Effect of supplemental trace mineral level and form on peripubertal bulls
by Whisper Lynn Alexander

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Animal and Range Sciences
Montana State University

Abstract:
The objectives were to determine if different supplemental trace mineral levels and /or forms (sulfate and metal amino acid complexes) influenced age at puberty, semen quality, endocrine status and scrotal circumference in peripubertal bulls. Forty-eight crossbred, prepubertal bulls were blocked by age (258 + 8.9) and scrotal circumference into five different treatment groups: 1) lx sulfate form (1S); 2) lx complexed form (1C); 3) 1S + 1C (2SC); 4) 1S + 2x 1C (3SCC); and 5) 3x 1S (3S). Each lx supplementation level contained 360 mg Zn, 125 mg Cu, 200 mg Mn and 12.5 Co. Liver biopsies were collected on d -21 and d 100, and were analyzed for Zn, Cu, and Mn. Scrotal circumference, semen, and blood samples were collected on d -14, 14, 42, 70, and 98. Semen was collected by electroejaculation and spermatozoa evaluated for morphology and motility. All bulls were deficient in Cu yet adequate in Zn on d -21. All animals except 2 were adequate in Mn. Following 100 d on treatment, liver Zn concentrations decreased ($P < 0.01$) and liver Cu concentrations increased ($P < 0.01$) in bulls regardless of treatment. Day 100 liver Mn concentrations were not different ($P > 0.01$) across any treatments or when compared to d -21. Day 100 liver Zn concentrations were similar ($P = 0.59$) across treatments, but liver Cu concentrations were greater ($P = 0.07$) in 3SCC and 3S bulls compared to 1C and 1S bulls, whereas 2SC bulls were intermediate. On d 42, m ore ($P = 0.03$) ulls fed complexed trace minerals (1C, 2SC, 3 SCC; 79%) were pubertal compared to those fed only sulfate trace mineral (1S, 3S; 47%). Bulls fed complexed supplement tended to reach puberty after fewer ($P = 0.11$) days on treatment (43.9 ± 5.7 d) than bulls fed only sulfate supplement (58.5 ± 6.7 d). We conclude that NRC recommendations for Zn may be inadequate for peripubertal bulls. Supplementing complexed Cu and Zn to prepubertal bulls may lower the age at puberty, however, no differences ($P > 0.41$) in semen characteristics were observed at one year of age.

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ABSTRACT

The objectives were to determine if different supplemental trace mineral levels and /or forms (sulfate and metal amino acid complexes) influenced age at puberty, semen quality, endocrine status and scrotal circumference in peripubertal bulls. Forty-eight crossbred, prepubertal bulls were blocked by age (258 ± 8.9) and scrotal circumference into five different treatment groups: 1) 1x sulfate form (1S); 2) 1x complexed form (1C); 3) 1S + 1C (2SC); 4) 1S + 2x 1C (3SCC); and 5) 3x 1S (3S). Each 1x supplementation level contained 360 mg Zn, 125 mg Cu, 200 mg Mn and 12.5 Co. Liver biopsies were collected on d -21 and d 100, and were analyzed for Zn, Cu, and Mn. Scrotal circumference, semen, and blood samples were collected on d –14, 14, 42, 70, and 98. Semen was collected by electroejaculation and spermatozoa evaluated for morphology and motility. All bulls were deficient in Cu yet adequate in Zn on d -21. All animals except 2 were adequate in Mn. Following 100 d on treatment, liver Zn concentrations decreased ($P < 0.01$) and liver Cu concentrations increased ($P < 0.01$) in bulls regardless of treatment. Day 100 liver Mn concentrations were not different ($P > 0.01$) across any treatments or when compared to d -21. Day 100 liver Zn concentrations were similar ($P = 0.59$) across treatments, but liver Cu concentrations were greater ($P = 0.07$) in 3SCC and 3S bulls compared to 1C and 1S bulls, whereas 2SC bulls were intermediate. On d 42, more ($P = 0.03$) bulls fed complexed trace minerals (1C, 2SC, 3SCC; 79%) were pubertal compared to those fed only sulfate trace mineral (1S, 3S; 47%). Bulls fed complexed supplement tended to reach puberty after fewer ($P = 0.11$) days on treatment (43.9 ± 5.7 d) than bulls fed only sulfate supplement (58.5 ± 6.7 d). We conclude that NRC recommendations for Zn may be inadequate for peripubertal bulls. Supplementing complexed Cu and Zn to prepubertal bulls may lower the age at puberty, however, no differences ($P > 0.41$) in semen characteristics were observed at one year of age.

CHAPTER 1

INTRODUCTION

The use of yearling bulls has gained widespread popularity. Producers use yearling bulls as a vital tool in an attempt to accelerate genetic turnover of economically important traits. A 1993 survey of beef cow/calf management practices revealed that 67% of beef operations in the northwestern United States use yearling bulls (Dargartz, 1993). Approximately 320,000 yearling purebred beef bulls are utilized for breeding each year in the United States (Kasari et al., 1996). Economic analyses of accumulated bull ownership and breeding costs associated with calf production per cow exposed, revealed greater profitability by using yearling bulls over 2-yr old bulls, given comparable genetic merit (Kasari et al., 1996). This economic advantage was only applicable if yearling bulls had reached puberty by breeding time. To allow for bulls to reach full reproductive potential, they should reach puberty three to four months before turnout into the breeding pastures (Gosey, 1983). Earlier puberty would also be beneficial for superior sires that are utilized in artificial insemination systems. Semen collection of bulls at an early age is advantageous both for semen sales and earlier analysis of progeny for the assessment of an AI sire's value (Jimenez-Severiano, 2002).

Although there are several advantages to utilizing bulls as yearlings, there are also some disadvantages, which must be considered. Spitzer et al. (1988) and Kennedy et al. (2002) reported that 20 to 24% of all yearling bulls tested did not pass a Breeding Soundness Exam (BSE). In a review of 1,276 BSE records, 43% of bulls less than 15 months old were classified as unsatisfactory breeders or recommendation deferred

(Carson and Wenzel 1997). Bulls that have not reached puberty by the time of a BSE and thus fail an evaluation, may be slaughtered, regardless of their genetic merit or phenotype. Elmore et al. (1975) re-evaluated 45 bulls less than two years of age that scored questionable or unsatisfactory on an initial BSE and reported 69% of these bulls' scores improved to satisfactory status 75 d later. Elmore et al. (1975) concluded that the primary cause of these yearling bulls' failure to pass the initial BSE was due to immaturity.

The initiation of puberty is dependent on the development of several interrelated systems. The most influential systems, which must develop for the initiation of spermatogenesis, are the testis, supporting glands and the endocrine system. As mentioned above, it is economically beneficial for a bull to reach puberty at an early age. Many of the factors that influence age of puberty in bulls are under direct control of management. It has been well documented that nutrition is an important factor in achieving reproductive maturity (Maas, 1987; Rice, 1991; Brown, 1994). In prepubertal life, under nutrition often will retard sexual development, delay the age of puberty and suppress spermatogenesis (Brown, 1994). The energy and protein requirements for peripubertal bulls has been investigated and debated (Flipse and Almquist, 1961, Coulter et al., 1997), but very little has been reported regarding the impacts of trace minerals on bull reproduction.

There are twenty-two mineral elements known to be essential for animal life. However, Corah (1996) reported that Cu and Zn were the two trace minerals most likely to be deficient in a large range of forages. Copper and Zinc deficiencies have both been reported to have negative effects on reproduction (Mertz, 1986; Corah and Ives, 1991)

however, the effects Cu and Zn deficiencies have on peripubertal bulls have not been investigated. Zinc has long been known to have many functions in the male reproductive system (Mertz, 1986; Corah and Ives, 1991; Arthington et al., 2002). Although Cu is not known to have a large impact on the male, it is known to have antagonistic and synergistic relationships with Zn, thus Cu levels are also of interest.

In addition, trace mineral supplement recommendations (NRC, 1996) were established for animals in maintenance status. Increased demands and stress applied to future potential sires to achieve excellent rates of gain, feed efficiency, and conformation, all while maturing sexually, may increase mineral requirements (Hutchenson and Cole, 1986).

Currently, trace minerals have gained considerable attention due to the wide range of different forms commercially available. The availability of these different forms of trace minerals further complicates the issue, as each form differs in bioavailability and absorption rates.

CHAPTER 2

LITERATURE REVIEW

Introduction

The purpose of this chapter is to critically review the scientific literature in the following areas 1.) testicular structure and function, resulting in spermatogenesis 2.) puberty and the factors affecting puberty 3.) Zinc absorption, molecular properties and the effect of Zn deficiencies on male reproduction 4.) Zinc supplementation.

Male Reproductive System

The testes are the site of sperm production and maturation. Their production is tightly regulated by an endocrine system involving the hypothalamus and pituitary.

Testis Structure

The testes, which are the paired male gonads, lay within an extension of the abdominal wall called the scrotum. The scrotum's pendulous nature, along with the tunica dartos muscle, allows for critical temperature regulation and support of the testes. Each testis is covered by the testicular capsule, composed of two layers, the visceral tunica vaginalis and the tunica albuginea (Senger, 1999). The interior of the tunica albuginea borders the highly vascular surface called the tunica vasculosa, which encompasses the lobules. Along with the layers of connective tissue within the testicular capsule, smooth muscle fibers contribute to sperm transport from the site of

spermatogenesis in the seminiferous tubules to the rete tubules and efferent ducts by contracting and relaxing when stimulated by neurotransmitters, acetylcholine and norepinephrine (Knobil and Neill, 1994; Senger, 1999).

