To the Graduate Council:

I am submitting herewith a thesis written by Heather E. Blackmon entitled "Effects of Administration of Ergotamine Tartrate on Fertility of Yearling Beef Bulls". I have examined the final paper copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirement for the degree of Master of Science, with a major in Animal Science.

F. Neal Schrick, Major Professor

We have read this thesis
and recommend its acceptance:

Dr. J. Lannett Edwards

Dr. Fred M. Hopkins

Accepted for the Council:

Vice Provost and
Dean of Graduate Studies

Effects of Administration of Ergotamine Tartrate on Fertility of Yearling Beef Bulls

A Thesis

Presented for the

Master of Science

Degree

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Abstract

Sixteen Angus crossbred bulls were used to investigate effects of ergotamine tartrate administration on semen parameters, endocrine profiles and the use of in vitro fertilization as a means of predicting developmental competency of oocytes fertilized with sperm from bulls fed ET. Bulls, approximately 350 kg body weight and 270 days of age, were assigned to one of two groups. Within each group, bulls were allotted by breed, weaning weight, hip height, scrotal circumference, and age. All bulls were fed a diet of cracked corn, corn silage, and soybean meal. Bulls restricted to this diet served as controls (**CON**, n = 8). Additional topdressing of 40 μg/kg body weight of ergotamine tartrate (ET) was provided to the treatment group (**ET**, n = 8). ET dose remained constant throughout experimental period. In order to maintain dosage of 40 μg/kg body weight, amount of ET administrated was altered as body weight increased. Administration of ET began in mid-November and continued through the end of June. Blood samples, body weight, and rectal temperatures were recorded every two weeks beginning at ET administration and continued through experimental period. Semen samples were collected from all bulls approximately every sixty days by electroejaculator. During May and June, testicular core temperatures were measured by scrotal thermography. From both groups, collected semen from bulls (n = 2 trt) having the largest and smallest scrotal circumference was extended at the location and returned to the laboratory for further assessment such as performance of sperm using in vitro fertilization (IVF). Comparisons between **ET** and **CON** for IVF parameters were determined using PROC FREQ

procedure. All other variables were analyzed by the Mixed procedure. Administration of ergotamine tartrate increased rectal temperature (39.3 ± 0.05 °C) compared to **CON** bulls (39.1 ± 0.05 °C; P = 0.02). However, concentrations of prolactin did not differ between **ET** (79.7 ± 9.5 ng/mL) and **CON** bulls (56.2 ± 9.6 ng/mL; P = 0.10). Neither scrotal circumference (P = 0.68) nor serum concentrations of testosterone (P = 0.17) differed throughout the experimental period between groups. Semen motility and morphology were similar in bulls administered ergotamine tartrate and controls (P = 0.83; P = 0.51, respectively). However, bulls exposed to ergotamine tartrate had a significant decline in testicular core temperatures (31.4 ± 0.3 °C) compared to **CON** bulls (33.0 ± 0.3 °C; P = 0.004). Cleavage rate of oocytes cultured with sperm from bulls fed ergotamine tartrate 51% was reduced compared to **CON** (69%; P = 0.001). Ability of cleaved embryos to develop to 8-16 cells (P = 0.11) or to blastocyst (P = 0.96) did not differ between groups. In conclusion, extended exposure of bulls to ergotamine tartrate appears to reduce fertilization ability of sperm; possible through the vasoconstrictive action associated with the treatment.

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I. Introduction

Fescue toxicosis results from ingestion of tall fescue infested with the endophyte, *Neotyphodium coehophialum.* Prevailing symptoms such as elevated body temperature, vasoconstriction of capillaries, and reproductive problems associated with fescue toxicity have contributed to massive declines in overall herd health. Fescue toxicosis affects reproductive performance, growth, and lactation (Porter, 1995). Numerous studies have shown how fescue toxicity negatively affects female reproduction. Fescue toxicosis has been linked to reduced LH concentrations (Browning et al., 1997), compromised embryonic development (Hockett et al., 2002), and reductions in pregnancy rates (Seals et al., 2002). Hoveland (1990) calculated that producers loose $560 million dollars annually due to lowered conception rates associated with fescue toxicosis. While research has well documented how fescue toxicity affects female reproduction, limited studies have examined if fescue toxicosis affects male reproductive performance.

If reproductive performance of the herd bull were compromised by ingestion of endophyte-infected tall fescue, genetic gains produced through use of superior genetics would be negated. Non-return rates would dramatically decline. An increase in cows not conceiving on first service directly impacts number of days cows are open, herd calving interval, and seasonal marketability of calves; thus, resulting in a monetary lose to producers. Ultimately, the number of calves born each calving season defines herd fertility.

In attempts to predict individuals that could increase fertility, producers have utilized the most current techniques to analyze breeding soundness. Unfortunately, estimation of bull fertility has been limited to semen analysis, which includes motility estimates and morphological observations (Spitzer and Hopkins, 1997). Few single sperm parameters show significant correlation with actual pregnancy rates; therefore, results of breeding soundness exams (BSE) may not represent the ability of bulls to produce pregnancies (Larsson and Martinez, 2000). When considering that between 5 and 15 percent of all bulls that pass BSE are unable to produce pregnancies (Spitzer et al., 1988), the challenge becomes identifying factors unidentifiable to BSE that further affect early embryonic development.

The objective of this thesis was to investigate effects of simulated fescue toxicosis with administration of ergotamine tartrate (ET) on semen parameters, endocrine profiles, and the use of in vitro fertilization as a means of predicting developmental competency of oocytes fertilized with sperm from bulls fed ET. It has been hypothesized that bulls fed ET would have compromised sperm quality and that increasing ambient temperature would affect symptoms of simulated fescue toxicity.

II. Review of Literature

Majority of research efforts in the field of reproductive physiology has concentrated on factors that affect female reproduction. Most recently, research focus has shifted to the male's contribution at conception and emphasis has been placed on environmental factors that could affect male reproductive performance. One such factor is fescue toxicity. The following review of literature discusses fescue toxicosis, symptoms associated with this condition, treatment recommendations, effects on reproduction, and a review of spermatogenesis.

The History of Fescue Toxicosis

Tall fescue is a well-adapted perennial grass that is grown throughout the United States, with heaviest concentrations found within the southeast (Hemken et al., 1984). Due to climate and annual rainfall within this region, tall fescue is not only used for grazing of livestock, but also for lawn and roadside management (Hemken et al., 1984). It is estimated that between 7 and 14 million hectares of land within the United States have stands or mixed stands of tall fescue (Hammond et al., 1982). Endophytes, associated with tall fescue, provide protection against overgrazing, drought resistance, and improve insect tolerance (Read and Camp, 1986; Rowan and Trapper, 1989). Residing within the cell walls of the plant stalk, stem, and seedpod, endophytes also aid in mineral balance and soil neutralization (Morgan-Jones and Gams, 1982; Malinowski and Belesky, 2000). While endophytes infecting tall fescue are most

beneficial to the plant, they produce detrimental symptoms to livestock routinely grazing tall fescue.

More than 40 different ergot alkaloids have been isolated from tall fescue and are collectively considered derivatives of the tetracyclic skeleton (Mantegani et al., 1999). Studies have shown that ergopeptide alkaloids, primarily ergovaline, are the causative agents of fescue toxicosis (Belesky et al., 1988; Porter, 1995). The first indications that the endophyte, *Neotyphodium coehophialum,* was associated with fescue toxicosis occurred when its presence was found in tall fescue grazed by cattle experiencing visual signs of fescue toxicosis. Signs included roughened hair coat, increased respiration, and lameness (Bacon et al., 1977; Schmidt et al., 1982; Hemken et al., 1984).

Symptoms of Tall Fescue Toxicosis

Fescue toxicity associated with ingestion of endophyte-infected tall fescue produces numerous signs and symptoms (Hemken et al., 1984). Pathophysiological conditions resulting from ingestion of endophyte-infected tall fescue are collectively classified as fescue toxicosis. Common conditions associated with fescue toxicosis are fescue foot and summer syndrome (Hemken et al., 1984). Symptoms of each condition are similar, such that one condition may not be distinguishable from the other.

Typical signs of fescue foot are swelling accompanied with soreness and lameness of feet and rear legs. In cases where there is extreme fescue toxicity, dry gangrene of extremities may occur such that a necrosis condition of the

dewclaw and hooves become present. Necrosis, due to lack of blood flow, can become so severe that hooves of affected animal may slough off (Read and Camp, 1986). Considering that tall fescue is a cool season grass, growth occurs predominately in early spring and late fall. Fescue foot is most often observed in February due to the abundance of new grass growth. Only a few days of grazing are required before the condition of fescue foot occurs. Rapid onset of symptoms may be seen in animals grazing infected pastures heavily fertilized with nitrogen (Read and Camp, 1986). Nitrogen, a nutrient required for optimal growth, stimulates plant growth and simultaneously promotes endophyte concentrations (Read and Camp, 1986).

Fescue foot is caused by the vasoconstrictive properties of endophytes restricting blood flow to the hind legs (Read and Camp, 1986). An elevated endophyte concentration, specifically ergovaline, has been shown to produce vasoconstriction of the capillaries (Hemken et al., 1984; Osborn et al., 1992; Oliver et al., 1993, Oliver, 1997; Browning, 2000; Hockett et al., 2002). Whittow (1962) first reported endophyte-induced vasoconstriction reduced blood flow to the hind legs in cattle and linked vasoconstriction to fescue foot. Other extremities affected by capillary vasoconstriction resulting from ingestion of high concentrations of ergovaline are the ears, nostrils, and tail. Vasoconstriction of the capillaries leading to these extremities has been shown by reduction of skin temperatures at each location (Oliver, 1997; Browning, 2000; Hockett et al., 2002). Vasoconstriction due to ingestion of endophyte-infected tall fescue has

been shown to produce tail discoloration and eventual lose of the tail switch (Osborn et al., 1992; Hockett et al., 2002).

Summer syndrome is defined by an overall reduction in livestock performance associated with elevated ambient temperatures (Hemken et al., 1984). Proposed theory by Hemken et al. (1984) suggested that elevating ambient temperatures increased sensitivity of animals to the endophyte toxin. Summer syndrome associated with fescue toxicosis has been linked to reduced weight gains, increased rectal temperatures, roughened hair coat, increased respiration, lowered milk production, and greatly decreased reproductive performance (Garner and Cornell, 1978; Hammond et al., 1982; Hemken et al., 1984).

