for average concentrations of plasma testosterone during the 6 h prior to administration of hCG. Wagyu bulls had greater ($P<0.05$) average plasma testosterone concentrations during the 6 h prior to hCG administration than Tuli, Bonsmara, Angus, Brahman and Romosinuano bulls (Table 19).

There was no significant breed influence ($P > 0.10$) for highest concentrations of plasma testosterone during the 6 h prior to hCG administration; however, Wagyu bulls tended ($P<0.07$) to have higher plasma concentrations of testosterone than did the Romosinuano bulls during this period (Table 19).

No breed differences ($P > 0.10$) were detected for lowest plasma testosterone concentrations during the 6 h period prior to hCG administration (see Table 19).

A significant ($P<0.05$) breed influence was detected for area under the curve for the 6 h prior to hCG administration. Wagyu bulls had greater ($P<0.05$) area under the curve prior to hCG administration than Tuli, Bonsmara, Angus, Brahman and Romosinuano bulls (Table 19).

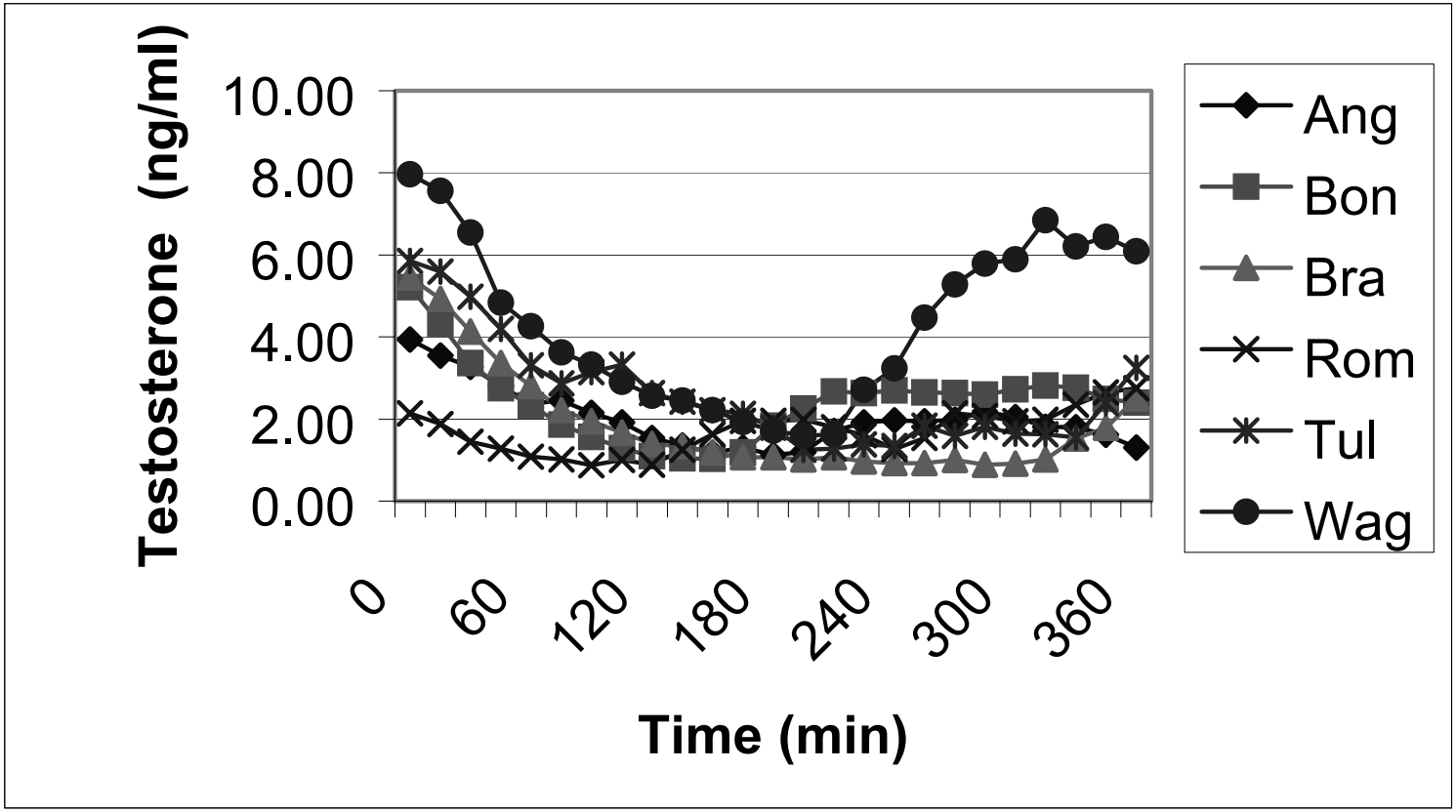

Figure 5. Plasma concentrations of testosterone prior to hCG administration in sexually mature Angus, Bonsmara, Brahman, Romosinuano, Tuli, and Wagyu bulls. Plasma samples taken every 15 min during the 6 h interval to determine testosterone concentrations. Time 0 was the initial sample taken.

Table 19. The range (maximum, average, and lowest) and area under the curve for testosterone concentrations (ng/ml; LS mean ± SE) prior to administration of hCG in sexually mature Angus, Bonsmara, Brahman, Romosinuano, Tuli, and Wagyu bulls

Breed	Mean testosterone concentration (ng/ml)	Highest testosterone concentration (ng/ml)	Lowest testosterone concentration (ng/ml)	Pre area under the curve (arbitrary units)
Wagyu	4.33±0.59 [b]	10.63±1.40	1.24±0.45	1525.9±214.1 [b]
Tuli	2.62±0.59 [a]	7.86±1.40	1.78±0.45	912.7±214.1 [a]
Bonsmara	2.41±0.66 [a]	7.47±1.56	0.71±0.50	865.6±239.4 [a]
Angus	2.02±0.70 [a]	5.16±1.67	0.76±0.53	717.5±256.0 [a]
Brahman	1.88±0.66 [a]	6.68±1.56	0.72±0.50	646.9±239.4 [a]
Romosinuano	1.70±0.59 [a]	4.71±1.40	0.61±0.45	596.8±214.1 [a]