Within the testicular capsule is the parenchyma, the major cellular mass of the testis. The parenchyma is composed of two main components, the seminiferous tubules and the interstitial tissue. In mature bulls, the majority of the parenchyma consists of the seminiferous tubules arranged within lobules. These convoluted loops are open ended with both ends empting into the rete tubules. Functionally, the seminiferous tubules are divided into two compartments, facilitating the two main types of cells contained within the seminiferous tubules, sertoli cells and germ cells. The first compartment is the basal compartment, which is the attachment site of the sertoli nurse cells and the site where germ cell formation begins. In the adluminal compartment, germ cells surrounded by sertoli cells develop into spermatocytes, spermatids and spermatozoa that are released into the rete tubules. The rete tubules transport spermatozoa to the efferent ducts, which then empty into the epididymis. Spermatozoa undergo final maturational changes in the epididymis (caput and corpus) and are stored in the cauda epididymis. Sertoli cells establish a blood-testis barrier, maintain high intratesticular testosterone concentrations, and nourish the developing germ cells (Berndston and Desjardins, 1974; Moura and Erickson, 2001).

The parenchyma also consists of the interstitial tissue, occupying the space between the seminiferous tubules. The interstitial tissue consists of blood, connective tissue, lymphatics, nerves and Leydig cells. The Leydig cells occupy up to 50 % of the total interstitial tissue (Knobil and Neill, 1994). These hormone secreting, polyhedral

cells are found in clusters and consist of extensive smooth endoplasmic reticulum (Amann and Schanbacher, 1983). The primarily function of these cells is to synthesize, secrete, and bathe the seminiferous tubules in testosterone (Zirkin et al., 1980; Amann and Schanbacher, 1983).

Testis Development

Curtis and Amann (1981) reported a four-fold increase in testicle weight of Holstein bull calves between 12 and 32 wks of age (Figure 2.1). This increase in weight was credited to the increase in diameter and total length of the seminiferous tubules. At 12, 16, 20, 24, 28, and 32 wks of age, seminiferous tubules occupied 44 ± 2, 47 ± 2, 53 ± 2, 62 ± 2, 73 ± 2 and 81 ± 1 % of the parenchyma, respectively (Curtis and Amann, 1981). Compared to 12 wks of age, bulls castrated at 32 wks of age experienced a seminiferous tubule diameter increase from 65 to 208 μm and an increase in length of seminiferous tubules from 830 to 2010 m/testis (Curtis and Amann, 1981).

Hochereau-de Reviers et al. (1987) reported a five-fold increase in the number of Sertoli cells between the time of Sertoli cell differentiation and puberty in rams and bulls. Curtis and Amann (1981) reported that indifferent cells began to differentiate into Sertoli cells at about 20 wks of age at a testis weight of 31 grams (Figure 2.1). While the literature differs in the time at which Sertoli cells first appear (between 20-28 weeks of age), all research seems to point to Sertoli cell formation beginning when testis weight is between 30-35 grams (Abdel-Raouf, 1960; McCarthy et al., 1979; Curtis and Amann, 1981).

Moura and Erickson (1997) reported 4.9 ± .3 x 10^9 sertoli cells per testis in 12 month-old Angus bulls. The number of A-spermatogonia was positively correlated with the number of Sertoli cells (Curtis and Amann, 1981; Knobil and Neill, 1994). It is likely that each Sertoli cell has the capability to facilitate development of only a given number of germ cells (Berndston and Desjardins, 1974). Berndtson and Desjardins (1974) investigated dairy bulls and reported that the number of Sertoli cells was correlated to the total daily sperm production (R^2 = 0.68) and testis mass (R^2 = .56). The Sertoli cell appears to be a main determinate of mature testicle size and total daily sperm production (Moura and Erickson, 2001). Thus, adequate formation of Sertoli cells during peripubertal development is a vital determinate of total sperm production in the adult bull (Amann, 1983; Berndtson et al., 1987, Hochereau-de Reviers et al., 1987). Both formation and maturation of Sertoli cells seems to be hormonally regulated by FSH (Knobil and Neill, 1994; Senger, 1999). Studies have found, however, that testosterone is also involved in Sertoli cell differentiation (Moura and Erickson, 2001).

Endocrine Involvement with Testicular Structures

The interaction of testicular structures and their resulting production of male germ cells are dependent on the balanced, endocrine interplay of the hypothalamus, the pituitary and the testis.

GnRH Gonadotropin releasing hormone (GnRH) is a neuropeptide product of hypothalamic neurons and is considered the master hormone of reproduction in both the male and female. Gonadotropin releasing hormone is transported to the pituitary by the portal vascular system where it interacts with receptors to initiate release of the

gonadotropin hormones, follicle stimulating hormone (FSH) and luteinizing hormone (LH; Griffin and Ojeda, 1988). Release of GnRH in the mature male is regulated by a negative feedback loop incorporating testosterone and estradiol (Senger, 1999).

LH Luteinizing hormone is released into the bloodstream from the anterior pituitary as a result of GnRH stimulation. Luteinizing hormone travels to the interstitial tissue of the testis to react with membrane bound receptors on Leydig cells where it stimulates conversion of cholesterol to testosterone. Luteinizing hormone is released from the anterior pituitary in a pulsatile fashion, three to eight times per day, causing a similar pattern of testosterone release. It is thought that LH and testosterone are released in this wave-like fashion for two reasons. First, high levels of testosterone are needed for normal spermatogenesis, however, not at a continual basis. Second, Leydig cells become less responsive to LH after continual exposure (Hochereau-de Reviers et al., 1987; Knobil and Neill, 1994; Senger, 1999).

FSH Like LH, FSH is released from the anterior pituitary in response to GnRH; however, the target cells for FSH are the Sertoli cells of the seminiferous tubules. Follicle stimulating hormone binds to receptors on Sertoli cells promoting protein secretion, as well as energy metabolism. Follicle stimulating hormone plays a key role in the development of the immature testis, particularly by controlling Sertoli cell proliferation (McLachlan et al., 2002).

Testosterone Testosterone is essential for the maintenance of normal spermatogenesis and fertility (Knobil and Neill, 1994; McLachlan et al., 2002; Parks et al., 2002). The Leydig cells of the interstitial tissue produce testosterone from five

enzymatic steps beginning with the precursor steroid, cholesterol. About half of the cholesterol used by the Leydig cells is produced *de novo* beginning with acetate; the remainder is taken up from circulation (DeRobertis and DeRobertis, 1980; Griffin and Ojeda, 1988). Most of the enzymes used for testosterone synthesis are stored in the smooth endoplasmic reticulum and mitochondria of the Leydig cells (Zirkin et al., 1980). The main rate-limiting step in the production of testosterone is found in the conversion of cholesterol to pregnenolone within the mitochondria. Once testosterone is produced it will have one of several fates. Testosterone may be converted to 5α-dihydrotestosterone and by aromatization to estradiol which will enter the bloodstream and inhibit gonadotropin secretion (Griffin and Ojeda, 1988). Testosterone may also enter the seminiferous tubules where after conversion to estradiol, by the sertoli cells, estradiol will bind to Sertoli cell receptors and promote spermatogenesis (Senger, 1999). Testosterone greatly contributes to the later stages of spermatogenesis by quantitatively maintaining meiosis and spermiogenesis and in conjunction with FSH facilitates spermiation (Courot and Ortavant, 1981; McLachlan et al., 2002). Testosterone may also prolong the lifespan of epididymal spermatozoa, as well as promote growth, development, and secretory activity of the accessory sex organs. The horse produces testosterone in the seminiferous tubules and epididymis which may aid in growth development and secretory activity of accessory sex glands (Hafez, 1993).

Inhibin Inhibin is a polypeptide hormone composed of a α subunit covalently linked to either a β_A subunit (inhibin A) or β_B subunit (inhibin B). Inhibin A and B are both produced by the Sertoli cells, however, inhibin B is the only form present in the

circulation of men (Chada et al., 2003). Inhibin B regulates synthesis and secretion of FSH in a negative feedback loop, thus controlling the number of sperm produced. An injection of inhibin B to male mice, decreased the number of B-spermatogonia entering meiosis (Van Dissel-Emilian et al., 1989). Allenby et al. (1991) reported that when spermatids are experimentally depleted from the seminiferous epithelium, blood FSH levels increased and inhibin levels decreased. From this, Knobil and Neill (1994) have speculated that there is a mechanism by which numbers of mature spermatozoa regulate the number of differentiating spermatogonia entering meiosis.

Spermatogenesis

Spermatogenesis is the process by which the most primary germ cells in the basal compartment of the seminiferous tubules, called the spermatogonia (2n), divide and develop into a haploid germ cell, the spermatozoa (1n), located in the adluminal area of the seminiferous tubule. Spermatozoa are cells very different from any other type of cell found in the body. They have a compact head within which the nuclear DNA is condensed and inactive (Knobil and Neill, 1994). Spermatogenesis is divided into three continuous phases: spermatocytogenesis, a meiotic division, and spermiogenesis. The beginning phase of spermatocytogenesis incorporates several mitotic divisions. Within the basal compartment of the seminiferous tubule, three sequential types of spermatogonia are formed, A – spermatogonia, I – spermatogonia, and B – spermatogonia. Continual sources of A – spermatogonia are also produced to replace the stem cell population, allowing for the process to continue indefinitely. When cells progress to B – spermatogonia status, they will then undergo the necessary nuclear changes to duplicate their DNA in the first stage of meiosis called prophase. The

completion of prophase results in a secondary spermatocyte. These cells will then finish the meiosis divisions producing haploid cells (1n) known as spermatids. Not only are the cells maturing and dividing, they are physically progressing from the basal compartment to the adluminal compartment toward the center of the seminiferous tubule. The entire process of spermatocytogenesis takes approximately 21 d in a bull (Senger, 1999). As there are no more mitotic divisions, the number of daughter cells emerging into the next phase, spermiogenesis, represent the number of spermatozoa that will eventually be released from the seminiferous epithelium. This is assuming that there is no subsequent cell degeneration (Hafez, 1993; Knobil and Neil, 1994; Senger, 1999).