Overall Impact of Fescue Toxicosis on Reproduction

The ergot alkaloids are responsible for reproductive failure (Lishman et al., 1979; Hammond et al., 1982; Hemken et al., 1984; Porter and Thompson, 1992; Oliver et al., 1993; Browning et al., 1998b). There are several causes for reproductive problems associated with endophyte toxicity. Endophytes found within tall fescue seed, hay, and green forage dramatically reduced feed intake of Holstein cows (Hemken et al., 1979). Fescue toxicosis suppressed body weight gains in prepuberal heifers such that onset of puberty was delayed (Washburn et al., 1989). Effects of fescue toxicosis on onset of puberty could result from reduction in LH concentrations within blood. Lishman et al. (1979) reported that weight loss associated with fescue toxicosis resulted in a decline in serum LH

concentrations in postpartum cows, absence of the preovulatory LH surge, and failure to ovulate. Ingesting infected tall fescue may further impair puberty and subsequent ovulation.

Inadequate hormone levels due to ingestion of endophyte-infected tall fescue have been shown to further impair ovulation and pregnancy (Boissier, 1978; Oliver, 1997; Browning et al., 1998a). Ergot alkaloids and synthetic derivatives such as ergotamine tartrate are dopaminergic agents that suppress GnRH-induced secretion of LH (Boissier, 1978). Further observations by Mckenzie and Erickson (1991) demonstrated that heifers fed endophyte-infected hay exhibited a reduction (23%) in basal LH concentrations, reduced estradiol-stimulated LH concentrations, and subsequent decline in folliculogenesis.

Endophytes, specifically ergovaline and ergonovine, have been shown to stimulate prostaglandin release during the luteal phase in cows resulting in the death of the corpus lutum (Oliver, 1997; Browning et al., 1998b). Estienne et al. (1990) observed luteal function was altered in heifers grazing endophyte-infected tall fescue. Heifers with a corpus lutum exhibited a decrease (62%) in circulating progesterone concentration. This study suggests endophytes affect CL's ability to secrete progesterone and may dramatically affect the ability to maintain pregnancy. Pregnancy rates were reduced in beef heifers administered ergotamine tartrate (ET) to simulate fescue toxic effects, with no alterations in progesterone concentrations (Seals et al., 2002).

Endophyte infestation decreased conception rates by 3.5% for each 10% increase in endophyte concentrations in cows grazing endophyte-infected

pasture (Porter and Thompson, 1992). Conception rates declined by 46% in heifers grazing pastures where 80 to 90% of plants were infected with the endophyte compared to heifers grazing pastures with endophyte infestation rates between 0 to 5% (Porter and Thompson, 1992). Embryo quality could contribute to reduction in conception rates. Hockett et al. (2002) reported recovered embryos, from beef heifers administrated ET, were of lower quality and reduced development compared to embryos recovered from control heifers. Good quality embryos were transferred into heifers administrated ergotamine tartrate to serve as controls. Pregnancy rates did not differ between ET and control heifers (Hockett et al., 2002).

Effects of toxic fescue on male reproduction have been far less studied. Zavos et al. (1988) reported that feeding endophyte-infected tall fescue seed to male rats decreased daily sperm production by 50%, decreased testicular parenchyma weight, and reduced epididymal weights. Lowered prolactin levels, characteristic of exposure to infected tall fescue, influenced gonadotropin release and growth of male accessory reproductive glands in rams (Bartke, 1980). Further study by Alamer and Erickson (1990) found endophyte ingestion though pasture grazing reduced GnRH levels and testicular Sertoli cells numbers of beef bulls approximately 3 months of age. Continued research by Alamer and Erickson (1990) reported reduced Sertoli cells numbers in bulls ranging between 8 and 12 months of age; thus, linking fescue toxicosis to permanently impaired testicular function. However, Evans et al. (1988) reported administering endophyte infected hay to Holstein bulls beginning at 2 months of age until 13

months produced no negative effects on weight and length of the testicles. In the same lot of bulls, prolactin and testosterone concentrations did decline when fed endophyte-infected hay. Reproductive problems associated with fescue toxicosis are not species specific; however, severity of symptoms and overall detrimental effects to the animal tends to be species dependent.

Ewes affected by *N. coenophialum* exhibited delayed conception, but other factors such as body weight gains, gestation lengths, average number of lambs born, and lamb survival rates were not altered (Bond et al., 1988). Negative effects of fescue toxicosis in horses seem to be confined to reproductive performance (Porter and Thompson, 1992). In mares, symptoms commonly associated with ingestion of endophyte-infected tall fescue include increased foal mortality, placental thickening, and agalactia (Poppenga et al., 1984). Zavos et al. (1988) found a diet consisting of endophyte-seed prolonged estrus in female rats as well as decreased litter weights, average number of pups produced per litter, and individual pup weight.

How does the Endophyte exert its Effects

Ergot alkaloids are known to have dopaminergic, antiserontonergic, and vasoconstrictive tendencies (Berde and Schild, 1978). Moreover, lysergamide has been identified as an alkaloid similar to ergonovine and has also been shown to produce vasoconstriction within bovine blood vessels (Oliver et al., 1993). Other ergopeptide alkaloids including ergotamine, ergovaline, and ergonovine have also been shown to cause peripheral vasoconstriction (Oliver, 1997;

Browning, 2000). Temperature reduction and increased blood pressure are the main signs vasoconstriction has occurred (Hammond et al., 1982; Hemken et al., 1984). Peripheral temperatures at specific extremities such as the tail, ear, and pastern have been examined in steers grazing endophyte-infected pastures and temperatures at all locations have been reported to decrease when compared to rectal temperature (Osborn et al., 1992; Browning, 2000). Studies have shown that animals affected by fescue toxicosis have decreased ability to shuttle blood away from the body core due to capillary vasoconstriction. Thus, body temperature and blood pressure increase while peripheral skin temperatures decline (Osborn et al., 1992; Oliver, 1997; Browning, 2000).

Clark et al. (1978) found vasoconstrictive properties associated with ergotamine increased blood pressure within the capillaries by as much as 10 times. Constriction of peripheral capillary beds decreased the amount of blood flowing to extremities; thus, decreasing peripheral temperatures (Browning, 2000). A study by Browning (2000) illustrated variation in skin temperature at two locations on the tail after injecting ergotamine tartrate. Skin temperature was significantly lowered at both locations; however, the sharpest decline was detected at the tip of the tail. These findings agree with early studies by Carr and Jacobson (1969) illustrating that skin temperature in cattle were reduced when exposed to ergot alkaloids. Reduction in the temperature was attributed to vasoconstriction and subsequent reductions in blood flow to the periphery. Osborn et al. (1992) observed steers to have a reduction in peripheral temperatures at the ear and pastern when consuming infected tall fescue.

Use of synthetic compounds to simulate fescue toxicosis has successfully created fescue toxicity symptoms such as elevated rectal temperature and vasoconstriction (Hockett et al., 2002; Seals et al., 2002). Ergotamine tartrate commonly used to produce "fescue toxic" effects mimics the action of ergovaline, but with only 10 percent of the potency (Seals et al., 2002). Dose response studies have been conducted using ET in order to determine concentrations required to accurately exert typical endophyte effects (Schrick, 2002).

Treatment and Preventative Measures

The most effective treatment of fescue toxicosis is complete removal of animals from infected pastures; however, this is not always feasible for producers (Hammond et al., 1982). In cases where grazing infected tall fescue is inevitable, pastures should be supplemented with mixed grasses, clover and other legumes to dilute concentrations of the endophyte (Hammond et al., 1982). Immediate, but temporary relief of symptoms associated with fescue toxicosis, may be alleviated by fluid therapy with normal saline or 5% dextrose. Application of a cool water bath over the head and body of the animal has been recommended to lower body temperatures (Hammond et al., 1982).

Preventative measures are preferred over use of therapeutic drugs because of potential drug residue and the withdrawal time required before slaughter (Rice et al., 1997). Development of a vaccine to protect cattle against fescue toxicosis could potentially save producers millions of dollars annually (Rice et al., 1997). Not only would a vaccine increase monetary yields by

increasing average daily gains, but would also drastically decrease cost of land management associated with pasture renovation and replanting (Rice et al., 1997).

Previous attempts at developing a vaccine to prevent fescue toxicosis have failed. Studies by Cox (1985) concluded that vaccines, which induced IgG antibodies against plant or fungal toxins, resulted in various degrees of animal protection. Rice et al. (1997) concluded that repeated vaccination would be required to induce the adequate systematic anti-ET titer in order to protect cattle against fescue toxicosis. The amount of vaccine required to adequately protect cattle from fescue toxicosis would be extremely expensive and labor intensive. Knowing that ergot alkaloids are dopamine D2 receptor agonist, Hill (1994) infused monoclonal antibodies, which act as a D2 receptor antagonist, and reversed toxicosis depressed serum prolactin. Unfortunately, the quantity of antibody needed to neutralize ingested ergot alkaloids or the duration of antibody administration required to relieve symptoms were not determined. Other non-medicinal practices are currently under investigation.

Varieties of tall fescue are being engineered with lowered levels of endophyte fungus as well as strains of fungus free tall fescue (Jackson et al., 1984). Unfortunately, due to cross-pollination, isolated strands of fungus-free tall fescue are impossible to maintain.

Process of Spermatogenesis in the Male

Prior to birth, primordial germ cells colonize the urogential ridge where cells divide and form undifferentiated gonocytes. At puberty or slightly before, gonocytes differentiate to form sperm cells known as spermatogonia. Through a cascade of divisions, spermatogonia form spermatozoa. As reviewed by Johnson, (1995), Keer, (1995), and Escalier, (1999), researchers have mapped out the course in which spermatozoa are produced within the seminiferous tubule of the testis. Three main processes must take place in order to produce viable sperm: spermatocytosis, meiosis, and spermatogenesis.

Characteristic spermatocytogenesis occurs at the basement membrane of the seminiferous tubules and is dependent on follicle stimulating hormone (FSH; reviewed by Johnson, 1995). Spermatocytogenesis is the process by which stem cell spermatogonia undergo numerous mitotic divisions providing a constant supply of stem cells. During this time, spermatogonia are undergoing morphological changes within the nuclei. Prior to entering meiosis, spermatogonia are diploid and divide to form primary spermatocytes (reviewed by Johnson, 1995). Meiosis is classified as the time in which genetic material is passed between homologous chromosomes of primary spermatocytes and reduction of haploid spermatids (reviewed by Johnson, 1995).