Different superscripts within a column differ $P<0.05$

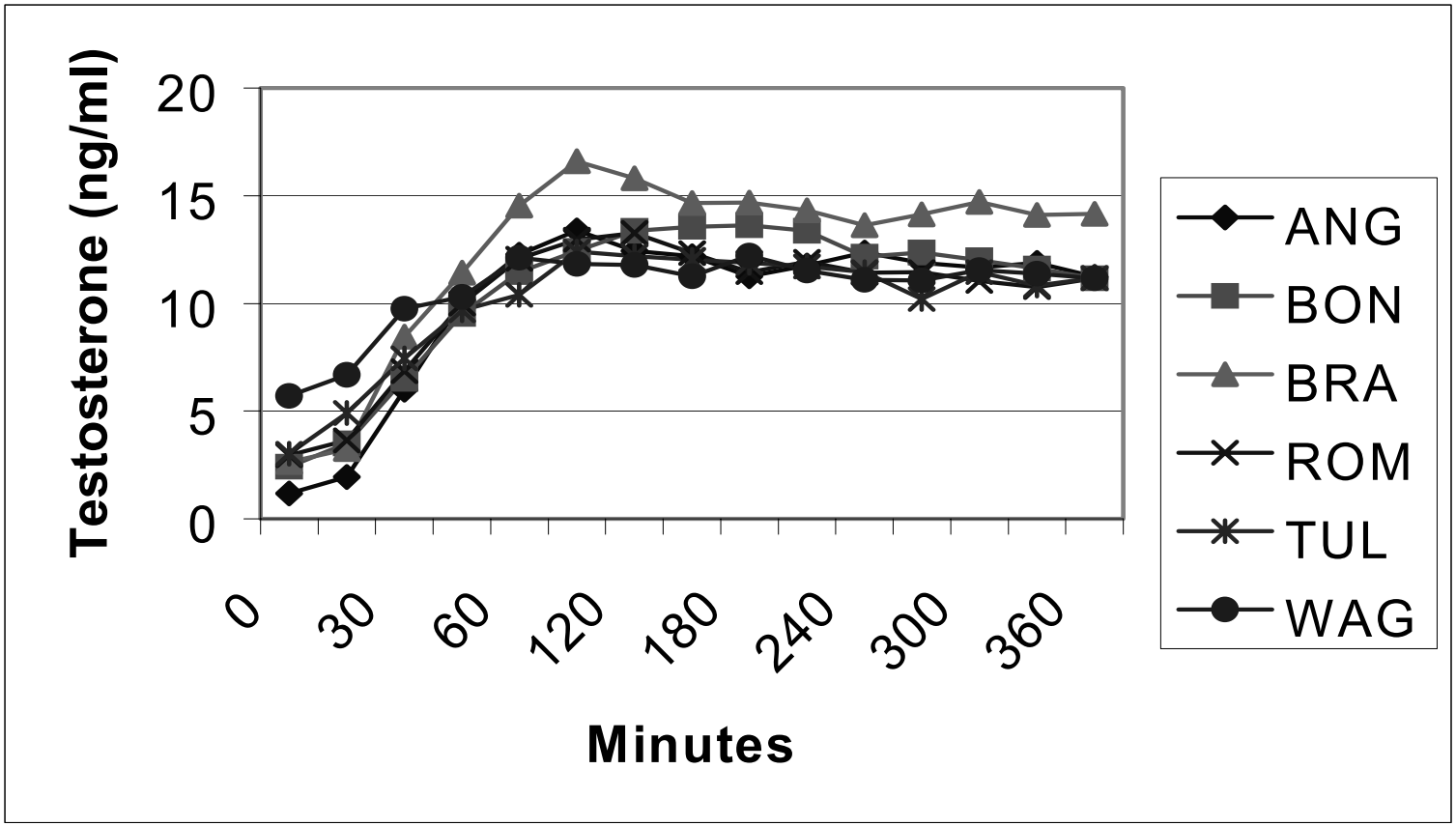

Figure 6. Plasma testosterone concentrations following hCG administration in sexually mature Angus, Bonsmara, Brahman, Romosinuano, Tuli, and Wagyu bulls. Time * breed interaction ($P<0.0002$). Plasma samples taken every 15 min during hour 1 and every 30 min during next 5 h to determine testosterone concentrations following hCG administration. hCG was administered immediately prior to Time 0 which was the initial sample taken.

There was no significant (P>0.10) breed influence over time following hCG administration; however, there was a significant (P<0.0002) time* breed interaction (Figure 6).

The maximum concentration of plasma testosterone as well as area under the curve following hCG administration did not differ (P > 0.10) among breeds (Tables 20).

Table 20. Maximum plasma concentration of testosterone (ng/ml; LS mean ± SE) and area under the curve following administration of hCG in sexually mature Angus, Bonsmara, Brahman, Romosinuano, Tuli, and Wagyu bulls

Breed	Maximum plasma testosterone (ng/ml)	Area under the curve (arbitrary units)
Brahman	17.46±1.14	4888.20±311.92
Bonsmara	15.14±1.14	4179.88±311.92
Angus	14.77±1.22	3997.88±333.46
Romosinuano	14.26±1.02	3961.60±278.99
Tuli	13.81±1.02	3878.85±278.99
Wagyu	13.20±1.02	3990.81±278.99

Results, Experiment # 2

Slaughter Characteristics

A significant (P<0.0002) breed influence was detected for BW at slaughter and hot carcass weight (HCW). Brahman were heavier (P<0.003) at slaughter and had a heavier (P<0.003) hot carcass weight than Angus and Romosinuano bulls (Table 21). There was a significant (P<0.003) breed influence on dressing percent. Brahman and Angus bulls had higher (P<0.05) dressing percentages than Romosinuano bulls (Table 21).

Table 21. Slaughter weight, hot carcass weight, and dressing percent (LS mean ± SE) of Angus, Brahman, and Romosinuano bulls at 69-111 d following sexual maturity

Breed	Slaughter weight (kg)	Hot carcass weight (kg)	Dressing percent (%)
Brahman	527.39±19.04[a]	318.08±13.25[a]	60.25±0.63[x]
Angus	431.37±19.97[b]	254.11±13.93[b]	58.81±0.65[x]
Romosinuano	385.27±17.03[b]	220.55±11.85[b]	57.02±0.57[y]

Different superscripts within a column differ [a,b] P<0.003, [x,y] P<0.05

Tissue Weights

There was a significant breed influence for whole pituitary gland weight (P<0.015) as well as whole pituitary gland weight on BW basis (P<0.0002). Romosinuano and Angus had heavier (P<0.02) whole pituitary gland weight than Brahman bulls (Table 22). On a BW basis, Romosinuano had heavier (P<0.03) whole

pituitary gland weights than Angus which had heavier (P<0.03) pituitary weights than Brahman bulls (Table 22).

Table 22. Whole pituitary gland weights and whole pituitary gland weight on a BW basis (mg/kg BW; LS mean ± SE) for Angus, Brahman, and Romosinuano bulls 69-111 d following sexual maturity

Breed	Whole pituitary gland weight (g)	Whole pituitary gland weight (mg/kg BW)
Romosinuano	2.05 ± 0.09^{a}	5.32 ± 0.17^{x}
Angus	2.02 ± 0.11^{a}	4.70 ± 0.21^{y}
Brahman	1.65 ± 0.10^{b}	3.16 ± 0.19^{z}

Different superscripts within a column differ ^{a,b} P<0.02, ^{x,y,z} P<0.003

A breed difference was not detected (P > 0.10) for anterior pituitary gland weight (Table 23), but anterior pituitary gland weight on a BW basis was significantly influenced (P<0.0002) by breed. Romosinuano had heavier (P<0.04) anterior pituitary glands on a BW basis than Angus which were heavier (P<0.04) than Brahman bulls (Table 23).

Table 23. Anterior pituitary gland weights and anterior pituitary gland weight on a BW basis (mg/kg BW; LS mean ± SE) for Angus, Brahman, and Romosinuano bulls 69-111 d following sexual maturity

Breed	Anterior pituitary gland weight (g)	Anterior pituitary gland weight (mg/kg BW)
Romosinuano	1.56±0.09	4.05±0.17[a]
Angus	1.49±0.11	3.45±0.21[b]
Brahman	1.33±0.10	2.52±0.19[c]

Different superscripts within a column differ [a,b,c] P<0.04

There was significant breed influence for paired testis weights (P<0.0002) and paired testis weight on a BW basis (P<0.024). Brahman had heavier (P<0.0002) paired testis weights and heavier (P<0.02) paired testis weights on a BW basis than Angus and Romosinuano bulls (Table 24).