The spermiogenesis phase of cell development describes the sequential morphological changes of the round spermatids into spermatozoa. These developmental progressions are categorized into four stages: the Golgi Phase, Cap Phase, Acrosomal Phase, and Maturation Phase. The spermatids physical appearance and the internal mechanics change drastically as they develop through these stages. Cells develop tails, an acrosome develops and the nuclear chromatins condense, to form fully developed spermatozoa. The release of the spermatozoa into the lumen of the seminiferous tubule is referred to as spermiation. The full cycle of spermatogenesis, which is the progression from A-spermatogonia to spermatozoa, takes 61 d in a bull (Amann, 1983; Senger, 1999). The release of the spermatozoa into the seminiferous lumen marks the beginning of the migration to the epididymis. During transport though the epididymis, spermatozoa undergo additional morphological and physiological changes necessary for their transport after ejaculation to the ova and fertilization. Travel through the caput, corpus, and cauda epididymis takes 14 d in the bull (Senger, 1999). Only spermatozoa, which are stored in

the distal cauda epididymis, are mature enough for fertilization (Hafez, 1993; Senger, 1999).

Puberty

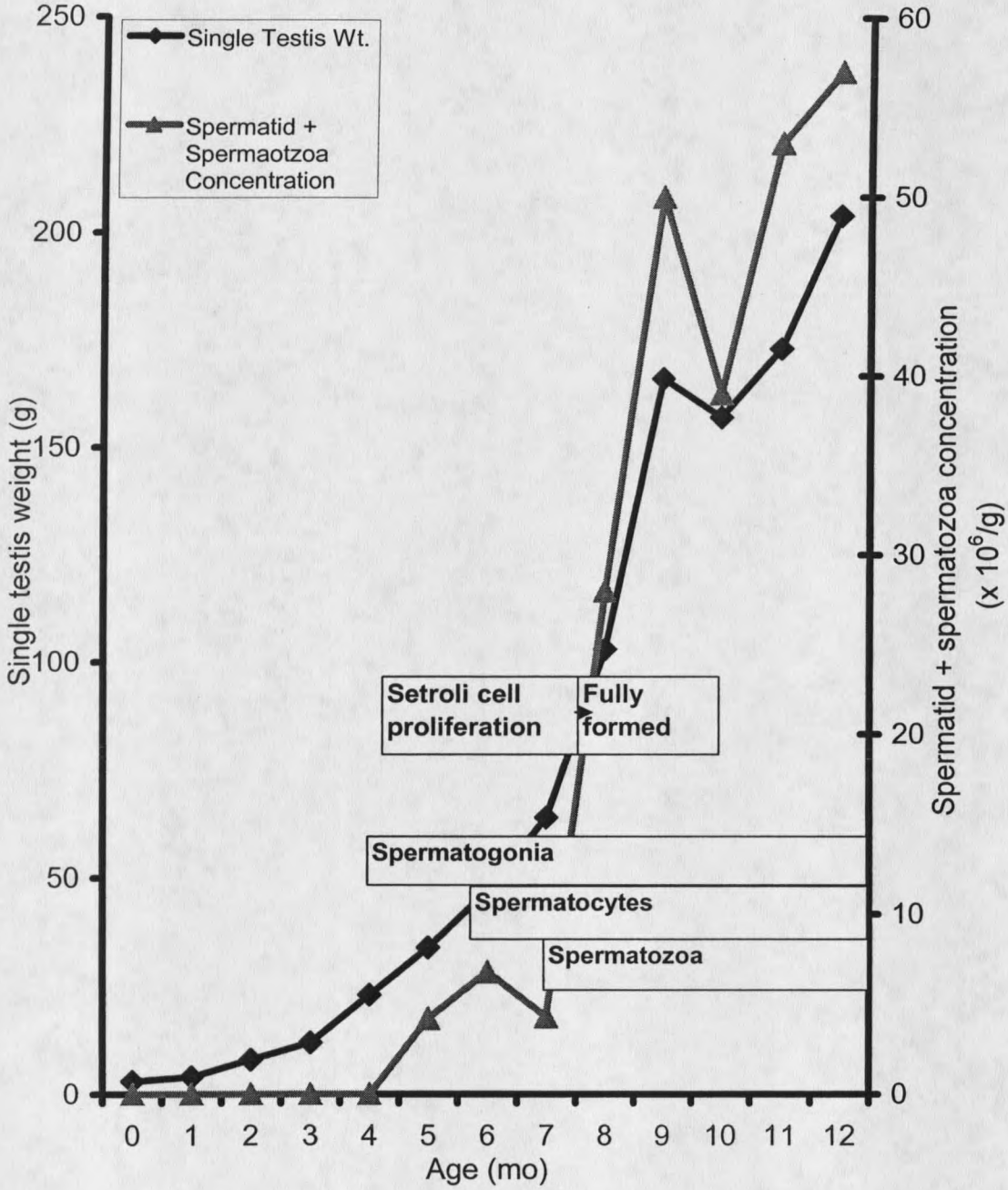
Measurements of Puberty Puberty is the maturational process of reproduction. There are several different ways in which puberty has been defined. A basic definition is the age at which an individual has the first capability to participate in reproduction. Brown (1994) defined puberty in the male as the maturation of physiological, biochemical, and behavior systems, supporting completion of spermatogenesis and accomplishment of an ejaculate capable of fertilizing an ovum. This however is difficult to measure, thus a criteria by which an ejaculate can be quantitatively measured, is necessary.

Currently, the most commonly utilized working definition of puberty in bulls is when an ejaculate contains 50×10^6 sperm of which $\geq 10\%$ are progressively motile (Lunstra et al., 1978; Amann, 1983; Amann and Schanbacher, 1983). This definition provides a specific endpoint in which to measure, however it is also important to note that the attainment of puberty does not signify full reproductive capacity. There is however, a close relationship between age at puberty and age of sexual maturity (Abdel-Raouf, 1960). The attainment of puberty is an obvious indicator of normal development of all involved systems. Puberty is characterized by a large increase in testis size and weight, changes in LH secretion patterns, increase in blood testosterone concentration, and the initiation of spermatogenesis, all of which will be explored in the following discussion (Amann and Schanbacher, 1983).

Initiation of Spermatogenesis Several vital events, although not fully understood, must occur before the final completion of spermatogenesis. These events include masculinization and maturation of the reproductive tract, multiplication of Sertoli cells, multiplication of the early stages of germ cells, and development of the endocrine system (Pelletier et al., 1981, Moura and Erickson, 1997, Knobil and Neill, 1994).

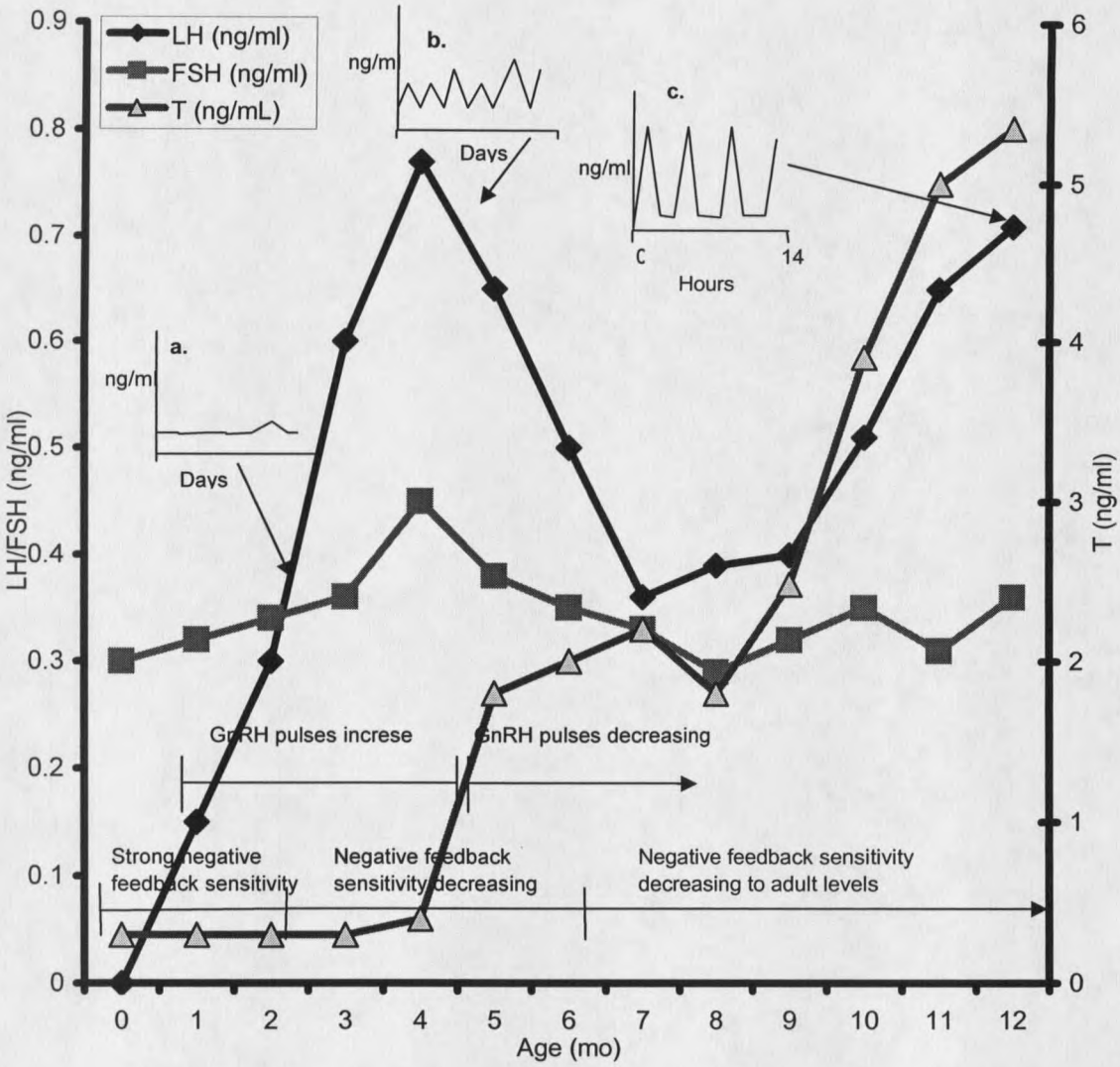
Histological examinations have provided evidence that the establishment of spermatogenesis in the developing bull calf is a progressive process that occurs over a number of weeks (Fig. 2.1; Abdel-Raouf, 1960; Curtis and Amann, 1981; van Den Dungen, 1990, Evans et al., 1996). Curtis and Amann (1981) evaluated cell progression in comparison to age of developing Holstein bulls. At 12 wks of age, all cross sections of the seminiferous tubules still contained only undifferentiated cells. Only a few A-spermatogonia were present in the tubules at 16 wks of age. By 20 wks of age however, A-spermatogonia were still the most mature cell types present however at greater numbers compared to 16 wks of age. These cells were joined by the presence of primary spermatocytes at 24 wks of age, and spherical spermatids by 28 wks. By 32 wks of age, 42% of the tubules contained elongated spermatids (Curtis and Amann, 1981).