The last step in production of a functional sperm is spermiogenesis. At this point, the sperm production process becomes testosterone dependent and is responsible for the major morphological changes associated with transformation into spermatozoa (reviewed by Johnson, 1995). Key changes that occur during

spermiogenesis include condensation of chromatin and formation of both the tail and acrosomal cap (reviewed by Johnson, 1995). Development of the spermatid is identified with development of acrosome produced by the Golgi apparatus. The acrosome is a covering surrounding the nucleus of the spermatozoon. It contains enzymes, such as hyaluronidase and zona lysine, responsible for aiding sperm entrance into the oocyte by softening the cumulus surrounding the zona pellucida (reviewed by Johnson, 1995). Development of the flagella is necessary for motility. Movement is accomplished by the infolding of the plasma membrane to produce a flagellar canal from which the flagellum can extend from the spermatid cell body (reviewed by Johnson, 1995). Maturation of the spermatids is the final phase of spermiogenesis. Spermatozoa are considered mature when released from seminiferous tubules (Amann, 1989). Fertilization capacity is not acquired until completion of epididymal maturation. At that time, the cytoplasmic droplet is lost and progressive motility is established (Johnson et al., 1980).

Throughout the process of sperm formation, somatic cells, consisting of Sertoli cells and myoid cells, as well as Leydig cells each provide specialized function assuring the integrity of developing spermatozoa. Sertoli cell act as nurse cells providing nourishment and means of maintaining waste; while Leydig cells aid in testosterone production (reviewed by Johnson, 1995). Myoid cells are responsible for maintaining seminiferous tubules and assisting in movement of the spermatozoa (Fawcett, 1975). Myoid cells also function to regulate Sertoli cell secretion, further aiding in preventing breakdown of tight junctions between Sertoli cells and germ cells (Hettle et al., 1988). Known as nurse cells, Sertoli

cells function to provide nutrition necessary to promote growth and development of spermatids and remove waste (reviewed by Johnson, 1995). Sertoli cells also function by providing structural support and communication to developing germ cells by means of gap junctions (Russell, 1977). The blood-testis barrier is composed of Sertoli cells regulating serum components; thus, assuring an optimal environment for production of spermatozoa (Waites, 1977). Sertoli cell numbers directly relates to spermatogonia, spermatid, and spermatozoan numbers (Berndtson et al., 1987). Due to the presence of large amounts of smooth endoplasmic reticulum, Leydig cells are responsible for converting cholesterol and pregnenolone to testosterone (De Kretser and Kerr, 1988). Testosterone is absolutely required for spermatogenesis (reviewed by Johnson, 1995).

Factors Altering Sperm Production

When considering factors affecting sperm production, it is important to examine length of time needed to produce functional sperm (reviewed by Johnson, 1995). Spermatozoa are released from any point of the seminiferous epithelium. Therefore, the length of spermatic cycle is defined as the time between consecutive releases of spermatozoa and is dictated by frequency of committed spermatogonia entering spermatocytogenesis (Leblond and Clermont, 1952). Determining the point at which *A* spermatogonia enter the process is difficult; therefore, spermatogenesis is estimated to be 4.5 times the cycle length.

The duration of spermatogenesis varies and is dependent on species (Table 1; Amann, 1989).

Depression of hormone secretion is a main cause of impaired spermatogenesis (Greep et al., 1936). Several hormones such as testosterone and FSH are responsible for driving spermatogenesis. Alterations in hormone secretions during the process of spermatogenesis could subsequently affect fertility. The pituitary gonadotropins, FSH and LH, are required for spermatogenesis to be initiated and maintained (Greep et al., 1936). Testosterone secreted by Leydig cells has been recognized as the major intratesticular hormone regulating spermatogenesis (Walsh et al., 1934). Sertoli cells have been shown to contain receptors for testosterone (reviewed by Johnson, 1995). Sar et al. (1993) illustrated that testosterone supports germ cell development indirectly through secretory function of Sertoli cells.

Table 1: Differentiation of Spermatogenesis between Species

	Human	Bull	Rat	Stallion	Ram
Cycle length (days)	16	13.5	12.9	12.2	10.4
Spermatogenesis (days)	74	61	60	57	47

The pituitary hormone, prolactin, plays a critical role in reproductive efficiency and function in rats (Guillaumot and Benahmed, 1999). Impaired prolactin concentrations have been shown to alter testicular function by inhibiting LH and FSH biosynthesis, interfering with Leydig cell receptor mediated steroidogenesis, and decreasing binding affinity of Sertoli cells receptors; therefore, decreasing FSH mediated spermatogenesis (Guillaumot et al., 1996). Receptors for prolactin have been located on both Leydig and Sertoli cells of the rat, indicating prolactin's role in steroidogenesis and regulation of hormones secretion involved in testicular development (Guillaumot et al., 1996).

Temperature Regulation within the Testes

The scrotum provides a thermoneutral environment for normal spermatogenesis. Regulation of testicular temperature is accomplished by heat carried into the testes by arterial blood flow, metabolic heat produced by the testes, and heat released through scrotal skin (Purohit et al., 1985). Scrotal size and shape change due to fluctuation in environmental temperature. The tunica dartos and the cremaster muscles are responsible for heat loss. Each contract when scrotal temperature declines; thus, drawing the testicles closer to the body and relaxing when temperatures are elevated. Relaxation allows testicles to hang further from the body allowing for cooling (Waites, 1977; Purohit et al., 1985). Thermoregulation within the testis plays an important role in spermatogenesis; therefore, physiological mechanisms of the scrotum regulate temperature.

Ambient temperature receptors within the scrotum register skin temperature changes and initiate reflexes that alter testicular temperature. Normal scrotal surface skin temperature (SST) of the testis increased as ambient temperature increased. The bottom SST is more significantly affected by temperature change, while the top of testicle remained less affected (Kastelic et al., 1996). Most animals maintain scrotal temperature between 2 and 8°C lower than body cavity temperature (Purohit et al., 1985).

Testicle cooling occurs as blood flows through the pampiniform plexus. This tight intertwined countercurrent blood flow mechanism allows for hot arterial blood to be cooled as it travels into testicles. Arterial blood flows in very close proximity to the much colder blood leaving the testicles through the testicular veins (Purohit et al., 1985).

Testicular temperature must be maintained between 30 and 33° C in order not to impair spermatogenesis (Harrison and Weiner, 1948). Blood flow to the testicles is important in heat distribution. Disruption in blood flow is responsible for cooler testicle temperatures (Purohit et al., 1985). Impaired thermoregulation of the scrotum and testicles can cause infertility (Gazvani et al., 2000). As previously discussed, vasoconstrictive properties of ergotamine tartrate are responsible for decreased blood flow. Classified as extremity, effects of ergot alkaloids on the testicular core temperature are of significant importance in spermatogenesis.

Key Role in Fertilization

Fertilization is the complex process by which spermatozoon binds the oocyte to form an embryo (Figure1). Once ejaculated spermatozoa are actively motile; however, they are unable to fertilize oocytes (Yanagimachi, 1990). Spermatozoa must undergo two main processes in order to penetrate the zona pellucida of an oocyte. The first process that must occur is the physiological change that allows spermatozoa to become competent for fertilization known as capacitation (Parrish et al., 1988; Yanagimachi, 1990). Capacitation occurs within the female reproductive tract following ejaculation (Parrish et al., 1988; Overstreet and VandeVoort, 1990; Yanagimachi, 1990; and reviewed by Johnson, 1995). During capacitation, the glycoprotein layers surrounding the spermatozoa are shed exposing the area of the acrosome on the sperm head (Parrish et al., 1988; Yanagimachi, 1990). The acrosomal region being free from the glycoprotein layers allows for sperm receptors sites located on the acrosomal region of the spermatozoa to interact with receptors of the oocyte located on the surface of the zona pellucida (Parrish et al., 1988; Overstreet and VandeVoort 1990; Yanagimachi, 1990). During capacitation, motility of sperm also becomes hyperactivated in order to facilitate sperm transport (Yanagimachi, 1990). Once spermatozoa have become capacitated, the second main process leading to fertilization must take place.

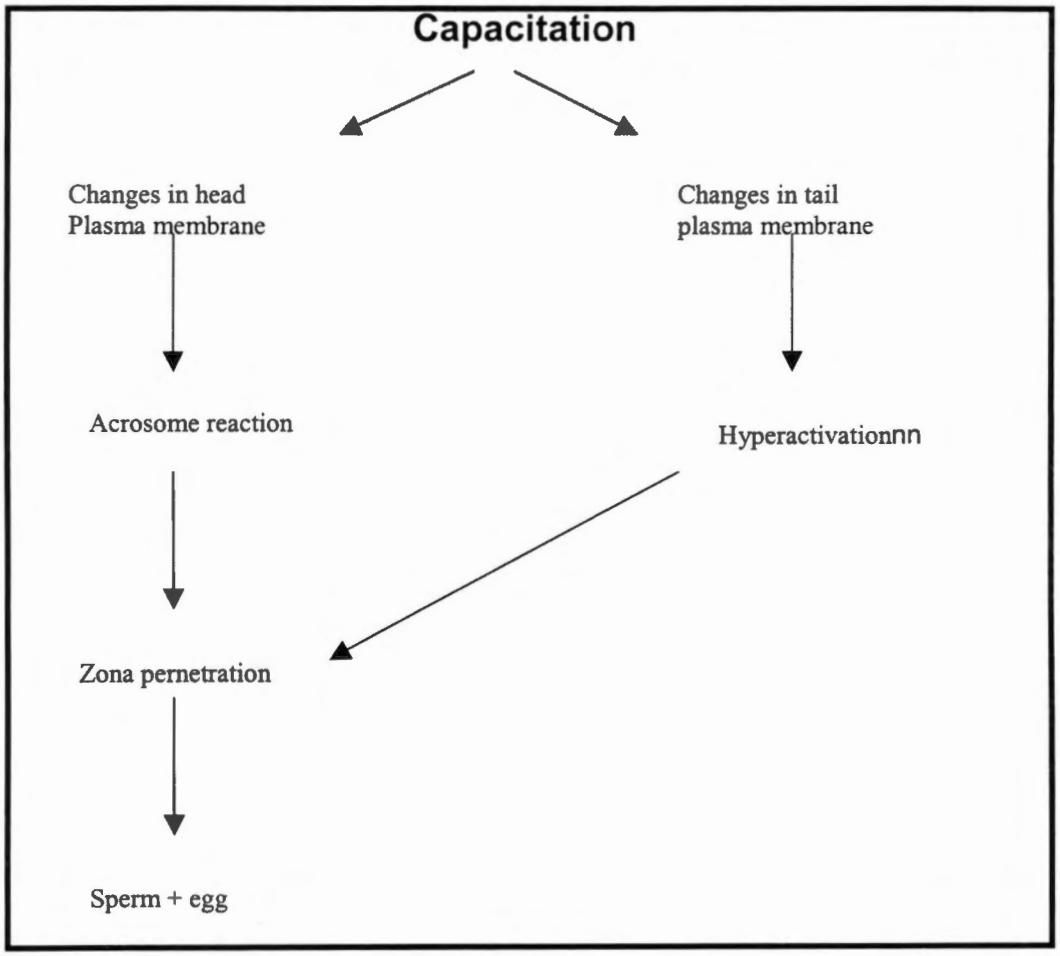

Figure 1. The relationships between capacitation, acrosome reaction, hyperactivation of spermatozoa and sperm-egg fusion.