Table 24. Paired testis weights and paired testis weight on a BW basis (g/kg BW; LS mean ± SE) for Angus, Brahman, and Romosinuano bulls 69-111 d following sexual maturity

Breed	Paired testis weight (g)	Paired testis weight (g/kg BW)
Brahman	315.18±16.96[a]	1.23±0.07[x]
Angus	213.30±18.13[b]	0.97±0.08[y]
Romosinuano	185.20±15.17[b]	0.98±0.06[y]

Different superscripts within a column differ [a,b] P<0.0002, [x,y] P<0.02

A significant (P<0.0002) breed influence was detected for length and diameter of the right testis. Brahman had longer (P<0.0007) testes and larger (P<0.0002) diameter of the testes than Angus and Romosinuano bulls (Table 25).

Table 25. Right testis length and diameter (mm; LS mean ± SE) for Angus, Brahman, and Romosinuano bulls 69-111 d following sexual maturity

Breed	Right testis length (mm)	Right testis diameter (mm)
Brahman	136.01 ± 4.05^{a}	69.43 ± 1.52^{x}
Angus	112.24 ± 4.32^{b}	58.09 ± 1.63^{y}
Romosinuano	108.44 ± 3.62^{b}	57.10 ± 1.36^{y}

Different superscripts within a column differ ^{a,b} P<0.0007, ^{x,y} P<0.0002

A significant breed influence was detected for paired epididymal weights (P<0.0002) and for paired epididymus on a BW basis (P<0.038). Brahman had heavier (P<0.0002) paired epididymal weight and heavier (P<0.04) paired epididymal weight on a BW basis than Angus and Romosinuano bulls (Table 26).

Table 26. Paired epididymal weights and paired epididymal weight on a BW basis (mg/kg BW; LS mean ± SE) for Angus, Brahman, and Romosinuano bulls 69-111 d following sexual maturity

Breed	Paired epididymal weight (g)	Paired epididymal weight (mg/kg BW)
Brahman	57.39±2.49[a]	110.46±5.47[x]
Angus	39.49±2.66[b]	91.07±5.85[y]
Romosinuano	35.57±2.23[b]	92.91±4.90[y]

Different superscripts within a column differ [a,b] P<0.0002, [x,y] P<0.04

Significant breed influences were detected for seminal vesicle weights (P<0.0004) and seminal vesicle weights on a BW basis (P<0.033). Brahman and Angus had heavier (P<0.004) seminal vesicle weights than Romosinuano bulls (Table 27). Angus bulls had heavier (P<0.01) seminal vesicle weights on a BW basis than Romosinuano with Brahman bulls being intermediate (Table 27).

Table 27. Seminal vesicle weights and seminal vesicle weight on a BW basis (mg/kg BW; LS mean ± SE) for Angus, Brahman, and Romosinuano bulls 69-111 d following sexual maturity

Breed	Seminal vesicle weight (g)	Seminal vesicle weight (mg/kg BW)
Angus	69.93±4.61[a]	163.20±8.92[x]
Brahman	76.80±4.31[a]	145.16±8.35[x,y]
Romosinuano	49.84±3.86[b]	130.15±7.47[y]

Different superscripts within a column differ [a,b] P<0.004, [x,y] P<0.01

Seminiferous Tubule Diameter, Testis Sperm Content and Daily Sperm Production

No significant (P<0.08) difference among breeds was detected for seminiferous tubule diameter or diameter of the lumen of the seminiferous tubule (Table 28).

Table 28. Seminiferous tubule diameter (μm) and seminiferous tubule lumen diameter (μm; LS mean ± SE) for Angus, Brahman, and Romosinuano bulls 69-111 d following sexual maturity

Breed	Seminiferous tubule diameter (μm)	Seminiferous tubule lumen diameter (μm)
Angus	215.43±5.79	88.91±3.87
Romosinuano	207.32±4.49	79.28±3.00
Brahman	197.21±5.02	76.89±3.35

Testis sperm content per g and total testis sperm content were significantly (P<0.02) influenced by breed. Romosinuano had greater (P<0.006) testis sperm content per g of tissue than Angus with Brahman bulls being intermediate (Table 29). Brahman had greater (P<0.009) total testis sperm content than Romosinuano and Angus bulls (Table 29).

Daily sperm production per g (DSP/g) of tissue and total daily sperm production (DSP) were significantly (P<0.02, P<0.002, respectively) influenced by breed. Romosinuano had greater (P<0.006) DSP/g than Angus with Brahman bulls being intermediate (Table 29). Brahman had greater (P<0.009) DSP than either Romosinuano or Angus bulls (Table 29).

Table 29. Testis sperm content per g of tissue, daily sperm production per g of tissue, total testis sperm content, and daily sperm production (LS mean ± SE) for Angus, Brahman, and Romosinuano bulls 69-111 d following sexual maturity

Breed	Testis sperm content per g of tissue (10^6)	Daily sperm production per g of tissue (10^6)	Total testis sperm content (10^9)	Daily sperm production (10^9)
Angus	56.86 ± 4.37^{b}	10.69 ± 0.82^{b}	20.61 ± 3.88^{y}	3.87 ± 0.73^{y}
Brahman	$64.61 \pm 3.78^{a,b}$	$12.15 \pm 0.71^{a,b}$	41.63 ± 3.36^{x}	7.82 ± 0.63^{x}
Romosinuano	73.80 ± 3.38^{a}	13.87 ± 0.64^{a}	28.48 ± 3.00^{y}	5.35 ± 0.56^{y}

Different superscripts within a column differ ^{a,b} $P<0.006$, ^{x,y} $P<0.009$

Caput, Corpus, and Cauda Epididymal Sperm Content

No breed differences ($P > 0.10$) were detected for caput epididymus sperm content per g of tissue (Table 30), but a significant ($P<0.004$) breed influence was detected for total caput epididymus sperm content (Table 31). Brahman had greater ($P<0.004$) total caput epididymus sperm content than Romosinuano and Angus bulls (Table 31).

There was a significant ($P<0.0002$) breed influence on corpus epididymus sperm content per g of tissue (Table 30) and total epididymal sperm content (Table 31). Brahman had greater ($P<0.006$) corpus epididymus sperm content per g than Romosinuano which were greater ($P<0.006$) than Angus bulls (Table 30). Brahman had greater ($P<0.0002$) total corpus epididymus sperm content than Romosinuano and Angus bulls (Table 31).

Significant breed differences were detected for cauda epididymal sperm content per g of tissue ($P<0.0004$, Table 30) and total cauda epididymal sperm content ($P<0.0021$, Table 31). Romosinuano had greater ($P<0.007$) cauda epididymal sperm content per g than Brahman and Angus bulls (Table 30). Brahman tended ($P<0.07$) to have greater cauda epididymal sperm content per g than Angus bulls (Table 30). Brahman and Romosinuano had greater ($P<0.002$) total cauda epididymal sperm content than Angus bulls (Table 31).