Endocrine Regulation of Puberty After birth, the bull enters the infancy period. This period is characterized by little to no secretion of gonadotropins from the anterior pituitary, and very limited steroidogenesis by the Leydig cells (Evans et al., 1994, Evans et al., 1996). In dairy bulls, the infancy s stage progresses to the prepubertal stage between 10 and 12 wks of age (Amann, 1983; Amann et al., 1985). Transition to the prepubertal stage of development is marked by increased frequency and amplitude of LH secretion. Circulating testosterone concentrations begin to rise around 15 to 20 wks of



(Adapted from Berardinelli, 2003 and Willett and Ohms, 1957; MacMillian and Hafs, 1968; Killian and Amann, 1972, Humphrey and Ladds, 1975, Foote et al., 1976, Curtis and Amann, 1981; Evans et al., 1996)

Figure 2.1. Approximate single testis weight and spermatid plus spermatozoa concentrations of typical dairy bull calves from birth to one year of age. Correlating events associated with initiation of spermatogenesis are diagramed.



(Adapted from Berardinelli, 2003 and MacMillan and Hafs, 1968; Lacroix and Pelletier, 1979; Curtis and Amann, 1981; Evans et al., 1996)

Figure 2.2. Approximate levels of LH, FSH, and testosterone of a typical dairy bull from birth to one year of age. Correlating events associated with the negative feedback mechanism responsible for puberty are diagramed. **a.** High sensitivity to the negative feedback loop causes low levels and amplitudes of LH. **b.** Prepuberty has inconsistent low amplitude, low frequency pulses of GnRH from the hypothalamus resulting in a similar pattern for LH and T. The sensitivity to the negative feedback loop is undergoing changes resulting in a more constant pattern of GnRH release, maturation of Leydig cells and promotion of Sertoli cell function. **c.** Puberty to postpuberty consists of hypothalamic secretions in an episodic pattern occurring every two to six hours. This will allow LH to be released in a high amplitude and high frequency pattern initiating spermatogenesis.

age in dairy bulls (Evans, 1996). This event is in response to the earlier LH stimulation, which will induce differentiation of the Leydig cells and thus increased secretion of testosterone (Amann, 1983, Aravindakshan et al., 2000). Testosterone is involved in the differentiation of Sertoli and germ cells within the seminiferous tubules (Knobil and Neill, 1994). The prepubertal stage is followed by the pubertal stage of development (Figure 2.2).

Knobil and Neill (1994) suggested that the primary events, which set into motion the onset of male puberty, originate within the central nervous system. Very little is know about the processes which turns "on the switch" to allow for the initiation of puberty, yet the endocrine system is most likely the primary factor limiting puberty (Schanbacher, 1982; Knobil and Neill, 1994).

Although LH, FSH and testosterone are being produced during the prepubertal stage, there are not sufficient amounts of each hormone to complete spermatogenesis. Certain endocrine criteria must be met before spermatogenesis is initiated and puberty can be reached (Schanbacher, 1982). First, GnRH must be produced from the hypothalamus in adequate amounts. This requires the development of hypothalamic neurons capable of producing GnRH at high amplitudes and high frequencies. This, in turn, allows for adequate amplitude and frequency of gonadotropin secretion, FSH and LH, from the anterior pituitary and finally increased secretion of testosterone. Serum levels of inhibin B reach a peak during early life and have a positive correlation with FSH, LH, and Testosterone in both boys and rams. By mid-puberty, inhibin serum levels lose the positive correlation with LH and testosterone and inhibin develops its adult role as a strong inhibitor of FSH (Maddocks and Sharpe, 1990; Sanford et al., 2000; Chada et

al., 2003). Chada et al. (2003) reports that elevated Inhibin B is a direct marker of the presence and function of Sertoli cells and appears to reflect testicular function in boys. These are the major endocrine events that must occur for the bull to reach puberty and produce viable spermatozoa (Figure 2.2).

It has been well established that the onset of puberty is not limited by the development of the anterior pituitary or the gonads, as these systems when individually manipulated are functional (Knobil and Neill, 1994; Guyton, 1986). The hypothalamus and GnRH secretion are considered the limiting factors in initiation of spermatogenesis (Amann and Schanbacher, 1983). When the prepubertal anterior pituitary is stimulated by exogenous GnRH, it will produce sufficient amounts of FSH and LH to initiate spermatogenesis (Amann et al., 1986). In addition, when exogenous LH and FSH stimulation was provided to the prepubertal lamb testis, final maturation of the testis occurred (Wood et al., 1991; Senger, 1999). These findings suggest that the onset of puberty is controlled by the sensitivity of the hypothalamic-pituitary unit to a steroid negative feedback system (Wood et al, 1991; Knobil and Neill, 1994; Guyton, 1996; Senger, 1999). This is due to the inability of the GnRH neurons in the hypothalamus to release GnRH in a pattern necessary for the release of sufficient gonadotropic hormones. As the bull approaches puberty, hypothalamus sensitivity to the androgen negative feedback system decreases allowing for sufficient gonadotropin release to initiate elevated testosterone levels (LH function), complete spermatogenesis and thus puberty (Fig. 2.2; Amann, 1983).

Factors effecting age at puberty Peripubertal bulls are expected to excel in rate of gain, feed conversion, conformation, and structural soundness at a time in which the body

is undergoing maturation of the endocrine, behavioral, and reproductive systems in order to reach puberty. The age in which bulls are capable of producing mature spermatozoa resulting in fertilization varies considerably with genetics, nutrition, and stress. Lunstra and Echternkamp (1982) reported an 88 d variation in puberty among 31 bulls monitored and a 62-d day variation between the six different breeds and breed combinations evaluated. Bos taurus breeds reached puberty younger than Bos indicus breeds (Aire and Akpokodje, 1975; Latimer et al., 1982; Brito et al., 2001). Among, Bos taurus cattle, the dairy breeds tended to reach puberty earlier than Continental beef breeds with British beef breeds being intermediate (Latimer et al., 1982; Grossman et al., 1995). Furthermore, inbreeding and line breeding delayed puberty, while crossbreeding hastened puberty (Mwansa et al., 1999).

It is generally accepted that nutritional management is the main limiting or controlling managerial factor affecting attainment of puberty (Short and Adams, 1988). In prepubertal life, under nutrition may retard sexual development, delay the time of pubertal onset, retard the growth of the external genitalia and suppress spermatogenesis (Brown, 1994). Reproductive function in young animals appears to be more susceptible to dietary restrictions of energy and protein than in adult animals and severe feed restriction may permanently damage gonadal and neural tissue (Brown, 1994). One important nutritional requirement, which has received little investigation, is the balanced intake of bioavailable trace minerals. The balanced intake of bioavailable trace minerals has important effects on the production and reproduction of the cow (Apgar, 1985), however, very little is known about the effect trace minerals have on peripubertal bulls.

In particular, further research on the availability of Zn (see role below) is warranted in the male.

Zinc Function and Relation to Male Reproduction

Molecular Aspects of Zn

Vallee (1983) stated that the biological abundance of Zn, its ability to interact with a wide variety of different structural formations, and its resistance to oxidation-reduction have generated selective evolutionary pressure for Zn to serve in many biological catalysis. For most of the 200 Zn-dependent metalloenzymes, Zn will bind with the protein enzyme often resulting in a spherical shape and catalytic function (Vallee, 1983; Mertz, 1986). Zinc not only has catalytic functions, but also structural, and regulatory roles, and often Zn will play more than one function within a single compound. For example, alcohol dehydrogenase from the horse liver, binds 4 molecules of Zn; two Zn ions are needed for catalytic activity, while two are needed for structure (Mertz, 1986). Zinc also contributes to the stability of RNA, DNA, ribosome's, and membranes (Eckhert and Hurley, 1977; Davies, 1984-85).

The Zn finger protein gene family, one of the largest gene families, is a class of regulatory genes that encode nucleic acid-binding proteins, causing the activation or repression of downstream target genes (Yong-Xin et al., 2002, Ishizuka et al. 2003). The resulting Zn finger protein contains a single Zn atom linked to 4 cystein residues or 2 histidine residues and 2 cystiene residues holding the polypeptide in a finger shaped loop. A number of Zn finger protein genes are specific to the testis (Ishizuka et al. 2003, Weissig et al. 2003).