Once capacitated spermatozoa come in contact with cumulus cells surrounding the oocyte, the glycoproteins of the zona interact with receptors on spermatozoa (Overstreet and VandeVoort, 1990; Yanagimachi, 1990; reviewed by Johnson, 1995). Interaction of the receptor sites activates the influx of calcium; thus, binding the two membranes. The binding of the two membranes further stimulates a massive influx of calcium allowing the zona pellucida to be penetrated (Overstreet and VandeVoort, 1990; Yanagimachi, 1990; reviewed by Johnson, 1995).

Factors that affect Fertilization

Both female and male partners affect the success of fertilization; however, the sperm's role is of utmost importance (Larsson and Martinez, 2000). Fertilization occurs in the oviduct of the female reproductive tract; therefore, the primary objective must be sperm motility. The ability of sperm to maneuver within the reproductive tract is imperative for fertilization to occur (reviewed by Johnson, 1995). However, Austin (1975) reported that sperm have the ability to remain motile much longer than their ability to fertilize an oocyte; thus, motility may remain high but sperm may be unable to penetrate the zona pellucida. Alone, motility is a poor prediction of fertility (Larsson and Martinez, 2000) and other factors such as morphology and acrosomal integrity must be considered. Motility does not guarantee that upon reaching the egg that fertilization will occur (Austin 1975).

Upon reaching the oocyte, fertilization is dependent upon sperm/egg recognition (Aitken and Irvine, 1990). Recognition may be impaired by sperm inability to capacitate (Aitken and Irvine, 1990). Lack of calcium, which is vital for the acrosomal reaction to occur, has also been linked to poor zona penetration (Aitken and Irvine, 1990; Overstreet and VandeVoort, 1990; Yanagimachi, 1990).

A large factor affecting successful fertilization is sperm morphology (Zamboni, 1990). Abnormal sperm morphology, virtually undetectable with basic morphological testing, is believed to be the causative agents for both infertility and subfertile conditions (Zamboni, 1990). Morphological "ultrastructural" damage to one of the major sperm components, such as the head plasma membrane, nucleus, acrosome, mitochondria, and flagellum could explain compromised fertilization. Unfortunately, "ultrastructural" defects are not noticed during normal semen analysis unless accompanied with a more gross morphological abnormality; therefore, further providing explanation why fertilization may still be compromised in individuals who pass semen analysis.

In Vitro Fertilization Utilized as a Tool in Predicting Bull Semen Fertility

A variety of techniques have been devised to test sperm fertilizing ability. Each of these techniques provides an estimate of actual fertilizing ability. Semen evaluation has been designed to aid producers in predicting male fertility. Artificial insemination (AI) most accurately indicates bull fertility (Larsson and Martinez, 2000). However, AI is expensive and time consuming when used for semen evaluation. Others techniques such as breeding soundness exams

(BSE) were developed as indicators of semen quality based on motility, and morphology (Spitzer et al., 1988). However, BSE are subjected to broad semen examination and have potential to give false positive results on ability to produce pregnancies. Few single sperm parameters, as measured in a BSE, have been correlated to *in vivo* fertility (Larsson and Martinez, 2000). A more efficient means of semen analysis is *in vitro* fertilization (Larsson and Martinez, 2000). In vitro fertilization (IVF) not only allows for motility and morphology testing, but other aspects associated with fertilization such as acrosomal membrane integrity and sperm/oocyte binding assays may be conducted (Barth, 1992; Holt et al., 1997; Zhang et al., 1998; Larsson and Martinez, 2000). By further examining sperm parameters, a more accurate prediction of a bull's ability to produce pregnancies may be made (Larsson and Martinez, 2000). Eyestone and First (1989) reported that results of IVF are largely related to semen quality.

Summary

Fescue toxicosis primarily affects reproductive performance, growth, and lactation in numerous species (Porter, 1995). Studies have attempted to explain, correct, and prevent its occurrence. When considering how fescue toxicosis impacts female reproduction, it seems logical that male reproduction may be compromised when exposed to fescue toxicosis. The objective of this thesis was to investigate effects of simulated fescue toxicosis with administration of ergotamine tartrate (ET) on semen parameters, endocrine profiles and in vitro fertilization.

III. Methods and Materials

Experimental Design

Sixteen Angus crossbred bulls were utilized to determine effects of ergotamine tartrate administration on semen parameters, endocrine profiles and in vitro fertilization. Bulls, approximately 350 kg body weight and 270 days of age, were assigned to one of two groups. Within each group, bulls were allotted by breed, weaning weight, hip height, scrotal circumference, and age. Prior to experimental period, bulls were weaned at the beginning of September and bunk fed corn silage. From mid-November through the end of June, bulls were bunk fed a diet of corn silage, cracked corn, and soybean meal (Figure 2). Bulls limited to this diet served as controls (**CON**, n = 8). Diet supplemented with 40 μg/kg body weight of ergotamine tartrate was provided to the treatment group (**ET**, n = 8; Figure 2; provided by Dr. Miroslav Flieger, Institute of Microbiology, Czech Republic). In order to maintain dosage of 40 μg/kg body weight, amount of ET administrated was increased as body weight increased. During experimental period, each individual bull received an average of 23 kg of feed on an as fed basis. This equates to approximately 16 kg of dry matter once a day. Water and minerals were available ad libidum. Ergotamine tartrate was given to simulate fescue toxicity since it mimics the action of the natural endophyte, ergovaline, but has only 10% of ergovalines' potency (Fluckinger et al., 1978). A guideline for ET dosage was derived from previous studies (Hockett et al., 2002; Seals et al., 2002).

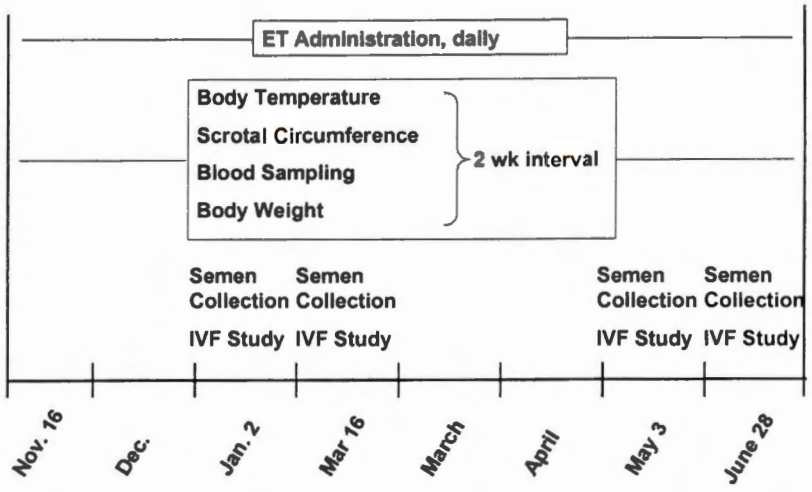

Figure 2. Representative timeline of experimental period

Individuals within similar treatment were housed together with 4 bulls completing a pen. No intermingling of treatments with controls was allowed; however, pens were in close proximity with access to outside grazing denied. Every two weeks, body weight, scrotal circumference, and rectal temperatures (GLA Agricultural Electronics, San Luis Obispo, CA) were measured (Figure 2). Ten mL blood samples were also collected via caudal venipuncture and placed on ice until centrifuged and serum frozen until assayed (Figure 2). Scrotal circumference was measured using a scrotal tape (Lane Manufacturing Co. Denver, CO). Backfat thickness was measured (Nov. 22, Feb. 14, June 14) by ultrasonography (Aloka 500V with 3.5 MHz backfat transducer; Corometrics Medical Systems Inc., Wallingford, CT). Testicular core temperatures were measured at an approximate 60-day interval using thermography (eMerge Vision

DTIS 500, eMerge International Inc., Sebastian, FL) on the days of semen collection in May and June.

Approximately 60 days from initiation of ET administration and at 60-day intervals, semen was evaluated (Jan. 2, March 16, May 3, and June 28, 2001). All bulls were collected individually with technician having no knowledge of treatment. Semen was collected using an electroejaculator (Lane Manufacturing Co. Denver, CO) and placed in 10 mL sterile conical tube until extended with specific Bioxcell extender. Both Bioxcell semen extender and Bioxcell extender-specific straws were generously provided by Dr. Gustave Hansen (IMV International Technologies, France). The Bioxcell extender was made on day of semen collection. Briefly, 100 mL of Bioxcell extender and 400 mL of sterile water were warmed to approximately 32° C in a water bath for 10 minutes prior to use. Once the temperature of water had equilibrated, 100 mL of extender was added to the water and solution was mixed thoroughly.

After motility and morphology examinations, semen was diluted with Bioxcell extender solution. Semen was extended at a 1:1 mL semen /extender ratio. Diluted semen remained at 32° C for 10 minutes and then at room temperature for 15 minutes. Extended semen was packaged in Bioxcell straws horizontally and placed in a cooler with cold packs for transport. At the laboratory, straws of extended semen were refrigerated at 4°C until in vitro fertilization (IVF) of oocytes approximately 26 hours post collection. Prior to extension, an estimate of progressively motile sperm was obtained using a light microscope at 400X. Approximately 25 μL of sperm was placed on a warmed

slide and covered with a cover slip in order to assess motility. Another sample of sperm (approximately 25 μL) was placed on a slide, mixed with eosin-nigrosin strain, smeared and allowed to dry. Morphology was evaluated under oil immersion at x 1000 using a light microscope (Spitzer et al., 1988). Sperm were randomly classified as having normal cells or cells with primary or secondary abnormalities.