Table 30. Caput, corpus, and cauda epididymus sperm content per g of tissue (LS mean ± SE) for Angus, Brahman, and Romosinuano bulls 69-111 d following sexual maturity

Breed	Caput epididymus sperm content per g of tissue (10^6)	Corpus epididymus sperm content per g of tissue (10^6)	Cauda epididymus sperm content per g of tissue (10^6)
Angus	426.08±72.99	179.64±51.78^{c}	852.95±174.12^{y}
Brahman	572.61±63.21	650.88±44.84^{a}	1307.90±150.79^{y}
Romosinuano	517.68±56.53	381.91±40.11^{b}	1915.30±134.87^{x}

Different superscripts within a column differ ^{a,b,c} P<0.006, ^{x,y} P<0.007

Table 31. Total caput, corpus, and cauda epididymus sperm content (LS mean ± SE) for Angus, Brahman, and Romosinuano bulls 69-111 d following sexual maturity

Breed	Total caput epididymus sperm content (10^9)	Total corpus epididymus sperm content (10^6)	Total cauda epididymus sperm content (10^9)
Brahman	6.02±0.53^{a}	1662.33±90.99^{m}	7.03±0.75^{x}
Romosinuano	3.69±0.47^{b}	427.43±81.38^{n}	6.87±0.67^{x}
Angus	3.36±0.61^{b}	219.52±105.06^{n}	2.84±0.86^{y}

Different superscripts within a column differ ^{a,b} P<0.004, ^{m,n} P<0.0002, ^{x,y} P<0.002

Plasma Testosterone Concentration at Slaughter and Testis StAR and P450 Content

Concentrations of plasma testosterone at slaughter were similar among breeds of bulls (Angus, 3.77±2.05; Brahman, 2.64±1.92; Romosinuano, 5.78±1.71). Significant breed influences for testis content of StAR ($P<0.0043$) and P450 ($P<0.05$) was detected. Romosinuano and Angus had greater ($P<0.006$) testis StAR protein content than Brahman bulls (Table 32). Angus had greater ($P<0.02$) testis P450 scc enzyme content than Brahman with Romosinuano bulls being intermediate (Table 32). A western blot of the testis StAR protein and P450 scc with three bulls representing each breed is shown in Figure 7).

Table 32. Testis StAR protein content (LS mean ± SE) for Angus, Brahman, and Romosinuano bulls 69-111 d following sexual maturity

Breed	Testis StAR content (IOD units)	Testis P450 content (IOD units)
Angus	0.155 ± 0.018^{a}	0.036 ± 0.007^{x}
Brahman	0.079 ± 0.17^{b}	0.012 ± 0.006^{y}
Romosinuano	0.156 ± 0.015^{a}	$0.028\pm0.006^{x,y}$

Different superscripts within a column differ ^{a,b} $P<0.006$, ^{x,y} $P<0.02$

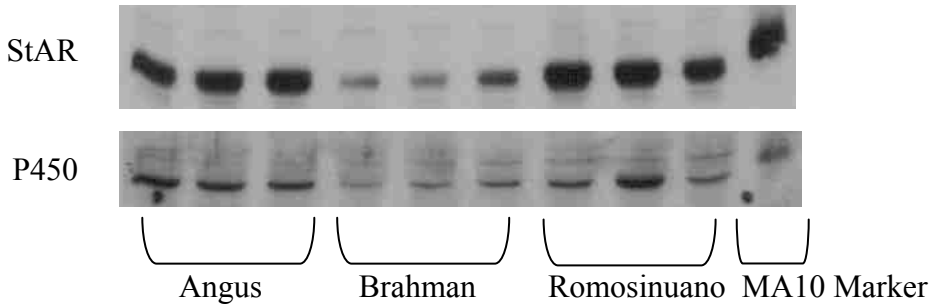

Figure 7. Western blots of testis StAR protein and P450 scc for Angus, Brahman, and Romosinuano bulls 69-111 d following sexual maturity.

Discussion

The intent of experiment # 1 was to determine if basal differences exist among concentrations of plasma testosterone as well as plasma testosterone concentrations following hCG administration in tropically-adapted *Bos taurus*, Sanga, temperate *Bos taurus* and tropically-adapted *Bos indicus* bulls. The intent of experiment # 2 was to determine if reproductive organ weights, testis sperm content, daily sperm production, epididymal sperm content and testis content of StAR protein and P450 scc enzyme of tropically-adapted *Bos taurus* bulls are more similar to those of temperate *Bos taurus* bulls or tropically-adapted *Bos indicus* bulls. Results from experiment # 1 indicate that differences were detected for average concentrations of plasma testosterone among breeds during the 6 h prior to hCG administration.

Basal concentrations of plasma testosterone during the 6 h interval were not influenced by tropical adaptation. The average basal concentrations of plasma testosterone during the six-hour sampling period were similar to those reported in Angus X Hereford bulls (Rawlings et al., 1978) and in Hereford and Brahman bulls (Godfrey et

al., 1990), but slightly lower than those reported in Norwegian Red bulls (Sundby and Tollman, 1978). Wagyu bulls had the greatest average and highest concentrations of testosterone during the 6 h sampling period with Tuli and Bonsmara bulls being intermediate and Brahman, Angus and Romosinuano bulls having the lowest testosterone concentrations. Differences in basal testosterone concentrations may be due to several factors. The first factor may be genetic differences in the hypothalamic-pituitary-gonad axis. A likely assumption may be that these differences are due to differences in LH concentrations; however, LH concentrations were not quantified in this study. In conjunction with LH, insulin-like growth factor 1 (IGF-1) acts to assist in Leydig cell maturation in mice (Wang et al., 2003). These authors suggest that this may ultimately affect testosterone production. Strauch et al. (2002) reported that sexually mature Angus bulls tended to have increased testicular IGF-1 mRNA expression compared to sexually mature Brahman and Romosinuano bulls. These same authors reported that no difference existed in testicular expression of LH-receptor mRNA between sexually mature Angus and Romosinuano bulls. Genetic differences in mean testosterone concentrations have been reported in African-American men and Caucasian men. African-American men have higher concentrations compared to Caucasian men (Ellis and Nyborg, 1992). The higher concentrations of testosterone in Wagyu bulls may be consistent with a rapid precocious puberty (Tatman, 2002) or a higher level of libido (Whitworth, 2002). During a 90 d controlled breeding season (April 15 – July 15, 1999), Wagyu bulls sired three times more offspring than Tuli bulls and nearly two times more than Bonsmara bulls (79 calves sired by Wagyu, 24 sired by Tuli, and 44 sired by

Bonsmara; Whitworth, 2002). A pronounced increase in concentrations of testosterone in Africander bulls is consistent with a higher level of fertility compared to Brahman cross and Hereford X Shorthorn bulls during natural mating in Australia (Post and Bindon, 1983).

Thibier (1976) reported that the average number of testosterone peaks during a 24 h period in mature dairy bulls ranged between 3 and 7 peaks. Similarly, Godfrey et al. (1990) reported an average of 1.3-2.3 testosterone peaks during an 8 h period in Brahman and Hereford bulls. In the present study, very few testosterone peaks were detected in the 53 bulls sampled during a 6 h sampling period; however, several bulls appeared to be on the decline of a testosterone peak at the commencement of sampling since the testosterone concentrations further declined until a basal concentration was achieved.