Obviously, Zn is an important aspect to the proper function of many biological processes and a Zn deficiency may alter these functions or the body's efficiency to perform these functions. When a Zn deficiency occurs, cell membrane stability will be altered through oxidative damage before intracellular components of a cell are affected. A review by Mertz (1986) reported that Zn deficiency altered function of specific receptors and nutrient absorption sites, activity of membrane bound enzymes, function of permeability channels, and function of carrier and transport proteins in the cell membrane.

Zn Absorption

Absorption of Zn is largely influenced by numerous host and dietary factors. In the bovine, 30% of Zn absorption occurs in the abomasum with the remaining absorption occurring in the duodenum, jejunum, and ileum (Miller and Cragle, 1965). In non-fasted rats, 60% of total absorption was from the ileum with 20% from both the duodenum and jejunum. Intestinal absorption begins with Zn moving from the intestine to the mucosal cells, probably by a carrier-mediated process when Zn is in the chelated form. At the brush border, Zn absorption occurs in two distinct phases. The fast absorption phase (less than 30 minutes) is mediated by a carrier molecule. This is followed by a much slower absorption phase, which consists of binding to non-specific mucosal components. Steel and Cousins (1985) reported that saturation of Zn absorption occurs in both Zn deficient rats as well as Zn adequate rats. In Zn deficient rats, however, the rate of absorption is more rapid and saturation levels higher (Steel and Cousins, 1985). At the brush border, many low molecular weight binding ligands including some amino acids enhance Zn mucosal uptake and absorption (Oestreicher and Cousins, 1982; Cousins, 1985; Mertz,

1986). These ligands can be influenced by nutritional and physiological status contributing to homeostatic control of absorption (Cousins, 1985). Miller and Cragle (1965) reported differences in daily net absorption of Zn between newborn Jersey calves, 5-12 mo old Jersey calves and mature Jersey cows (55%, 20%, and 12% respectively). These groups, however, were fed different rations that may have contributed to the large variation in absorption. Pregnant females become more efficient in absorbing, recycling, and storing Zn (Davis and Williams, 1977; Underwood, 1976; Mertz, 1986; Swenson, 1999). Davies and Williams (1977) reported a two fold increase in duodenum absorption of Zn in the pregnant rat. Endogenous Zn excretions into the intestine from pancreatic and bile secretions are important in keeping homeostasis of Zn for some species (Cousins, 1985). Cousins (1985) reported pancreatic Zn secretions increased by 25% in Zn-deficient pigs and that bile and pancreatic duct obstruction decreased the zinc absorption in rats. In contrast, pancreatic secretions are not essential for adequate Zn absorption in human studies (Cousins, 1985).

Once Zn is transferred to the mucosal cells it is then transferred to the portal blood system were it has a high affinity for albumin. Some amino acids may also facilitate transportation in the blood. When adequate Zn levels are present, binding to albumin occurs slower than absorption by the mucosal cells from the intestine and may contribute to the homeostatic control of the body. In the bloodstream, Zn enters red blood cells and /or is carried to soft tissues within hours of being bound to albumin. The main organ involved in Zn metabolism is the liver. As the blood passes through the liver, 30-40% of the Zn is absorbed by the liver (Cousins, 1985). Intercellular Zn is distributed among various metalloproteins, organelles, macromolecules, and membranes. High

intercellular Zn will activate the metallothionein gene producing metallothionein polypeptides that bind intracellular Zn after transport into the cell (Aggett, 1985; Cousins, 1985; Krebs, 2000).

Zinc Deficiencies

Zinc is essential for normal body growth and maintenance. Severe Zn deficiencies affect many systems in the body and thus, a wide spectrum of clinical signs may occur. Food intake and appetite are severely decreased by Zn deprivation (Neathery et al., 1972, Georgievskii et al., 1981, Mertz, 1986; Underwood and Suttle, 1999). As Zn is important in cell growth and replication, suppressed appetite may be a result of an adaptive or metabolite-driven control of substrates needed for normal cell function (Underwood and Suttle, 1999). If there is an impairment of nutrients for further cell growth, the requirements for Zn are decreased. However, Underwood and Suttle (1999) also suggest that there is an over production of cholecystokinin, an appetite depressing hormone, due to the lack of a Zn finger which will depress production. Often, other clinical signs are compounded by the inability or desire to eat. Pair-feeding studies report that the effects common to severe Zn deficiency are secondary to a loss of appetite (Neathery et al., 1974; Martin and White, 1992). Excessive salivation is an early sign of Zn deprivation due to a combination of excess saliva production and reluctance to swallow (Apgar, 1985).

With Zn deficiencies, animals have decreased feed efficiencies, which partially contribute to retarded body growth. In ruminants the microorganisms of the rumen also have Zn requirements and a deficiency will, therefore upset

metabolism in the rumen (Ruckebusch and Thivend, 1980). Skeletal development, as well as production of hair, wool, and feathers is also negatively influenced in Zn deficient animals. Zinc also has a role in wound healing and thus, delayed or failure of a wound to heal is a common clinical sign of Zn deficiency. Long term, severely deficient animals will develop parakeratosis. With parakeratosis, lesions are most severe in the hind limbs, nostrils, teats, head, and the neck (Underwood, 1999). In addition, joints may stiffen, and the extremities may swell with Zn deficiencies (Stevenson and Earle, 1956; Underwood, 1999; NRC, 1996). As with any deficiency, Zn deficiencies can occur in a variety of different levels and severities, thus resulting in different symptoms.

Clinical vs. subclinical deficiencies Underwood and Somers (1969) described two types of Zn deficiencies in growing rams. First are the severe deficiencies, which induce clinical signs, as discussed above. Second are the mild or subclinical deficiencies in which there are no clinical symptoms and body growth is normal, but testicular development and sperm production are markedly reduced. National Research Council (2000) recommends 30 ppm of Zn in all bovine diets. If no Zn antagonists are present, 30 ppm is considered sufficient to diminish clinical signs. However, specific amounts of Zn required to support optimal production have not yet been established. Animals experiencing high stress or highly demanding physiological states may have higher requirements than recommended for Zn (NRC, 1996). All of the clinical signs can be easily detected and diagnosed but subclinical deficiencies are not easily diagnosed and may pose a more serious threat to producers (Swenson, 1999). Subclinical deficiencies often result in thriftless or

suboptimal growth, fertility, or overall reduced production and performance. Subclinical deficiencies may present a greater problem as genetic selection for increased production continues. Adjustments have not been made by NRC (1996) for cattle experiencing high production stress. For example, adjustments in Zn requirements have not been made for situations such as yearling bulls in a bull test station where they are expected to grow to their maximum genetic potential, have superb feed efficiencies, reach puberty, and achieve superior semen production.

Mineral Interactions Mineral deficiencies may occur either as gross deficiencies or excesses or as 'conditioned' deficiencies or excesses. A conditional deficiency relates to other elements or compounds negatively interacting with the metabolism of the mineral of concern. These interactions may interfere with absorption, excretion, retention, or utilization (Underwood, 1976). This makes the basic trace mineral requirement equation even much more complex. Not only is the level of a mineral important in assessing an animal's requirements but the interactions of other compounds in the diet must also be considered.

These types of interactions have been reported between Zn and Cu in several species. Pigs are often supplemented with high levels of Cu to increase weight gains and feed efficiencies. Suttle and Mills (1966) fed Cu levels up to 750 ppm to pigs and found serious mineral imbalances. However, when 500 ppm of Zn and 750 ppm Fe were fed with the 750 ppm of Cu no signs of toxicity were present. Wellington et al. (1998) demonstrated the Cu-Zn relationship among heifers fed different combinations of Cu and Zn. Heifers fed 21 ppm Cu and 90 ppm Zn together experienced higher daily gain and were more efficient on feed when

compared to heifers fed only Cu or only Zn. The heifers fed Cu plus Zn also stored more liver Cu compared to heifers supplemented with only Cu. A negative impact on liver Cu concentrations (-42ppm) for heifers supplemented with only Zn was reported (Wellington et al., 1998). Hatfield et al. (2001) reported supplemental Cu (15.4 ppm) tended to increase concentrations of liver Zn in ewes, without any negative impact from high supplementation of Zn (102.2 ppm) on liver Cu concentrations. The interaction between Zn and Cu, as well as with Fe, may be due to competition for protein binding sites during absorption from the intestine (Underwood, 1976; Cousins, 1985; Hatfield et al., 2001).

High levels of sulfur and molybdenum will form thiomolybdates, which bind to Cu in the rumen to form insoluble complexes that are not absorbed or are stored in an unavailable form by the animal. This subject was reviewed by Suttle (1991) who reported that it is possible that thiomolybdates have a negative effect on the amount of Cu stored bound to metallothionein. The intravenous administration of ^{35}S-labeled thiomolybdates to cattle shifted Cu bound to metallothionein to a ^{35}S-labeled protein, thus making it unavailable to the animal. Under these circumstances, measurements of total blood, plasma, or tissue Cu would not reflect the true ability of the body to utilize the stored Cu. Suttle (1991) also suggested that thiomolybdates may also exert toxic effects on estrogen metabolism. Swenson (1999) reported a negative reproductive response to a trace mineral sulfate treatment when supplemented to first calf heifers. More heifers fed either complexed mineral or no mineral were bred (AI) compared to heifers fed only

sulfate trace minerals. Smart et al. (1985) reported that a diet with 0.3% sulfates resulted in a decrease in plasma Cu yet high sulfates had no affect on plasma Zn.