In Vitro Evaluation of Semen

From both groups, collected semen from bulls (n = 2/trt) having the largest and smallest scrotal circumference was extended at the location and returned to the laboratory for further semen assessment. Scrotal means were 39.4 cm and 39.5 cm for CON and ET, respectively. The procedures utilized for the *in vitro* production of embryos (IVP), were modifications of procedures previously described by Edwards and Hansen (1996). The majority of chemicals and reagents necessary for IVP of bovine embryos were purchased from Sigma Chemical Inc. (St. Louis, MO). Medium 199, gentamicin, and penicillin-streptomycin were purchased from Specialty Media, Inc. (Phillipsburg, NJ). Fetal bovine serum (FBS) was obtained from BioWhittaker (Walkersville, MD). FollitropinV was provided by Vetrepharm Canada, Inc. (London, Ontario) while LH was provided by the National Institute of Health (USDA, Beltsville MD). Ovaries were purchased from an abattoir in Gaffney, SC. Media (HEPES-TALP, IVF-TALP, and SPERM-TALP; Parrish et al., 1988) and KSOM (Biggers et al.,

2000) with modifications generously provided by Dr. John Hasler were prepared in the laboratory or purchased from Specialty Media.

Ovaries were packaged in thermoses and contained within a cooler during air transport to the laboratory. Upon arrival to the laboratory, ovaries were immediately washed with warm water equilibrated to ovary arrival temperature. Extraneous tissue was removed and ovaries were again washed. In a cross-wise fashion, follicles approximately 3-8 mm in diameter were sliced using a scalpel blade. Ovaries were vigorously washed in oocyte collection media (OCM) in order to remove oocytes contained within the follicles. Upon retrieval, oocytes were selected based on quality. Oocytes used for study were deemed uniform in shape, color, and texture. Only oocytes with substantial amounts of cumulus surrounding the oocytes were selected and allowed to mature in oocyte maturation media (OMM) until time of fertilization (approximately 22 hours after placement in culture). OMM was prepared the morning of collection and allowed to equilibrate in humidified air for a minimum of 2 hours. Oocyte maturation occurred in a humidified atmosphere.

Collected semen was filtered through a layered Percol density gradient in order to remove excess extender, debris, and dead sperm prior to fertilization. Semen from Bioxcell straw was expressed directly to the top of Percol contained with in a 15 mL conical tube. Percol and semen were centrifuged at 2200 rpm of 15 minutes. Sperm pellet was aspirated from bottom of conical tube and expressed into conical tube containing 10 mL of Sperm TALP. Tube was centrifuged at 1100 rpm for 8 minutes. Sperm concentration was determined

after Percol-purification using a grided hemacytometer. A sperm concentration of 375,000/500 μL IVF TALP collected from bulls within in each group was added to the oocytes. A minimum of 50 oocytes was used for each bull on each collection date. Based on previous IVF results, a bull known to have high motility, as well as producing high cleavage and blastocyst percentages, served as a laboratory control. Putative zygotes were vortexed for 4 minutes and washed extensively in HEPES-TALP for the removal of cumulus and sperm. Culture of putative zygotes occurred in KSOM at humidified atmosphere of 5.5 CO_2, 7% O_2, and 87.5% N_2. Cleavage was assessed on day 3-post fertilization. Embryos with development further than one division were transferred into KSOM medium supplemented with both essential and non-essential amino acids until blastocyst evaluation on day 9 post fertilization.

Blood Collection and Radioimmunoassays

Blood samples were centrifuged at 2000 x g for 30 minutes and serum decanted and stored at –20°C until assayed for prolactin and testosterone. Radioimmunoassays (Coat-A-Count; Diagnostic Products Corporation; Los Angeles, CA) were performed to determine concentrations of testosterone (Hockett et al., 2002;Towns et al., 2002). Sensitivity of the testosterone assay was 0.04 ng/mL with intra- and inter-assay coefficients of variation (CV) of 10% and 2%, respectively. Prolactin radioimmunoassay was performed as described by Seals et al. (2002). Sensitivity of prolactin assay was determined to be 0.05 ng/mL. Intra- and inter assay CV were 11% and 10%, respectively.

Statistical Analysis

Body weight, backfat, body temperature, prolactin, scrotal circumference, testosterone, and testicular core temperatures were determined by analysis of variance using the Mixed procedure (SAS, 2000). Motility and morphology of semen were also determined by analysis of variance using the Mixed procedure. Comparisons between ET and CON treatments for the *in vitro* fertilization studies were determined using PROC FREQ procedure (SAS, 2000).

IV. Results

Body weight, Rectal temperature, and Prolactin

Throughout duration of the experimental period, diets were maintained such that body weight did not differ between groups (P = 0.7; Figure 3). There was no significant difference in backfat thickness between **ET** and **CON** bulls (P > 0.05; Figure 4). An overall increase in rectal temperatures in **ET** (39.9 $\pm$ 0.05 °C) compared to **CON** bulls (39.1 $\pm$ 0.05 °C; P = 0.02; Figure 5) indicated that bulls were affected by administration of ergotamine tartrate to simulate fescue toxicosis. Bulls administered **ET** had similar prolactin concentrations compared to **CON** bulls (P = 0.10; Figure 6).

Testicular Response

Scrotal circumference was not affected in response to ergotamine tartrate administration (P > 0.05; Figure 7). Concentrations of testosterone did not differ between **ET** and **CON** bulls (P = 0.17; Figure 8). Furthermore, sperm motility was not compromised by administration of ergotamine tartrate (P =0.83; Figure 9). However, due to the age of bulls at first collection, motility of first ejaculate was significantly impaired compared to later collection dates. Morphological evaluation of semen indicated that extended exposure to ergotamine tartrate did not alter this sperm parameter (P = 0.51; Table 2).

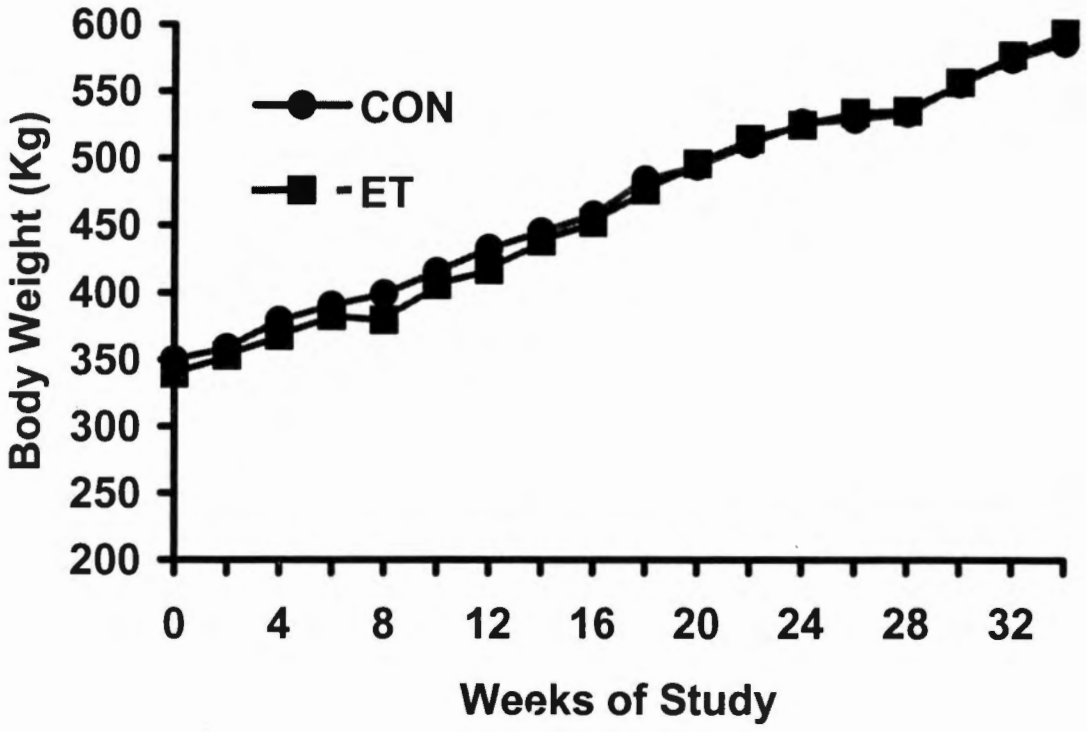

Figure 3. Body weight measurements between groups collected during experimental period did not differ during administration of ergotamine tartrate. Pooled SEM = 9.02 kg.

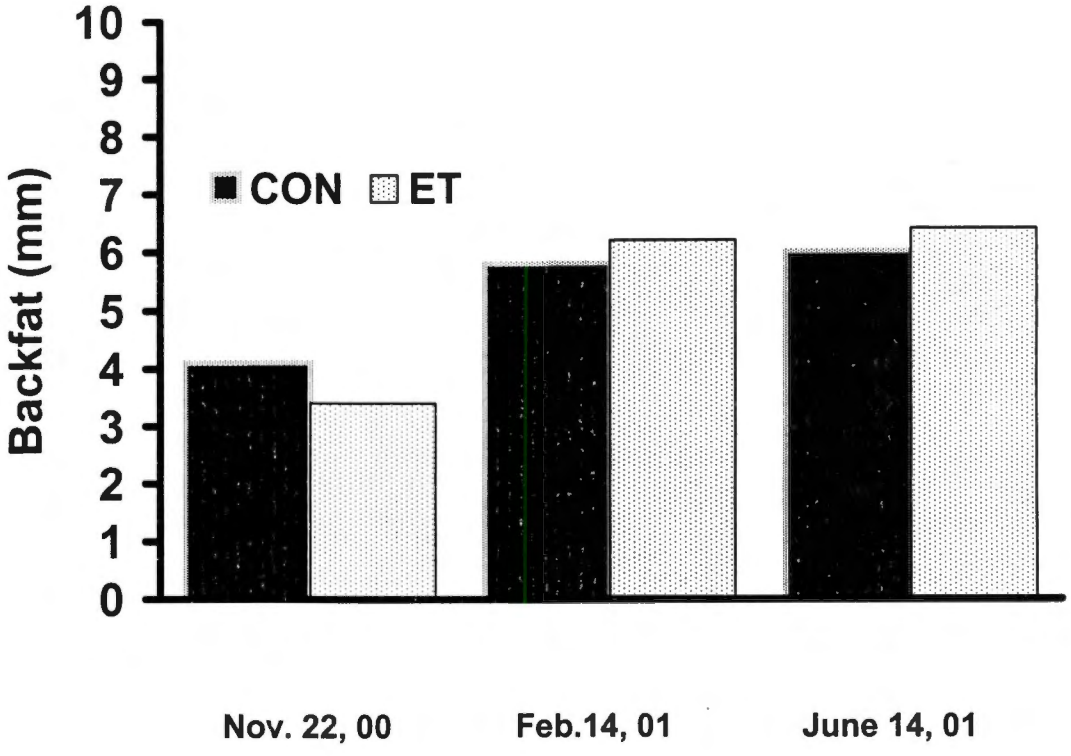

Figure 4. Backfat thickness measured during the experimental period of ergotamine tartrate administration did not differ. Pooled SEM = 0.42 cm.