A possible explanation as to why very few peaks of testosterone were detected in bulls in the present study may be due to stress and high concentrations of plasma cortisol encountered by the bulls when they were placed in the alleyway and during the period when extensions were added to the cannulas and when the cannulas were checked to ensure proper functioning. However, this should have only been a temporary suppression and should not have affected testosterone peaks later in the 6 h sampling. Increased serum cortisol concentrations as a result of administration of ACTH (Johnson et al., 1982) or electroejaculation (Welsh and Johnson, 1981) have been reported to suppress secretion of testosterone. Testosterone suppression was also reported in bulls following administration of dexamethasone (Thibier and Rolland, 1976). The

suppression of testosterone was a result of LH suppression due to increased concentrations of corticosteroids and possibly increased concentrations of progesterone (Welsh and Johnson, 1981; Johnson et al., 1982). Peaks of testosterone occurred 6 h following ACTH treatment (Johnson et al., 1982) or electroejaculation (Welsh and Johnson, 1981) after cortisol concentrations were back to a basal concentration.

Testosterone concentrations following hCG administration were not influenced by tropical adaptation. The testosterone concentrations following hCG administration found in bulls in the present study were similar to those reported in 7 mo old (Sundby, 1981) and 1.5-3 year old (Sundby et al., 1975) Norwegian Red bulls. These data as well as the data from the present study indicate that various breeds challenged with hCG have similar capabilities to produce and secrete testosterone. A possible explanation for this is that a similar number of Leydig cells were stimulated by the hCG. Another possible explanation is that the hCG quantity was such that it saturated all the Leydig cells in the testis of all the bulls, thus resulting in similar testosterone production and secretion.

Results from experiment # 2 indicate that the weights of the reproductive organs of tropically-adapted *Bos taurus* bulls are more similar to that of temperate *Bos taurus* bulls but that testis and epididymal sperm concentrations are intermediate to tropically-adapted *Bos indicus* bulls and temperate *Bos taurus* bulls. Consequently, testicular and epididymal sperm concentrations were influenced by tropical adaptation. This would be expected since a key concept of tropical adaptation is the ability to reproduce in tropical or subtropical climates. As expected from weights at slaughter, Brahman had a heavier hot carcass weight than Angus and Romsinuano bulls. Chase et al. (2001) reported that

Brahman X bulls had heavier slaughter and hot carcass weights than Tuli X Angus and Senepol X Angus bulls. The heavier weight at slaughter can be attributed to the Brahman bulls being older and thus heavier at sexual maturity. Dressing percentages of Brahman X Angus were greater than Tuli X Angus and Senepol X Angus bulls (Chase et al., 2001); however, these were slightly lower than that reported in the present study.

Organ weights were not influenced by tropical adaptation. When obtaining organ weights from animals of different breeds it is important to consider the BW and to examine the organ weights on a BW basis since some animals may be older and heavier at similar physiological endpoints. This ensures that all animals are compared on an equal basis. The whole pituitary gland weight reported for Romosinuano bulls in the present study was similar to those reported in British X Continental bulls and steers (Doornenbal, 1974). The anterior pituitary gland weight has been reported to be heavier in ¾ Angus compared to ¾ Brahman steers at slaughter weights (Bruner et al., 1996; Carroll et al., 1996). Larger anterior pituitary gland may result in the ability to produce and secrete more LH. This differs from data of the present study since no differences were detected in anterior pituitary weight between Angus and Brahman bulls. A possible explanation for these different findings may be that the ¾ Angus and ¾ Brahman steers were slaughtered at a common weight whereas the bulls in the present study were slaughtered after reaching a common physiological endpoint (sexual maturity). Consequently, there was approximately 100 kg difference in BW between Angus and Brahman bulls in the present study.

The similarities in actual paired testis weight, corrected paired testis weight, testis length and testis diameter of Romosinuano and Angus bulls is likely due to the similarity in bodyweight at slaughter. This indicates that these parameters of the Angus and Romosinuano bulls are proportionate to their body size and BW. This is also true for the Brahman bulls as they had heavier BW and thus larger and heavier testes. The paired testis weights of the Angus bulls in the present study are similar to those reported in yearling Angus bulls (Coulter and Bailey, 1988). Paired testis weight of the Brahman bulls in the present study were slightly higher than those reported in Brahman bulls by Chase et al. (1994). This is likely due to the Brahman bulls in the present being slightly older and heavier than those reported by Chase et al. (1994). In the present study, Angus and Romosinuano bulls had similar epididymal weights (actual and adjusted for BW), which was due to the proportionality of the size of the testis. This was also seen in Brahman bulls which had heavier paired epididymal weights due to having larger testes. Paired epididymal weights previously reported in Brahman (Chase et al., 1994) and Angus (Weisgold and Almquist, 1979) were similar to those in the present study.

The rate-limiting step in steroidogenesis is the transfer of cholesterol from the outer to the inner mitochondrial membrane by the StAR protein (Stocco and Clark, 1996). Differences in testis StAR content among breeds may influence the ability of an animal to produce androgens. Green (1999) reported that testis StAR protein and P450 scc enzyme of fetal Angus and Brahman bull calves during various time points of gestation in which fetal Angus bull calves had greater StAR and P450 scc enzyme content at some time points and fetal Brahman having greater StAR and P450 scc

enzyme concentrations at other time points. This differs from the present study where sexually mature Angus had greater testis StAR and P450 content compared to sexually mature Brahman bulls. An explanation for the differences between the present study and that of Green (1999) is that in the present study StAR protein and P450 scc enzyme were measured at one time point and in the study by Green (1999) examined 7 different time points. One reason for the inconsistency our data and that reported by Green (1999) is likely due to the low numbers compared at each time point during gestation. The concentrations of plasma testosterone reported for bulls at the time of slaughter in the present study were variable between bulls within breed. The problem may not only be genetic related but also hormonally related. Wang et al. (2003) reported that IGF-1 stimulates maturation of adult Leydig cells in conjunction with LH by increasing steroidogenic enzyme expression and decreasing androgen-metabolism by 5-alpha reductase-1.

Testicular sperm content is an estimation of the total number of sperm cells in the parenchyma of the testis. Daily sperm production is the amount of sperm an animal is capable of producing each day. Daily sperm production per g of tissue in Angus bulls in the present study was similar to that reported in 7 yr old Angus bulls (Weisgold and Almquist, 1979) but lower than that reported in 15 mo old Angus bulls (Coulter et al., 1987). Daily sperm production in Angus bulls in the present study was approximately 50% of that reported for 15 mo old Angus bulls (Coulter et al., 1987). Angus bulls in the present study as well as those evaluated by Weisgold and Almquist (1979) and Coulter et al. (1987) were slaughtered between May and September. The lower daily sperm

production in Angus bulls in the present study may be due to the high environmental temperatures experienced in Texas from May to September. High ambient temperatures have been shown to have detrimental effects on spermatogenesis in Angus (Meyerhoeffer et al., 1985) and Hereford bulls (Austin et al., 1961). This is particularly important in beef cattle operations that have a controlled breeding season from May to July or in situations where the bulls is with the cows year around and expected to breed females that come into heat during the summer months. Testis sperm concentrations and daily sperm production by the Romosinuano bulls in the present study indicate that indeed this breed is tropically-adapted and are not greatly affected by high ambient temperatures.