Male Reproduction and Zn

Not all of the specific functions of Zn are fully understood, however it has been well documented that Zn is essential for spermatogenesis and development of primary and secondary sexual characteristics. There is a peripubertal rise in the Zn concentration of the testis and prostate followed by a decrease to adult concentrations after puberty (Parizek, 1966). Zinc is two to three times higher in the testis and prostate compared to other tissues in the body (Crichton et al., 1982). In the testis, Zn plays a role in regulating dihydrotestosterone and functions as a scavenger of reactive oxygen species in the seminal plasma (Srivastava et al., 1983, Griffin and Ojeda, 1988, Irvine, 1996). The Zn found in the seminal plasma originates from the prostate (Leissner et al., 1980; Henkel et al., 2003). In ejaculated sperm, more than 93% of the Zn still bound to the spermatozoa is located in the flagellum with the remainder located in the head of spermatozoa functioning to stabilize the chromatin (Henkel et al., 1999; Henkel et al., 2003). The Zn found in the spermatozoa tail is especially concentrated in the outer dense fibers where it is bound to the sulfhydryl groups of cysteine. Over 60% of the Zn found in the flagellum is discarded during the epididymal maturation process (Henkel et al., 2003). This allows for the sulfahydryl groups to oxidize, thus stabilizing and stiffening the outer dense fibers allowing for efficient movement (Crichton et al., 1982; Srivastava et al., 1983; Henkel et al., 1999). In humans, the flagella Zn content of ejaculated spermatozoa is negatively correlated with motility (Henkel et al., 1999). The hereditary, sterilizing defect of the sperm tail called the 'Dag-Defect' has been associated with the epididymis's inability to

remove Zn during transit (Bloom and Wolstrup, 1976). It is probably essential that Zn be bound to spermatozoa flagellum during spermiogenesis to inhibit it metabolically yet some Zn removal is absolutely necessary for sperm to obtain motility later in the maturation process (Srivastava et al., 1983; Henkel et al., 2003).

More than 200 Zn-dependent enzymes have been identified in all the main biological pathways including the reproductive process. The testes are the most rapidly growing tissues in the body and many vital enzymes involved in nucleic acid and protein synthesis are Zn metalloenzymes (Bedwal and Bahuguna, 1994). Specific to male reproduction is the role Zn plays in androgen production (Abbasi et al., 1980, Underwood and Suttle, 1999). Om and Chung (1996) reported that in Zn deficient animals, Leydig cells can transport cholesterol across the cell membrane; however the cells are incapable of converting them into sex steroids. A study conducted by Taneja and Nirmal, (1980) supported this by investigating cholesterol accumulation within the cells of the testis. After two weeks of Zn depletion in mice, Leydig and Sertoli cells accumulated a large number of cholesterol-rich bodies. As the Zn deficiency continued, the cholesterol bodies also accumulated around the germ cells and the lumen of the seminiferours tubules. It was hypothesized that the lack of Zn impaired the ability of the Leydig cells to convert cholesterol to testosterone. Zinc is also essential in 5α–reductase activity, the enzyme which converts testosterone to dihydrotestosterone (DHT, the more active androgenic metabolite). Zinc is also known to be an important aspect in hormone receptor function. Minetti et al. (1992) reported that Zn deficient mice showed a destabilization of the interactions of the androgen-receptor complexes. Androgen

receptors were reduced in these Zn deficient mice compared to the adequately Zn fed mice. Some of the Zn metalloenzymes which have been reported to play a large part in testis function and growth are listed in the table below.

Table 2.1. Zinc dependent enzymes related to male reproduction and development (Mertz, 1986, Heder et al., 1989, Mathews and Holde, 1991, Bedwal and Bahuguna, 1994).

Zn Dependent Enzyme	Function
5α–Reductase	Conversion of testosterone to DHT
Lactic, Malic, and Alcohol Dehydrogenase	Catalytic, energy production
Alkaline Phosphatase	Catalytic, growth
Adenylate Cyclase	Catalytic, synthesis of AMP from ATP
DNA and RNA Polymerases	Catalytic, DNA and RNA synthesis
Aspartate Transcarbamylase	Structural, DNA synthesis
Nucleoside Phosphorylase	Structural, Nucleotide metabolism
Ribonuclease	Zn inhibits this enzyme from degradation of RNA
Angiotensin-Converting Enzyme	Proteolysis, testicular development
Neutral Metalloendopeptidase	proteolysis
Leucine Aminopeptidase	proteolysis
Sorbitol Dehydrogenase	Catalytic, Reduces sugar to sorbitol, correlated to spermatozoa motility
LDH-X	Contributes to stability of mitochondria region of the spermatozoa midpiece

More than 20 Zn finger protein encoding genes located both on sex chromosomes and on autosomes have been proposed to play a regulatory role in

spermatogenesis (Yong-Xin et al., 2002). The expression of many Zn finger genes are specific only to the testis and in addition to this, many are specific to different stages of spermatogenesis (Ishizuka et al. 2003). Several of these Zn finger genes and their functions are reported in the table below.

Table 2.2. Zinc finger proteins and their functions as associated with spermatogenesis.

Zinc Finger Proteins	Function
Zfp 95 (Weissig, et al., 2003)	Meiosis of spermatocytogenesis
Zfp 96 (Weissig, et al., 2003)	Late spermiogenesis
Zfp 36 (Shyu, et al., 2003)	Round spermiogenesis stage, possible role in germ cell homeostasis
Zfp 4 (Yan, et al., 2002)	Late spermiogenesis,
Zfp 32 (van Baren et al., 2002)	Meiosis of spermatocytogenesis and spermiogenesis
Zfp 202 (Xing and Sairam 2002)	Negative regulatory role in expression of FSH receptors
Zfp – L and ZfpF (Ishizuka et al., 2003)	Regulates gene activity during meiotic prophase of spermatocytogenesis
Zipro1/Zfp 38 (Qiu et al., 2003)	Round spermiogenesis stage
Zfp59 (Qiu et al., 2003)	Morphological changes in maturation

Zinc deficiency has resulted in lower scrotal circumferences, lower sperm counts, disorganization of Leydig cell formation, and spermatozoa abnormalities (Abbasi et al., 1980; Davies, 1984-85; Apgar, 1985; Underwood, 1999). Abbasi et

al. (1980) reported Zn deficiency in men to have a negative effect on gonadal function, with the most evident being on spermatozoa numbers. In mildly zinc deficient rats, in which testicular weight was not reduced, epididymal sperm numbered 35% of that from Zn adequate rats, motility was reduced in Zn deficient rats, and spermatozoa had various defects (Wallace et al., 1984). Sixty-day old mice were sterile after three weeks of low zinc intake (Taneja and Nirmal, 1980). Hesketh (1982) investigated Leydig cell formation of Zn deficient pigs. There was distinct loss and disorganization of the smooth endoplasmic reticulum that was often accompanied by disorganization of the peripheral cytoplasm. These changes were accompanied by a disordered distribution of the mitochondria. These organelles are important as they are the sites for synthesis of a number of enzymes involved in testosterone production (Hesketh, 1982).

Zinc content in the adult testis is high compared to infant animals. Testis Zn concentrations increased in men at puberty and reached a maximum level at the age of 34-40 years (Bedwal and Bahuguna, 1994). Thus, it is not surprising that Mertz (1986) reported low Zn intake by young males of several species, including the bovine, interfered with normal sexual development. Rats fed both severely and moderately deficient Zn diets reached puberty at a later age when compared to rats fed adequate Zn levels. Both groups of Zn deficient rats showed varied degrees of degeneration in the germinal epithelium. After the Zn depletion period, a Zn adequate diet was fed for 30 and 41 d, resulting in restored function of the testis and the epididymis. However, when post pubertal rats were depleted of Zn and then fed

the Zn adequate diet (30 and 41 d), the negative effects were not reversed (Mason et al., 1982).

As important as Zn and overall trace mineral balance is to bulls and reproduction, it is surprising this topic has not received more attention. In the last 15-20 years with the infusion of the continental breeds, and the use of more sophisticated population genetic selection procedures, the productivity of today's bull has increased tremendously (Corah, 1996). The production required of the typical bull before one year of age has also been elevated to dramatic standards, and thus alters the nutritional requirements of the body. This in turn has forced the industry to take a closer look at how specific nutrients can be fed more efficiently, including the involvement of organic vs. inorganic sources of trace minerals.

Forms of Zn Supplementation

There are a wide variety of sources and chemical forms in which trace minerals can be fed (Table 2.3). It is believed that these different forms vary in their ability to be absorbed and/or metabolized in the body. In order to prevent toxic or deficient states, it is not only important to assess the correct requirements of each animal but also the most biologically active forms. The absorption of trace minerals from the lumen of the intestine to the portal system is probably dependent on a ligand to carry the mineral across the brush border. It is essential that the trace minerals be presented in a chemical form suitable for that uptake and translocation to the portal blood and on to the soft tissues of the body (Aggett, 1985). Ruminants are fed high fiber rations and a large portion of Zn in forages is associated with the plant cell wall (Spears, 2003). Spears (2003) hypothesized that the binding of minerals to undigested fiber may reduce the

ability of Zn to be absorbed. It is also possible that minerals may be bonded to other insoluble molecules and thus, rendered unavailable to the body systems (Aggett, 1985; Cousins, 1985). Thus, attaching trace minerals, during processing, to a specific ligand to facilitate transport across the brush border is the basis behind the wide range of commercially available proteinated, chelated, and complexed trace mineral supplements. It is suggested that these supplements are superior in two ways compared to inorganic supplements. First, the "organic" supplements are already in a form stable enough to pass through the rumen without being bound by other antagonists. Second, these supplements are already in a form which allows for translocation from the intestinal lumen to the mucosal cells and may be transported in the blood in this original form (Davies, 1984-85; Mertz, 1986, Spears, 1996). The absorptive and utilization efficiency of inorganic forms is dependent on the animal's ability to convert the supplement into chemical forms, which can be absorbed and transported (Davies, 1984-85). If this were the complete story, it may be more economically feasible to simply feed larger amounts of inorganic mineral. Spears (2003) reported that supplementing some organic forms of Zn improved animal production responses such as growth, milk production, and/or reproduction. Increased absorption may not be the only mechanism allowing for the increased response to organic minerals. In fact, Oestreicher and Cousins (1982) have reported no difference in absorption rates while performance and reproduction were increased. Organic minerals may increase the efficiency of some biological processes or they may enter a more beneficial storage pool within the body (Neathery et al., 1972; Rojas et al., 1995). Spears (2003) suggested that Zinc Methionine and Zinc Oxide are metabolized differently after absorption, due to different clearance rates.