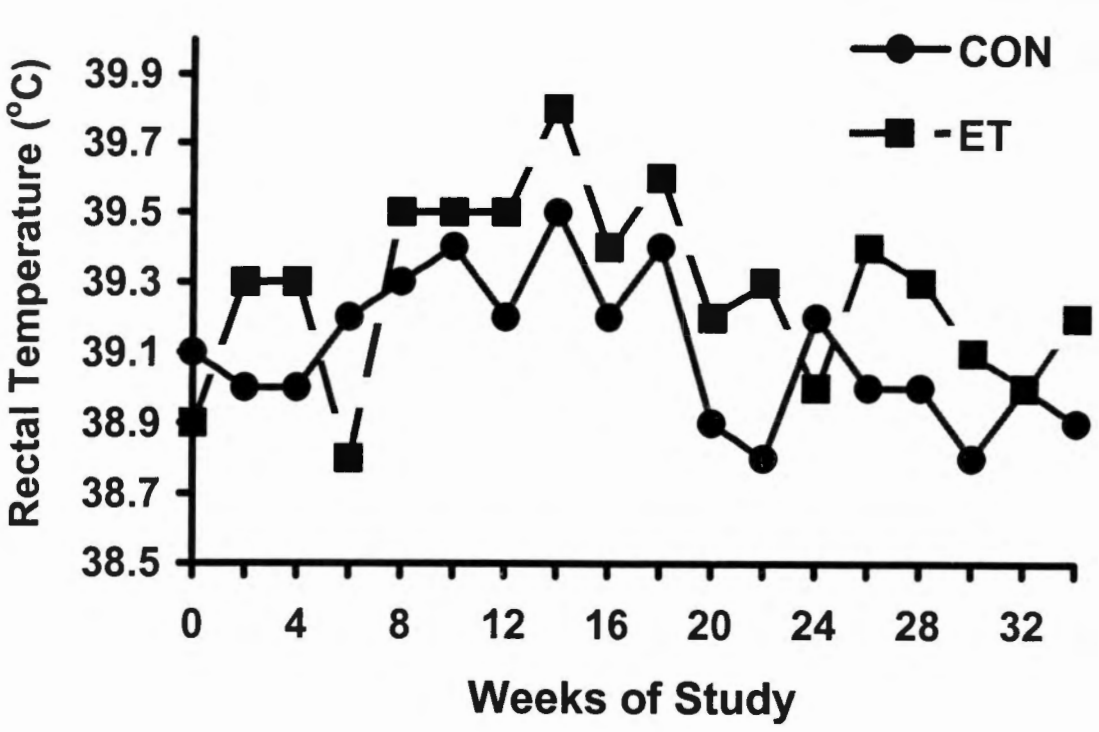

Figure 5. Rectal temperatures recorded during administration of ergotamine tartrate. Overall rectal temperatures were elevated in **ET** compared to **CON** bulls (P= 0. 02). Pooled SEM = 0.12 ° C.

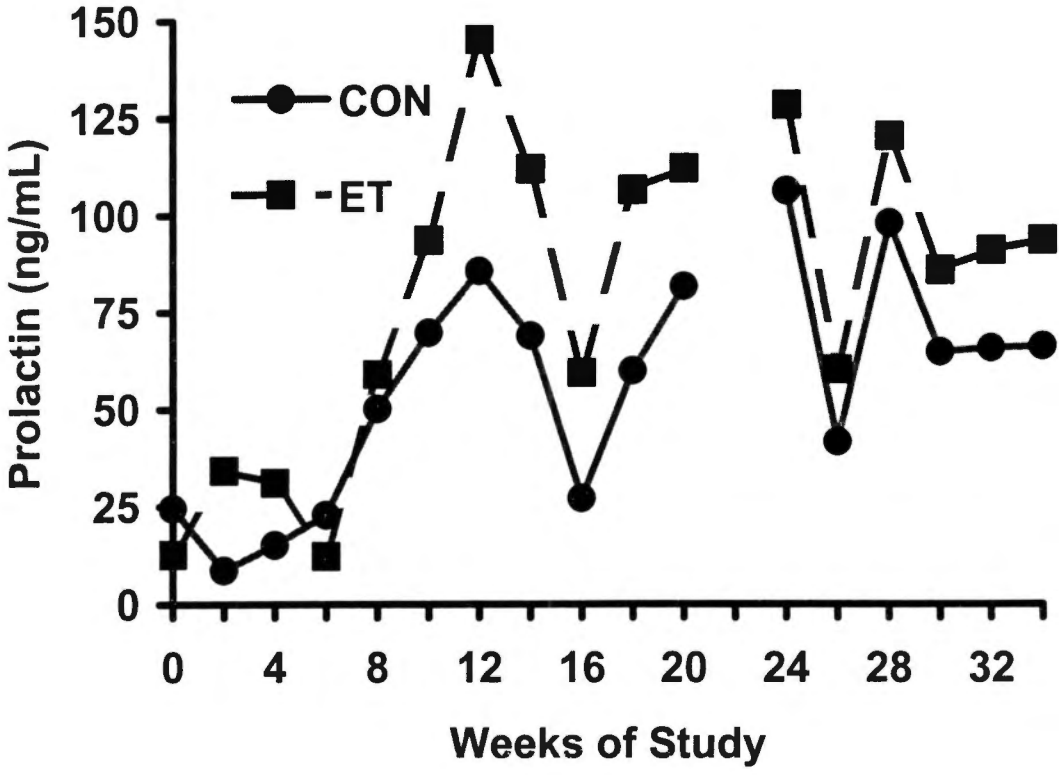

Figure 6. Concentrations of prolactin for **ET** and **CON** bulls collected during the experimental period. Overall prolactin did not differ between **ET** and **CON** bulls. Pooled SEM = 15.6 ng/mL.

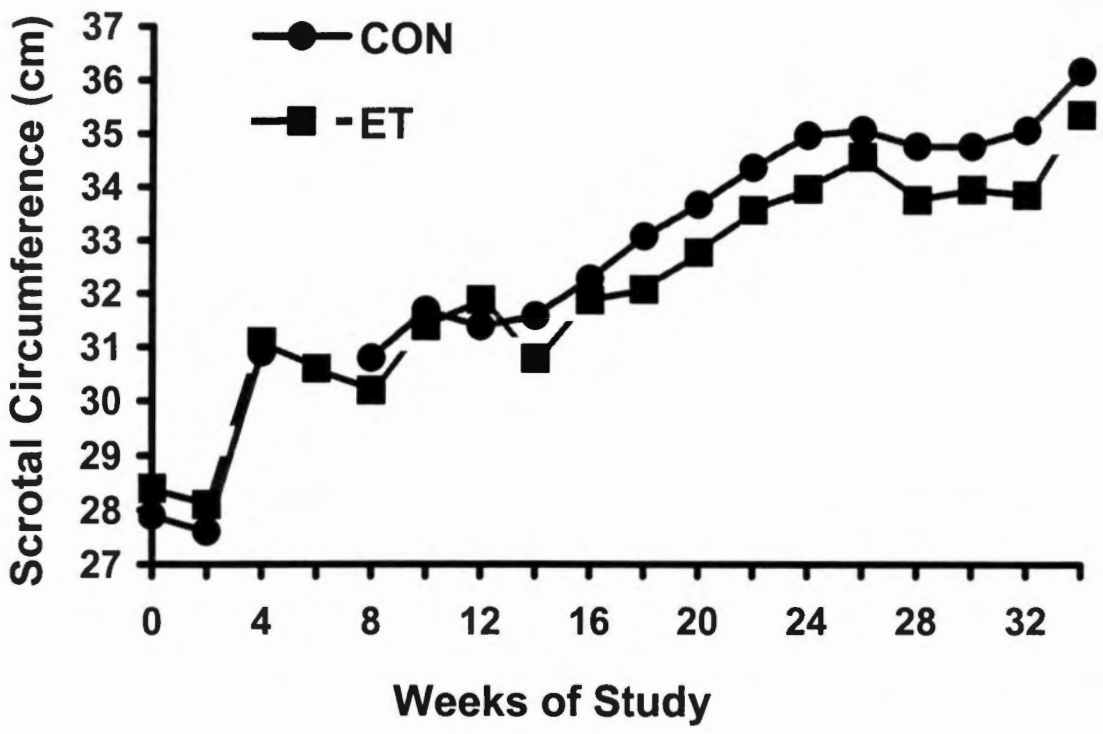

Figure 7. Scrotal circumference did not differ between an **ET** and **CON** bulls during administration of ergotamine tartrate. Pooled SEM = 0.90 cm.

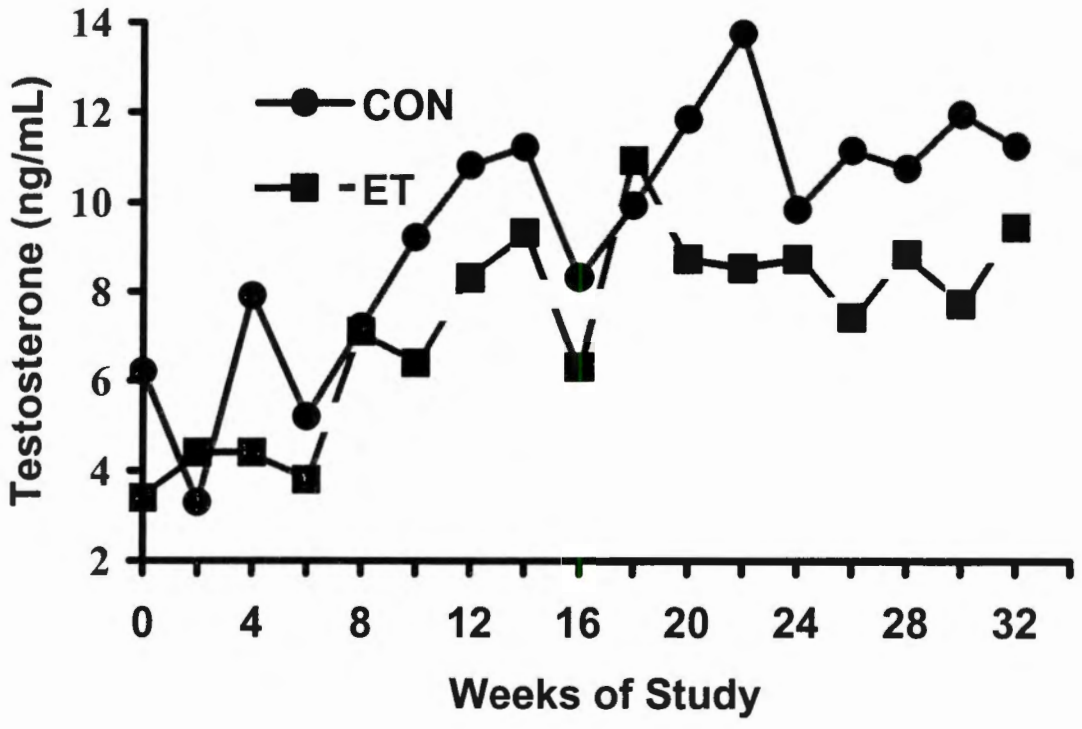

Figure 8. Concentrations of testosterone for **ET** and **CON** bulls collected during administration of ergotamine tartrate did not differ during experimental period. Pooled SEM = 1.64 ng/mL.