The lowered epididymal sperm concentrations detected in the Angus bulls in the present study could be a result of high ambient temperature affecting spermatogenesis in the testis and thus decreasing the concentrations of spermatozoa in the three sections of the epididymus. Epididymal sperm concentrations in the Romosinuano bulls indicate that they are tropically-adapted and are not greatly affected by high ambient temperatures.

Conclusion

It is concluded that various breeds of bulls have different basal concentrations of plasma testosterone; however, basal testosterone concentrations were not influenced by tropical adaptation. Various breeds of bulls have similar concentrations of plasma testosterone following administration of hCG which also was not influenced by tropical adaptation. In experiment #2, it is concluded that the weights of reproductive organs and

testis StAR protein and P450 scc enzyme of tropically-adapted *Bos taurus* bulls are more similar to tropically-adapted *Bos indicus* bulls than to temperate *Bos taurus* bulls and thus not influenced by tropical adaptation. Testis and epididymal sperm concentrations were influenced by tropical adaptation. Tropical adaptation is important in regards to reproduction in beef crossbreeding operations along the Gulf Coast.

CHAPTER V

CONCLUSIONS

The majority of beef cattle crossbreeding operations along the Gulf Coast utilize cattle that are tropically-adapted due to the hot and humid climate of that region. Brahman cattle are a tropically-adapted *Bos indicus* breed that has primarily been utilized in this region. However, newly introduced tropically-adapted breedtypes such as Bonsmara (*Sanga X Bos taurus* composite breed from Southern Africa), Romosinuano (*Criollo* breed from Colombia) and Tuli (*Sanga* breed from Southern Africa) as well as the temperate Japanese *Bos taurus* breed of Wagyu (which may be of economic value) need to be studied as viable options in crossbreeding systems in the Gulf Coast region.

In livestock production systems, management practices such as transportation and restraint elicit a response from the animals' hypothalamic-pituitary-adrenal axis. These practices act as stressors to the animal. The response of a breed to management stressors such as transportation and restraint may determine if that breed will be advantageous in crossbreeding systems. Additionally, functions of the reproductive system depend on the hypothalamic-pituitary-gonadal axis. The ability of an animal to reproduce in the hot, humid climate of the Gulf Coast region may determine if that breed can successfully be utilized in crossbreeding systems. The present studies were conducted to examine if tropical adaptation influences adrenal and reproductive function in bulls.

The cortical responses to transportation and restraint stress were not influenced by tropical adaptation. The response to both of these stressors could be categorized into

high responders (Angus, Brahman), intermediate responders (Romosinuano, Tuli) and low responders (Wagyu, Bonsmara). The tropically-adapted breedtypes were not categorized into a single group. Therefore, it was concluded that response to both transportation and restraint stress was influenced only by breedtype. This may be a result of some breedtypes being selected heavily for docility. This is only speculation and further research is required to better understand these findings.

Testosterone production and secretion is important in the process of spermatogenesis. All breedtypes except the temperate Japanese *Bos taurus* had similar concentrations of testosterone. Therefore, it was concluded that tropical adaptation did not influence basal concentrations of plasma testosterone, but that it was influenced by breedtype. The temperate Japanese *Bos taurus* bulls had greater basal concentrations of testosterone than other breedtypes examined. This was likely a result of two things, 1) the precocious puberty and rapid sexual maturation exhibited by this breedtype (Tatman, 2002) and 2) a higher level of libido amongst this breedtype (Whitworth, 2002).

While it was concluded that basal testosterone concentrations prior to hCG administration were affected by breedtype, testosterone production following hCG administration was similar between all of the breedtypes. Therefore, it was not influenced by breedtype or tropical adaptation. This may be due to similar number of Leydig cells being stimulated by hCG in all of the bulls or that the hCG quantity was such that it saturated all of the Leydig cells in the testis of all the bulls, thus resulting in similar testosterone production and secretion.

Most organ and gland weights (actual weight and weight corrected for BW) and the steroid precursors, StAR protein and P450 scc enzyme, were not influenced by tropical adaptation. It was concluded that these parameters were influenced only by breedtype since the tropically-adapted breedtypes were not similar.

Actual paired adrenal gland weight, total adrenal area, medullary and cortical areas were influenced by tropical adaptation. The tropically-adapted breedtypes had both lighter glands and smaller areas than their temperate *Bos taurus* counterpart breedtypes.

To better understand the stress response and how to manage it, future research should possibly include stress-related genes and markers and also the inclusion of catecholamines from the adrenal medulla and their involvement in stress responsiveness. The possibility of locating genes involved with temperament may warrant the attention of researchers in the future.

Testis and epididymal sperm concentrations were influenced by tropical adaptation. The tropically-adapted breedtypes had greater testicular and epididymal sperm concentrations than the temperate *Bos taurus* breedtype counterparts during the summer months. This would be expected since a key concept of tropical adaptation is the ability to reproduce in tropical or subtropical climates.

Future research should possibly focus on the growth axis, the ability to perform in feedlots located in the high plains (Midwest) and carcass characteristics of tropically-adapted breedtypes as well as the temperate Japanese *Bos taurus* Wagyu breedtype.

This would provide a better understanding if these breedtypes can economically fit into crossbreeding systems in the Gulf Coast region.

In summary, adrenal gland weight, adrenal gland area (total, medullary, and cortical), and testicular and epididymal sperm concentrations were influenced by tropical adaptation. However, cortical response to transportation and restraint, basal testosterone concentrations, other organ weights (actual and BW basis) and testis and adrenal StAR and P450 content were influenced by breedtype, not tropical adaptation.

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APPENDIX A

RADIOIMMUNOASSAY PROCEDURE FOR PLASMA CONCENTRATION OF CORTISOL

Assay Solutions

PBSG	1 L
NaCl	8.17 g
$NaH_2PO_4H_2O$	0.856 g
Na_2HPO_4	0.54 g
EDTA	3.72 g
Thimerosal Powder	0.1 g

1) Dissolve all of the above in 900 ml D-D H_2O in 1000 ml beaker.
2) Adjust pH to 7.4.
3) Weigh 1.0 g gelatin (Knox) and add to a 1 L bottle and add EDTA-PBS solution.
4) Dissolve gelatin by placing bottle on magnetic stirrer/hot plate on "low" heat and moderate stirring speed for approximately 2 h.
5) PBSG is ready to use after cooling to room temperature.
6) Store at 4°C.

Charcoal Dextran Suspension	500 ml
Charcoal Norit SPXX	3.125 g
Dextran Pharmacia T-70	0.3125 g
PBSG	500 ml

1) Combine dextran, charcoal and PBSG in 1000 ml beaker.
2) Make up charcoal the day before it is needed and allow mixture to stir for at least 6 h.
3) Stir mixture for 1 h prior to use.