Table 2.3. Definitions of various organic mineral products, adapted from Spears (1996) and Swenson (1999).

Metal amino acid complex	The product resulting from complexing a soluble metal salt bound to amino acid(s)
Metal amino acid chelate	The product resulting from the reaction of a metal ion from a soluble metal salt with amino acids with a mole ratio of one mole of metal to one to three moles of amino acids.
Metal proteinate	The product resulting from the chelation of a soluble salt with amino acids and/or partially hydrolyzed protein.
Metal polysaccharide	The product resulting from complexing of a soluble salt with a polysaccharide solution.

Literary Summary

Zinc has structural, physiological, catalytical, and regulatory functions in the body (Underwood, 1999) and is essential in the maintenance of normal male sexual function. Very little scientific work, however, has been conducted investigating the impact Zn has in the peripubertal bull's reproductive system and at what levels Zn is required by the bull. Providing clear-cut recommendations for bulls of this age is difficult, as many parameters in the formulation of a balanced trace mineral supplement must be considered. Requirements of the peripubertal bull must be assessed, and may include variations in species, age, breed, stress levels, and stage and level of production. All trace minerals, including Zn, are commercially available in several forms and trace mineral manufacture's all claim different levels of absorption and impact on the animal. Thus, mineral supplementation must not only be provided in correct amounts, but must also be balanced with other antagonistic and synergistic minerals, and in a form that is palatable and available to the animal.

CHAPTER 3

EFFECT OF SUPPLEMENTAL TRACE MINERAL LEVEL AND FORM ON PERIPUBERTAL BULLS

Introduction

Adequate levels of Zn and Cu are essential for development and maintenance of the peripubertal bull. Assessing the correct Zn and Cu requirements for bull calves is important both from a production and economic standpoint. The current National Research Council (NRC, 1996) guidelines do not make adjustments in mineral requirements for cattle based on growth potential, levels of productivity, physiological status, stress levels, breed, or sex. This is compounded by the fact that the trace mineral requirements can be confounded by metabolic or nutritional factors that bind specific trace elements, rendering them nutritionally unavailable to the animal (Corah, 1996). Sufficient Zn and Cu must be consumed in order for the bull to reach puberty and achieve his genetic and physiologic potential. However, over consumption has the potential to produce toxic affects or increase feed costs. Determining bull requirements for Zn and Cu are further complicated by the different forms of trace minerals available, especially with each manufacturer claiming different absorption rates and bioavailability.

Due to the importance of Zn in male reproduction and the synergistic relationship of Cu with Zn, we felt that further investigation into the role of different levels and forms of trace minerals would be beneficial to producers. The objectives of this study were to determine if form and/or level of supplemental trace minerals fed to peripubertal bull

calves influenced: 1.) Liver trace mineral storage 2.) Rate of sexual maturity 3.) Quantity and quality of semen production and 4.) Testicular development.

Materials and Methods

Experimental Design

This experiment was conducted at the USDA-ARS Livestock and Range Research Laboratory, Fort Keogh, in Miles City, MT. Fifty crossbred bull calves, sired by genetically similar Hereford sires, with an average initial body weight of 248 ± 31.5 kg were utilized. All bull calves had the same grandsire. Eighty-one days before initiation of the trial (d 0), all mineral supplements were removed from the bull calves and their dams. Bulls were allotted by puberty status, age (258 ± 8.9 d), and scrotal circumference (26.88 ± 2.3 cm) into five groups to evaluate different trace mineral supplementation treatments: 1) 360 mg Zn, 200 mg Mn, 125 mg Cu, 12 mg Co in sulfate form (1S), 2) 360 mg Zn, 200 mg Mn, 125 mg Cu, 12 mg Co in complexed form (1C), 3) 360 mg Zn, 200 mg Mn, 125 mg Cu, 12 mg Co in sulfate form plus 360 mg Zn, 200 mg Mn, 125 mg Cu, 12 mg Co in complexed form (2SC), 4) 360 mg Zn, 200 mg Mn, 125 mg Cu, 12 mg Co in sulfate form plus 720 mg Zn, 400 mg Mn, 250 mg Cu, 24 mg Co in complexed form (3SCC), and 5) 1080 mg Zn, 600 mg Mn, 375 mg Cu, 36 mg Co in sulfate form (3S).

The supplements were individually measured and fed daily in 0.45 kg of wheat middlings from d 0 to 100 before providing bulls with their basal diet. The basal diet of 75.5% corn silage, 10.5% corn, 7% alfalfa hay, and 7% protein supplement was fed to all bulls and was formulated to achieve 1.2 kg ADG. Basal diet nutrient composition was

Table 3.1. Zinc, Cu, Mn, and Co contained in 13.6 kg of diet plus respective supplement fed to peripubertal bulls.[a] Treatments 1S and 3S provided Zn, Cu, Mn and Co in sulfate forms. Treatment 1C contained all complexed mineral (Availa-4®; Zinpro Corporation, Eden Prairie, MN). Treatments 2SC and 3SCC contained both complexed and sulfate forms of minerals.

Treatments[a]	Zn ppm	Cu ppm	Mn ppm	Co ppm
1S	53	16	54	.93
1C	53	16	52	1.1
2SC	76	22	56	1.5
3SCC	118	35	58	2.8
3S	115	32	74	2.7

Table 3.2. Nutrient composition of basal ration fed to bulls. Diet was formulated to provide a 1.2 kg per day gain. Diet contained 7% alfalfa hay, 10.5% corn, 75.5% corn silage, and 7% protein supplement.

DM %	48.70
CP %	13.70
NE_g, Mcal/kg	.50
NE_m, Mcal/kg	.70
TDN %	66.60

Table 3.3. Zinc, copper, manganese and cobalt concentration in 13.6 kg of basal ration fed to bulls.

	Zn ppm	Cu ppm	Mn ppm	Co ppm
Alfalfa Hay	1.3	.5	1.5	< 0.5
Corn Grain	2.5	.4	3.2	< 0.5
Corn Silage	3.3	3.3	24.3	< 0.5
Total	7.1	4.2	29.0	< 0.5

analyzed and is reported in Table 3.2. The mineral analysis of the basal diet was analyzed by Animal Health Diagnostic Laboratory (Michigan State University, East Lansing MI) using coupled argon-atomic emission spectroscopy (Braselton, 1997). The results are reported in Table 3.3.

Bulls were assigned to one of ten pens with all feed provided in Calan gates and one animal per treatment per pen following an initial collection (-14 d) of semen, blood, scrotal circumference, and body weights. Bulls were allowed a 14 d acclimation period to adapt to individual feeding gates and automatic waterers. Two bulls (both treatment 1C) died during treatment (both unrelated to treatment). Thus, two pens contained only four bulls.

Water was supplied free choice in automatic waterers from a common source. Water was analyzed for mineral content by the Animal Health Diagnostic Laboratory (Michigan State University, East Lansing MI) using coupled plasma-atomic emission spectroscopy (Braselton, 1997). Sodium concentration was above recommended levels, however all other minerals were within acceptable ranges. Pertinent mineral concentrations in the water are reported in Table 3.4.

Table 3.4. Concentration of minerals in water, available free choice to all bulls.

Element	Concentration in Water, ppm
Fe	.0149
P	1.17
Ca	2.42
Mg	0.827
Zn	0.170
S	23.3
Cu	0.010
Mn	0.015
Na	499.0
Mo	<0.020

Liver Biopsies

Liver biopsies were collected on d –21 and 100 utilizing the Tru-cut® needle biopsy technique described by Corah and Arthington (1994). Liver samples were analyzed for Cu, Zn, Mn, and Co by the Animal Health Diagnostic Laboratory (Michigan State University, East Lansing MI) using coupled plasma atomic emission spectroscopy techniques (Braselton, 1997). From this analysis, all bulls were considered deficient in Cu and all adequate in Zn (Mertz, 1986) on d -21, thus bulls were not blocked by liver trace mineral status. Individual liver biopsy data is reported in Table 3.5.

Table 3.5. Zinc, Cu, and Mn concentration of each individual bull from liver biopsy analysis on d -21 and d 100.