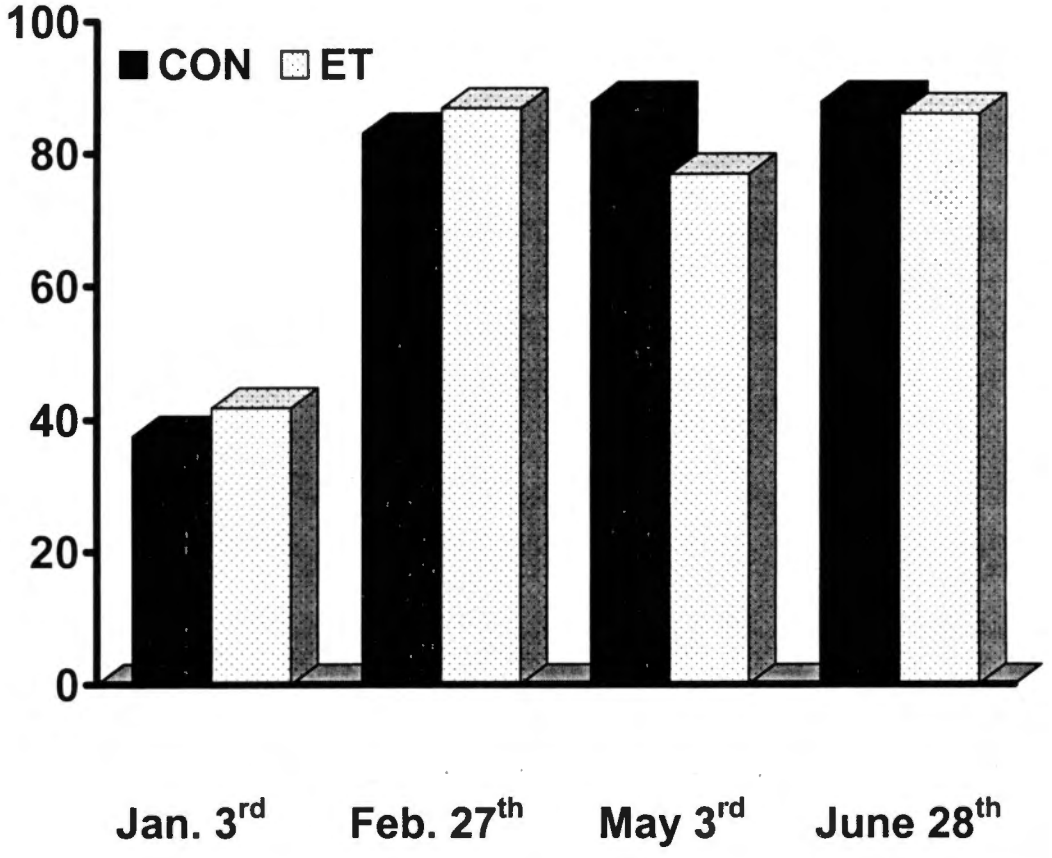

Figure 9: Sperm motility measured at the time of semen collection did not differ between **ET** and **CON** bulls (P= 0.83).

A date by replication effect was noted on the initial date of semen collection due to age of bulls at time of collection. The percentage of primary and secondary abnormal sperm was also not altered ($P > 0.10$; Table 2).

Bulls exposed to ergotamine tartrate had a significant decline (31.4°C ± 0.33°C) in testicular core temperatures compared to CON bulls (33.0°C ± 0.32°C; $P = 0.004$; Figure 10). Moreover, oocytes fertilized with semen from bulls administered ergotamine tartrate had reduced cleavage rate percentages compared to those fertilized with sperm from CON bulls ($P = 0.001$; Table 3); however, ability of cleaved embryos to develop to 8 to 16-cells was not different ($P = 0.10$; Table 3). The percentage of those embryos that did develop 8 to16 cells did not differ between treatments in their ability to further develop to blastocyst ($P = 0.59$; Table 3). Overall, blastocyst percentages also did not differ between ET and CON bulls ($P = 0.96$; Table 3).

Table 2: Sperm morphological differences at time of semen collection was not different between **ET** and **CON** bulls

Sperm	Normal Morphology %	Primary Morphology %	Secondary Morphology %
CON	76.9	13.5	9.5
ET	73.9	16.8	10.9
P-Value	0.51	0.33	0.54
SEM	3.6	4.3	2.9

CON: Sperm from bulls not receiving a diet of ergotamine tartrate
ET: Sperm from bulls receiving a diet supplemented with 40ug/kg body of ergotamine tartrate
Primary Abnormality: Abnormal head shape and presence of protoplasmic droplets
Secondary Abnormality: Detached heads, distal protoplasmic droplets, or bent tails

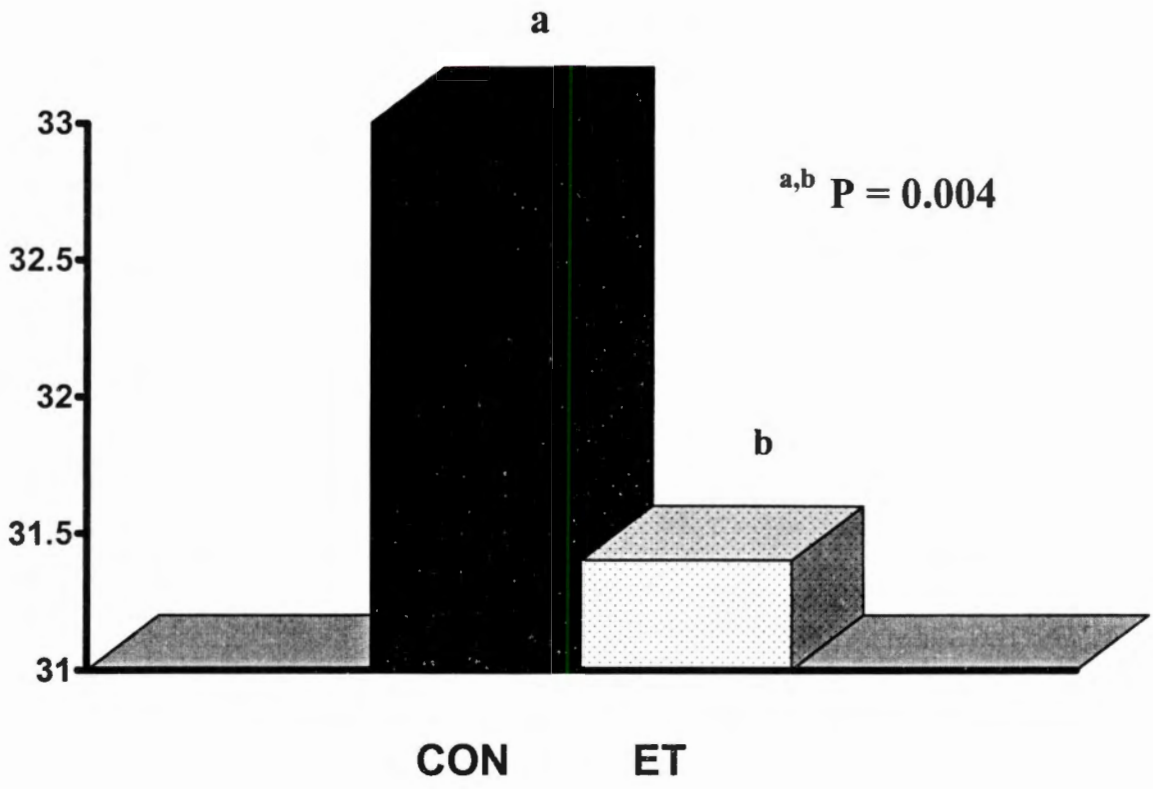

Figure 10. Testicular core temperatures declined in bulls receiving a diet of ET (P=0.004).

Table 3: Ability of sperm collected from bulls fed a **CON** or **ET** diet to fertilize bovine oocytes

TRT	REP (n)	Motility After Percoll	COC (n)	PZ (n)	Cleav (%)	8-16 (%)	Blast (%)
CON	2	59.5	200	169	69.2[a]	75.2	22.2
ET	2	59.3	200	143	51.1[b]	64.4	22.0
P-value		0.99			0.001	0.11	0.96

[ab]Least square means differ
Reps: total number of replications
Motility After Percol: proportion of motile sperm after Percoll-purification
COC: cumulus oocyte complexes
PZ: number of putative zygotes placed in culture after denuding of COC
Cleav: number of putative zygotes cleaved
8-16 cell: number of cleaved embryos developing to 8-16 cell
Blast: blastocyst; percentage of cleaved embryos developing to blastocyst

V. Discussion

Body Weight, Body Temperature, and Prolactin

Fescue toxicosis is derived from ingestion of over 40 different ergot alkaloids infesting tall fescue; therefore, administration of one synthetic compound (ET) may not be indicative of the full spectrum effects of fescue toxicosis on male fertility. Common signs of fescue toxicosis include: elevated rectal temperature, reduction in body weight gains, vasoconstriction of blood vessels and lowered prolactin concentrations during time of endophyte exposure. Bulls administered ergotamine tartrate exhibited suggestive signs of fescue toxicosis. ET bulls exhibited symptoms of elevated rectal temperature and reduced blood flow to the testicles. Surprisingly, endocrine function was not comprised. Neither prolactin nor testosterone concentrations declined during experimental period of ET administration in those bulls receiving ET supplementation compared to control bulls.

Weight loss associated with fescue toxicosis has been shown to impair reproductive performance in livestock (Hemken, 1979; Lishman et al., 1979; Washburn et al., 1989). During the experimental period of administration of ergotamine tartrate, diet was maintained such that nutrition did not play a role in reproductive events associated with simulated fescue toxicosis. Neither body weights or backfat thickness differed between treatment groups.