Sample Methodology

1) Sample Preparation
    a. Pipet 10 µl sample in duplicate into appropriately labeled plastic 12 x 75 mm tubes
    b. Add 490 µl of PBSG to each tube and vortex.
    c. Place in 70°C water bath for 1 h.
    d. Allow sample to cool down for 1 h to room temperature and then proceed with setting up the assay.
2) Setting up the Assay
    a. Pipet 800 µl, 600 µl, 500 µl PBSG in duplicate into appropriately labeled tubes (T, N, 0 respectively).
    b. Pipet 500 µl of standard into appropriately labeled plastic tubes in duplicate.
    c. Add 100 µl of tritiated hydrocorticosterone (approximately 12000-13000 cpm) to all tubes.
    d. Add 100 µl of antibody to all tubes except the T and N tubes and vortex.
    e. Store in refrigerator at 4°C for 12 to 18 h.

3) Day 2

   a. Add 5 ml Ecolume (ICN, Aurora, OH) cocktail to mini-vials and label caps for vials.

   b. Add 200 µl of charcoal dextran suspension to all tubes except T tubes (This step should be performed in the cold room).

   c. Vortex tubes and allow to sit for 15 min.

   d. Centrifuge (Sorvall Instruments, Model RC3C, Newton, CT) for 20 min at 2800 rpm (2282 x g).

   e. Keep tubes at 4°C after centrifugation.

   f. Decant supernatant into the mini-vials and count each vial for 1 min in beta counter.

APPENDIX B

RADIOIMMUNOASSAY PROCEDURE FOR PLASMA CONCENTRATION OF TESTOSTERONE

Assay Solutions

PBSG	1 L
NaCl	8.17 g
$NaH_2PO_4H_2O$	0.856 g
Na_2HPO_4	0.54 g
EDTA	3.72 g
Thimerosal Powder	0.1 g

1) Dissolve all of the above in 900 ml D-D H_2O in 1000 ml beaker.
2) Adjust pH to 7.4.
3) Weigh 1.0 g gelatin (Knox) and add to a 1 L bottle and add EDTA-PBS solution.
4) Dissolve gelatin by placing bottle on magnetic stirrer/hot plate on "low" heat and moderate stirring speed for approximately 2 h.
5) PBSG is ready to use after cooling to room temperature.
6) Store at 4°C.

Charcoal Dextran Suspension	500 ml
Charcoal Norit SPXX	3.125 g
Dextran Pharmacia T-70	0.3125 g
PBSG	500 ml

1) Combine dextran, charcoal and PBSG in 1000 ml beaker.
2) Make up charcoal the day before it is needed and allow mixture to stir for at least 6 h.
3) Stir mixture for 1 h prior to use.

B. Assay Methodology

1. Ether Extraction of Sample

   a. Pipet 200 µl of unknown sample into appropriately labeled 18 x 150 mm glass tubes.
   b. Label additional set of 12 x 75 mm glass tubes to match large tubes.
   c. Add 5 ml anhydrous ether (EM Science, Cincinnati, OH) to each 18 x 150 tube. Keep tubes under a fume hood.
   d. Vortex 4-6 tubes by hand (only vortex 12-24 tubes prior to extracting) for 1 min.
   e. Place 4 tubes into a rack in a styrofoam ice chest containing liquid nitrogen (usually 1.5-2 inches high to cover liquid in tube) for 10-15 s (until plasma freezes).
   f. Remove tubes from liquid nitrogen and decant liquid into appropriately labeled 12 x 75 glass tube.
   g. Place 12 x 75 mm tube into nitrous gas dryer (Multivap Analytical Evaporator, Organomation, South Berlin, MA) containing 37°C distilled water. Once dryer is full, tubes should be placed in a rack and kept under hood.

h. Rotate tubes into dryer as other tubes dry. Place dry tubes in rack and keep under hood until rack is full.

i. Add 500µl PBSG to each dried tube, vortex each tube and store at 4°C.

2) Setting up the Assay

f. a. Pipet 800 µl, 600 µl, 500 µl PBSG in duplicate into appropriately labeled tubes (T, N, 0 respectively).

g. Pipet 500 µl of standard into appropriately labeled plastic tubes in duplicate.

h. Pipet 50 µl of the extracted sample in duplicate into appropriately labeled 12 x 75 plastic tubes.

i. Add 450 µl of PBSG to each tube.

j. Add 100 µl of tritiated testosterone (approximately 12000-13000 cpm) to all tubes.

k. Add 100 µl of antibody to all tubes except the T and N tubes and vortex.

l. Store in refrigerator at 4°C for 12 to 18 h.

3) Day 2

g. Add 5 ml Ecolume (ICN, Aurora, OH) cocktail to mini-vials and label caps for vials.

h. Add 200 µl of charcoal dextran suspension to all tubes except T tubes (This step should be performed in the cold room).

i. Vortex tubes and allow to sit for 15 min.

j. Centrifuge (Sorvall Instruments, Model RC3C, Newton, CT) for 20 minutes at 2800 rpm (2282 x g).

k. Keep tubes at 4°C after centrifugation.

l. Decant supernatant into the mini-vials and count each vial for 1 min in beta counter.

APPENDIX C

PROCEDURE FOR DETERMINATION OF SPERM CONTENT FROM TESTICULAR AND EPIDIDYMAL HOMOGENATES

Homogenization Fluid:	For 10 L
150 mM NaCl	87.66 g
3.8 mM NaN_3	2.47 g
0.05 % Triton x-100	5.00 ml
Bring to volume with D-D H_2O	

Testis:

1) Obtain 1.5 to 1.7 g sample of thawed testicular parenchyma.
2) Mince very well with a scalpel blade followed by scissors.
3) Dilute with 150 ml homogenization fluid and homogenize for 2 min.
4) Pour mixture into 250 - ml Erlenmeyer flask, cover and refrigerate at 4°C for 24 h.

Epididymus:

1) Separate epididymus into caput, corpus and cauda sections.
2) Weigh each section.
3) Mince each section very well with a scalpel blade followed by scissors.
4) Dilute each section of the epididymus with homogenization fluid (caput = 150 ml, corpus = 150 ml, cauda = 200 ml) and homogenize separately for 2 min and 15 s.
5) Pour mixture into 250 – ml Erlenmeyer flask and refrigerate at 4°C for 24 h.

Determination of Sperm Content 24 h later:

1) Shake flasks vigorously so that homogenate is mixed together well.
2) Use a glass Pasteur pipette to obtain a sample of the homogenate and fill one side of hemacytometer chamber.
3) Repeat steps 1 and 2 to fill other side of hemacytometer chamber.
4) Place hemacytometer on top of two wooden sticks in a petri dish with a damp piece of filter paper (humidified chamber) for 2 to 3 min.
5) Using an inverted phase microscope, view hemacytometer grid at 400X.
6) Count the sperm in all 25 squares in both hemacytometer chambers.
7) Dilute as necessary if too many sperm are present to count.
8) If there is greater than 15 % difference in counts (from each chamber), repeat steps 1 through 6.
9) Calculate sperm content:

Testis: Sperm Content/g = $\frac{.01 \times (\text{volume} + \text{sample weight}) \times \text{ave. \# counts}}{\text{Sample weight}}$

Epididymal Regions: Sperm Content/g = $\frac{.01 \times (\text{volume} + \text{tissue weight}) \times \text{ave. \# counts}}{\text{Tissue weight}}$

Ave. # counts = adding counts from both chambers of the hemacytometer chamber and dividing by 2.

APPENDIX D

WESTERN BLOT ANALYSIS

FOR StAR AND P450

Stock Solutions

A. Acrylamide/bis (30% T, 2.67% C)
87.6 g acrylamide (29.2 g/100 ml)
2.4 g N'N'-bis-methylene-acrylamide (0.8g/100 ml)
Make to 300 ml with distilled water. Filter and store at 4°C in the dark (30 d maximum)

B. 1.5 M Tris-HCl, pH 8.8
27.23 g Tris base 918.15 g/100 ml)
~80 ml distilled water

Adjust to pH 8.8 with 1N HCl. Make to 150 ml with distilled water and store at 4°C.

C. 0.5 M Tris-HCl, pH 6.8
6 g Tris base
~ 60 ml distilled water

Adjust to pH 6.8 with 1N HCl. Make to 100 ml with distilled water and store at 4°C.

D. 10% SDS
Dissolve 10 g SDS in water with gentle stirring and bring to 100 ml with distilled water.

E. Sample Buffer (SDS reducing buffer) (store at room temperature)

Distilled water	4.0 ml
0.5 M Tris-HCl, pH 6.8	1.0 ml
Glycerol	.80 ml
10% (w/v) SDS	1.6 ml
2-b-mercaptoethanol	0.4 ml
0.05% (w/v) bromophenol blue	0.2 ml

Dilute the sample at 1:4 with sample buffer, and heat at 95°C for 4 min.

F. 5X electrode (Running) buffer, pH 8.3 (enough for 10 runs)

Tris base	9 g	(15 g/l)
Glycine	43.2 g	(72 g/l)
SDS	3 g	(5 g/l)
To 600 ml with distilled water		

Store at 4°C. Warm to 37°C before use if precipitation occurs.

Dilute 60 ml 5X stock with 240 ml distilled water for one electrophoretic run.

Procedure for Western Blot

1. Trim tissue from -80°C freezer to 200 mg.

2. Homogenize in 0.25 M sucrose buffer which contain 0.1mM EDTA and 10 mM Tris.

   TRIS-SUC-EDTA (for homogenizing tissue):

.607 g	trisbase
42.79 g	sucrose
.145 g	EDTA

   Adjust to pH 7.4
   Bring to 500 ml in volumetric

3. Freeze sample at -80°C.

4. Thaw sample to room temperature.

5. Run a regular 1 dimensional polyacrylamide gel; be sure to run prestained MW markers on each gel. MW STDS: 20 ul marker + 8 ul 4X sample buffer. Load 5 ul per well.

6. Remove the gel and notch accordingly. Remove the stacker.

7. Put the gel in cold 1X TRANSFER BUFFER for 5 minutes. While the gel is equilibrating, wearing gloves and using forceps, cut the PVDF membrane to the exact size of the gel.

TRANSFER BUFFER (1X)

20 mM TRIS (FW 121)	2.42 gm/liter
150 mM Glycine (FW 75.07)	11.24 gm/liter
10% MEOH	100 ml/liter
.01% SDS	.1 gm/liter

8. First – wet the PVDF in methanol
   Second – soak the PVDF in water until saturated
   Third – wet the PVDF in transfer buffer

9. Now you're ready to set up the transfer apparatus – make sure you follow the order exactly

   YOU

   Plastic grid (clear-mini)
   Sponge pad
   3 MM paper
   PVDF
   Gel
   3 MM paper
   sponge pad
   plastic grid (black-mini)

   LAB BENCH

   Wet thoroughly in transfer buffer
   Cut the 3 MM paper the same size as the sponge
   Very important – flip the gel over so the back side is facing up
   Notch the PVDF to match the notch in the gel
   After placing PVDF on gel, fill a test tube with transfer buffer and pour over PVDF, then steam roll the tube over the membrane to remove ALL bubbles; repeat after the 2nd 3 MM paper

10. Place grids into transfer apparatus making sure the current is running in the right direction: negative (black) to positive (red) – gel is next to black and PVDF is next to red.
    Fill transfer apparatus with cold transfer buffer, add stir bar and put on a stir plate and run in the cold.

11. Set at 100 volts and run for 2 h. (replace ice block after 1 hour)

12. After the transfer, remove the PVDF membrane and soak while shaking in blocking buffer overnight or for at least 1 h at room temperature.

BLOCKING BUFFER
4% milk soln. In PBS+TWEEn
(4 gms Carnation powdered milk + 100 mls PBS+TWEEN)

PBS+0.25% TWEEN
8 gm NaCl
0.2 gm KCl
1.44 gm Na2HPO4
0.24 gm KH2PO4
Bring up to 1 liter: pH 7.4 Add 2.5 mls TWEEN 20.

13. Make up PRIMARY Ab in 2% milk solution:
(2 gms powdered milk + 100 mls PBS+TWEEN)
We use a 1:1000 dilution – 100 ul Ab + 50 mls 2% milk solution
14. Remove the PVDF from the overnight blocking buffer and put into the primary Ab solution. Shake for 1 h at room temperature. You can discard the overnight 4% blocking buffer.

15. Remove primary Ab solution and save – can be stored in refrigeration and reused. Wash PVDF 3 times – 5 min each – with PBS+TWEEN (shake).

16. Pour off last wash and put PVDF into 2nd Ab solution and shake for 30 min at room temperature: we use 1:15,000 dilution
(3.3 ul + 50 mls 2% milk solution)

17. Remove 2nd Ab solution and save – can be stored in refrigerator and reused. Wash PVDF 2 times – 30 min each – with PBS+TWEEN (shake).

18. Remove PVDF from wash and proceed with Chemiluminescence:
- mix solutions according to directions (we use 4 mls of each)
- blot excess wash from PVDF
- add PVDF – protein side down – wash for 1 min
- blot dry
- put PVDF between protector sheet – protein side up

19. Place into cassette immediately with film.
Try different exposure times to get the best results.

PROCEDURE FOR STRIPPING AND RE-BLOTTING WESTERNS

Strip Buffer:

SDS	2 g	(2 % SDS)
Trizma Base	.757 g	(62.5 mm tris)
B-mercap	700 µl	(100 mm BME)

Bring up to 100 mls with distilled water; pH 6.8

Wash Buffer:

Trizma base	.12 g	(10 mm Tris)
NaCl	.876 g	(150 mm NaCl)

Bring up to 100 mls with distilled water; pH 7.4

1. Soak the PVDF membrane in strip buffer for 30 min at 70°C; shake or swirl every 10 min.

2. Then, wash the PVDF membrane in wash buffer 2X for 10 min each at room temperature, using a shaker or rotator of some sort.

3. Rinse the PVDF membrane with PBS-tween 1X for 5 min.

4. Block the membrane as usual with 4% milk solution and proceed as usual with the Ab staining procedure.

If you are planning to restain with your original first Ab, you can strip the membrane using .1 M Glycine at pH 2.6 for 5-10 min at room temperature; rinse with PBS-tween; block and restain as usual.

If the PVDF membrane has dried out considerably before you are ready to strip, just wet

the membrane in methanol very quickly and then begin soaking in strip buffer.

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