Fescue toxicosis has been shown to affect body weight by reducing average weight per day of age; furthermore, leading to complications in reproductive performance (Hemken et al., 1979; Lishman et al., 1979; Washburn

et al., 1989). Endophyte found within seed, hay, or green forage of tall fescue dramatically reduced feed intake in Holstein cows (Hemken et al., 1979). Further, conditions associated with fescue toxicity such as fescue foot are also responsible for weight loss.

A classic fescue toxic effect is elevated body temperature. When suffering from fescue toxicosis, ability to thermoregulate is impaired partly due to inability to completely shed (Hemken et al., 1979). Roughened hair coat, due to fescue toxicity, maintained throughout summer months contributes to elevated body temperature during periods of high ambient temperature. Ingestion of endophyte-infected tall fescue is believed to alter serum concentration of hormones that maintain body heat generation through metabolism (Browning et al., 1998a). Hormones such as thyrotropin (T4) and triiodothyronine (T3) are metabolic hormones intimately involved in thermoregulation. Heat intolerance resulting from ingestion of endophyte-infected tall fescue could be indicative of inadequate reduction of thyroid function, resulting in sustained high concentration of T3 and T4. Normal thyroid hormone secretion declines during periods of elevated ambient temperature, subsequently slowing metabolism. If thyroid hormones were unable to decrease, metabolism would remain high. Metabolism is an internal source of heat (Finch, 1986); therefore, ingestion of ergot alkaloids could increase metabolism and subsequently elevate body temperatures during periods of high ambient temperature.

Ergot alkaloids increase thyroid hormone secretion by acting directly on the hypothalamic-pituitary-thyroidal axis (Browning et al., 1998a). Acting both as

an agonist and antagonist, ergotamine exerts its effect on adrenergic, dopaminergic, and sertonergic receptors increasing T3 and T4 secretion; thus, negating effects of elevated temperature to reduce the rate of metabolism. By increasing metabolism through T3 and T4, internal heat is elevated directly affecting body temperature.

Other possibilities why rectal temperatures increased when exposed to ergotamine tartrate would be impaired ability to dissipate excess heat to extremities. Animals do not exhibit the same capacity to thermoregulate due to differences in hair and skin coat thickness. Under normal physiological conditions, mammals attempt to dissipate elevated body temperature by vasodilatation of the capillary beds. By increasing blood flow to the extremities, core temperatures would decline reducing overall body temperature. Vasodilatation, however slight, is the normal response to elevated temperature. *In vitro* studies confirmed that upon treatment with ergot alkaloids, vasoconstriction of the dorsal pedal veins occurred (Hill, 1994). Studies have shown that animals affected by fescue toxicosis have decreased ability to shuttle blood away from the body core. Elevated rectal temperatures observed in ET bulls were similar to increased rectal temperatures reported in steers administered ET (Osborn et al., 1992).

A consistent measurable effect of fescue toxicosis is decreased serum prolactin (Osborn et al., 1992; Porter and Thompson, 1992; Oliver, 1997; Browning, 2000; Seals et al., 2002). However, during the experimental period of ergotamine tartrate administration, prolactin secretion did not decline in bulls

receiving ET. When remembering that ergotamine tartrate is synthetic and only has 10% potency of natural ergovaline (Fluckinger et al., 1978), ET concentrations may need to be increased in order to produce symptoms in bulls. It must be remembered that fescue toxicosis is derived from ingestion of over 40 different ergot alkaloids; therefore, administration of single compound (ET) may not be indicative of fescue toxicosis. Prolactin concentrations have differed amongst studies utilizing ET to simulate effects of toxic fescue on serum prolactin concentrations (Browning et al., 1997; Rice et al., 1997; Seals et al., 2002). Species and mode of ET administration have been shown to influence ability of ergotamine tartrate to inhibit prolactin secretion (Browning et al., 1997; Rice et al., 1997; Seals et al., 2002).

The agonist and antagonistic properties exhibited by ergot alkaloids could offer another consideration why prolactin concentration did not decline in bulls administered ergotamine tartrate. Ergot alkaloids have been shown to work with adrengergic, serotonin and dopamine receptors (Solomons et al., 1986; Oliver et al., 1993). Stimulating dopamine inhibits prolactin secretion, while adrengergic receptors have been shown to stimulate prolactin concentration (Jabbour and Lincolin, 1999). Environmental factors such as day length and subsequent elevated melatonin increase prolactin secretion (Lincoln and Short, 1980); thus, offering further explanation why a synthetic compound could not suppress prolactin secretion. While serum prolactin concentrations did not differ between **ET** and **CON** bulls, other effects of ET such as elevated rectal temperatures and vasoconstriction of blood vessels flowing to extremities were still apparent.

Testicular Response to Administration of Ergotamine Tartrate

Receiving a diet of ergotamine tartrate did not alter scrotal circumference of treated bulls. Scrotal circumference is usually indicative of body weight; therefore, it is not surprising that scrotal circumference did not differ when considering body weight was maintained. Scrotal circumference in bulls and rams has been shown to be highly correlated with testicular weight and subsequently sperm production (reviewed by Coulter and Foote, 1979). Blood flow was reported to parallel testicular weight (Wang et al., 1993).

When considering that scrotal circumference did not differ between treatments, it is not surprising that serum testosterone concentrations did not significantly decline during administration of ergotamine tartrate. Testosterone concentrations paralleled results of Evans et al. (1988) reporting no difference in testosterone concentration in bulls calves fed endophyte infected fescue hay.

Apparent testicular alteration due to administrating ergotamine tartrate was seen in reduced testicular core temperatures. Testicles of bulls fed a diet containing ET were 2 degrees cooler than control bulls. Vasoconstriction occurs under the influence of ergot alkaloids; thus, inhibiting blood flow to the testicles resulting in cooler testicular temperatures in those bulls receiving ET. Clark et al. (1978) found ergotamine restricted normal blood flow within capillaries by as much as 10 times that of normal flow. Constriction of peripheral capillary beds would reasonably explain a decrease in peripheral temperatures including testicular core temperatures. Effects of ergotamine tartrate on testicular core

temperatures are of significant importance in spermatogenesis. Impaired thermoregulation due to reduced blood flow of the scrotum and testicles can cause infertility (Gazvani et al., 2000).

Vasoconstriction has been contributed to interaction of the ergot alkaloids with adrenergic, dopaminergic, and sertonergic receptors. Tall fescue also produces a chemical associated with the endophyte fungus known as Lysergamide (LAA) that also acts as a further agonist or antagonist to produce vasoconstriction (Bacon and DeBattista, 1991). Oliver et al. (1993) determined that LAA produced vasoconstriction *in vitro;* however, amount of contractility was dependent on the amount of LAA added.

Testicular temperature must be maintained between 30 and 34° C, which is between 2 to 4 degrees cooler than body cavity temperature, in order not to impair spermatogenesis (Harrison and Weiner, 1948; Purohit et al., 1985). Bulls administered ergotamine tartrate had significantly lowered testicular core temperatures than that of control bulls; however, testicular temperature remained within parameters for normal spermatogenesis.

Numerous studies have illustrated the detrimental effects of elevated temperatures on sperm (Hall et al., 1985; Wang et al., 1993; Setchell et al., 1995). Sperm exposed to elevated temperature within the testis have reduced motility, compromised acrosome integrity, and suffer extreme morphological change. Unfortunately, no studies have looked at effects of lowered testicular temperatures on sperm integrity and subsequent fertilization. Hall et al. (1985) reported that primary functions of rat Sertoli cells (protein synthesis, production of

cAMP, and lactate production) were greater at 38 °C compared to 34°C. This implies that Sertoli cells function more optimally at higher temperatures. The molecular aspects of the sperm affected by lowered testicular temperature must be examined.

Use of In Vitro Fertilization as an Indicator of Fertility

Utilization of IVF provided an optimal environment for fertilization to occur as well as aiding in maturation and penetration of the oocyte with sperm. Gross motility and morphology was not different in **ET** bulls compared to **CON** during experimental period; however, ability of embryos to cleave after fertilization with sperm exposed to ET may have been compromised. Due to the fact that oocytes fertilized with sperm from bulls suffering from simulated fescue toxicosis did not cleave as well as that of those fertilized with **CON** sperm, implied that **ET** could produce "ultrastructrual" damage to sperm undetectable with gross morphological diagnostics. Further illustrating that sperm damage may occur without recognition, Austin (1975) reported that sperm have the ability to remain motile much longer than their ability to maintain fertilization capacity; thus, motility may remain high but sperm may be unable to penetrate zona pellucida. However, IVF provides calcium required in penetration of the cumulus by the sperm; therefore, eliminating potential binding capacity problems associated with membrane integrity. Ability of putative zygotes (PZ) that cleaved to further progress to blastocyst, suggests that while administration of ergotamine tartrate may compromises morphology, sperm integrity was not completely destroyed.

IV. Summary and Implications

Bulls administered ergotamine tartrate exhibited classic "fescue toxic" effects; however, severity of symptoms was not indicative of fescue toxicosis. Rectal temperatures became elevated in bulls receiving ET. Due to experimental requirements, neither body weight nor backfat differed amongst treatment. Serum prolactin did not differ between bulls administrated ergotamine tartrate and controls. Scrotal circumference also did not differ between treatment groups; however, testicular core temperature declined with administration of ET. Temperature reduction in the testicles was presumably due to the vasoconstrictive properties of ergotamine. Testicular core temperatures remained within acceptable range for normal spermatogenesis to occur. Gross motility and morphology of sperm from bulls administered ET was not compromised. However, cleavage rates of PZ were depressed when utilizing sperm exposed to ET for fertilization; subsequent developmental competence was not compromised. Results suggest that while gross motility and morphology remained unchanged, possible undetected "ultrastructural" damage to sperm components may have been caused by ET administration. In conclusion, results imply that while fertility was only slightly affected, ET may damage sperm in ways undetectable with normal semen inspection. Further investigation in how fescue toxicosis affects sperm morphology is warranted.

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Vita

Heather Elizabeth Blackmon was born on December 31, 1976 in Tigerville, South Carolina. She attended Blue Ridge High School in Blue Ridge, SC from 1991 until graduation in June 1995. Heather received a NCAA scholarship to run track and cross- country for Coastal Carolina University in Conway, South Carolina where she majored in Biology. In the spring of 1999, Heather graduated from Coastal with a Bachelor of Science degree. Heather entered graduate school in January 2000, with extreme interest in beef cattle reproduction, under the direction of Dr. F. Neal Schrick. In May of 2002, Heather graduated with a Master of Science degree in Animal Science with concentration in Reproductive Physiology. She is currently pursuing a career in embryology with the Cleveland Clinic Foundation, Cleveland, OH.