Bull I.D.	Zn (d-21)	Cu (d-21)	Mn (d-21)	Zn (d100)	Cu (d100)	Mn (d100)
600	124	19.4	5.12	170	121	5.06
602	145	13.4	5.68	116	204	7.25
603	178	22	7.15	152	264	7.06
604	181	12.1	7.36	117	163	6.41
612	161	18.1	6.17	113	327	7.14
613	167	22.5	6.23	109	316	7.05
615	160	11.6	5.93	138	223	6.47
616	216	22.4	6.83	162	346	6.5
618	133	15.6	5.25	117	165	5.68
620	142	13.3	5.76	109	203	6.84
626	194	18.3	6.08	106	154	9
627	220	23.9	7.23	107	183	5.22
629	191	14	5.37	139	270	5.35
630	182	9.98	6.83	117	117	6.41
633	187	10.4	5.38	111	160	6.7
635	179	27.1	5.96	147	149	6.43
636	183	9.69	8.36	97.3	167	6.84

Bull I.D.	Zn (d-21)	Cu (d-21)	Mn (d-21)	Zn (d100)	Cu (d100)	Mn (d100)
639	187	28.3	5.46	115	204	7.43
642	242	44.5	6.12	126	108	7.14
645	171	52.1	1.21	113	248	5.21
646	221	9.47	1.3	150	199	6.26
647	222	10.5	5.6	107	157	7.3
650	144	49	6.37	123	211	6.62
656	124	26.9	5.76	123	174	6.41
659	143	17.9	5.86	124	351	5.81
660	203	9.56	5.68	126	127	5.95
661	205	19.4	7.44	124	122	6.45
666	200	16	6.07	147	293	7.23
667	161	26.4	5.51	133	195	6.96
668	219	19.3	6.26	137	198	6.72
669	247	44.8	6.23	120	226	6.91
671	118	13.6	5.72	119	231	6.04
672	169	16.6	6.21	143	236	7.89
674	153	31.8	6.85	137	203	7.43
675	198	11.4	4.92	131	207	6.34
676	193	28.3	6.93	84.3	81	4.58
679	178	35.4	7.42	121	284	6.78
680	165	21.2	6.84	131	139	6.16
685	213	10.4	6.27	162	295	5.74
689	166	10.1	7.05	134	185	7.02
691	299	13.9	6.46	102	175	6.72
692	283	15.4	5.16	117	279	4.93
694	158	19.7	4.54	113	185	5.98
700	181	17.2	5.4	163	305	5.64
702	285	12.7	4.54	121	300	5.56
703	193	11.3	6.31	97	198	5.56
704	181	58.9	6.04	130	259	6.52
02A21	175	13.2	6.52	121	165	6.01

Semen Evaluation and Scrotal Circumference

Semen, blood, scrotal circumference and weights were collected on d –14, 14, 42, 70, and 98. The same individuals performed scrotal measures, semen collections, and semen analyses over the entire study to eliminate variation. Scrotal circumference was measured at the widest circumference with a manual metal tape (Puls, 1995; Hammerstedt, 1996). Semen was collected by electroejaculation by two experienced technicians. Bulls failing to provide an ejaculate were allowed to rest for a short period of time and then another collection was attempted. If bulls did not provide an ejaculate, semen parameters were recorded with a zero value. Ejaculate volume was recorded and 10μl of raw semen was evaluated microscopically at 10 x magnification for gross swirl. Progressive motility and strength of motility (rate of movement across field of view) of diluted semen (1:5, v:v in phosphate-buffered saline, pH 7.4) were recorded at 40 x magnification on a warmed microscope slide. A sample of raw semen was mixed with an Eosin/Nigrosin morphology stain (Lane Manufacturing Inc., Denver Co.) for morphology and viability evaluation (Lunstra and Echternkamp, 1982). Spermatozoa morphology was evaluated by a counting 100 random cells and classifying them as normal or having head or tail abnormalities as described by Barth and Oko (1989). Tail abnormalities and classifications included proximal and distal cytoplasmic droplets, coiled and bent tails, plus any miscellaneous abnormalities. Head abnormalities included abnormal acrosomes and all types of abnormally shaped heads. Concentration of spermatozoa in each ejaculate was determined using a hemocytometer. Puberty was defined as the age at which an ejaculate contained a minimum of 50 x 10^6 total spermatozoa with at least 10% progressive motility (Lunstra et al. 1978).

On collection d 42, 1.1 ml of semen from each bull was stored for trace mineral analyses. Samples were stored at -20°C until mineral analyses were performed by the Animal Health Diagnostic Laboratory, (Michigan State University, East Lansing MI) coupled argon plasma emission spectroscopy techniques (Stowe, 1985) were utilized for analyses.

All procedures and protocols were approved both by the Fort Keogh and Montana State University Institutional Animal Care and Use Committees.

Blood Collection and Serum Assays

Blood samples were collected in vacutainer tubes (10 mL) via caudal venipuncture at 0, 30, and 60 minutes following an i.m. injection of GnRH (Fertagyl, Intervet Inc., Millsboro, DE, .22 μg/kg BW). Blood was allowed to clot and stored at 4° C overnight. Serum was harvested by centrifugation at 3000 x g for 20 minutes and stored at –20° C until hormone analysis. Serum concentration of testosterone was determined by radioimmunoassay (RIA) using a recently validated testosterone assay (Diagnostic Products Corporated, Los Angeles, CA). Briefly, 50 μl of serum and 500 μl of I^{125} labeled testosterone were incubated in antibody-testosterone coated tubes. Bovine serum standards and serum quality controls containing high and low concentrations of testosterone were included in all assays. Intra- and inter-assay coefficients of variation were 2 and 12% respectively and assay sensitivity for testosterone was .04 ng/mL.

The LH assays were conducted as described by Niswender et al. (1969). Assay sensitivity was 0.5 ng/mL and the intra- and inter-assay coefficients of variation were 9.6% and 11.9%, respectively. The FSH assay was validated and conducted in Dr. Jon

Wheaton's laboratory (University of Minnesota) according to the NIDDK procedure for radioimmunoassay of oFSH. Assay sensitivity was 0.3 ng/mL and the intra-assay coefficient of variation was 4.8%.

Statistical Analyses

Data was analyzed as a randomized complete block design using the bull as the experimental unit. Differences in spermatozoa morphology and concentration, scrotal circumference, Zn semen concentrations, age, and number of days to reach puberty were analyzed using the General Linear Model (GLM) procedure of SAS (1994). Differences in concentration of liver mineral levels and LH, FSH, and testosterone were analyzed using repeated measures using the GLM procedure of SAS (1994). Comparison of puberty status for bulls between different treatments was analyzed using categorical analysis of variance of SAS (1994). All differences were considered significant at $P < 0.10$. For all data, the bulls were grouped for two separate comparisons; first by the individual treatment and then by the mineral form (sulfate or complexed) of each treatment. We recognize that when comparing by form, there are different numbers of bulls and the levels are not equal between the two groups.

Results

Liver Mineral Concentrations

Liver Cu concentrations of all bulls were below the minimum requirements of 100 ppm at the initiation of treatment (Figure 3.1). After 100 d of treatment, liver Cu was increased ($P < 0.01$) to adequate concentrations (> 100 ppm, Puls, 1995) compared to initial concentrations, in all bulls regardless of treatment (Figure 3.1). On d 100, liver

concentrations of Cu were greater ($P = 0.07$) in 3SCC and 3S bulls compared to 1C and 1S bulls, whereas liver concentrations of Cu in 2SC bulls were intermediate.

Initial (d -21) liver Zn concentrations were adequate (100 ppm; Mertz, 1986; Puls, 1995) and after 100 d of treatment, all liver Zn concentrations were still within the adequate range (Figure 3.2). However, following 100 d of treatment, liver Zn concentrations of all bulls decreased ($P < 0.01$) across all treatments ($P = 0.50$).

Puberty

Five bulls (one from each treatment) were pubertal at the initiation of the trial and were removed from the puberty data set. Two bulls did not reach puberty by the end of the supplementation period and were assigned a puberty date of 126 d or an additional 28 d (time allotted between each collection day). Table 3.5 contains the number of bulls within each treatment that were classified as being pubertal by day. There was no effect ($P = 0.42$) of treatment on age at puberty (Figure 3.3) nor was there an effect ($P = 0.35$) of mean days on treatment to reach puberty (Figure 3.4) over the entire treatment period. Bulls fed complexed trace minerals (1C, 2SC, 3SCC) tended (P = 0.11) to reach puberty after fewer days on treatment (43.9 ± 5.7 d) than bulls fed only sulfate minerals (58.5 ± 6.7 d; 1S, 3S; Figure 3.5). On d 42, more ($P = 0.03$) bulls fed complexed trace mineral (79%) were pubertal compared to those fed only sulfate trace mineral (47%; Figure 3.6). In addition, there was a tendency for treatment to affect ($P = 0.12$) the percentage of bulls reaching puberty on d 42 (Figure 3.7).

Semen Evaluations

No differences in head or tail abnormalities were detected ($P > 0.10$) in spermatozoa from bulls receiving different levels or forms of mineral supplement (Figures 3.8 and 3.9). On day 42, 3S bulls had a greater ($P = 0.08$; 39.2%) percentage of proximal and distal droplets in ejaculates compared to all other treatments (1S, 1C, 2SC, 3SCC; 15%, 20%, 25%, 21.6% respectively). No differences were detected in ejaculate concentration or motility between treatments ($P > 0.10$). No treatment differences were detected in semen Zn (Figure 3.10) or Cu concentrations on d 42. Neither semen Zn (Figure 3.11) nor Cu concentration differed ($P > 0.10$) when bulls were grouped by the date of puberty or when all of the pubertal bulls were compared to all of the non pubertal bulls ($P > 0.10$; Table 3.6).

Scrotal Circumference

Scrotal circumference did not differ ($P > 0.10$) between bulls receiving different mineral treatments or forms throughout the trial (Figure 3.12 and 3.13). Scrotal circumference increased similarly across bulls in all treatments.

Hormone Assays

Testosterone, FSH and LH means did not differ ($P > 0.10$) between bulls receiving different mineral levels or forms of mineral. Figure 3.14 illustrates the difference ($P < 0.01$) between the testosterone concentrations measured in the serum collected at the collection day before puberty and the testosterone levels collected at puberty. When the prepubertal mean concentrations of LH and FSH in serum collected at the previous collection date were compared to the mean concentration in serum at the

pubertal collection date, no difference were detected ($P > 0.10$). Serum LH and testosterone concentrations were different ($P < 0.01$) between the 0, 30, and 60 minute time intervals after the GnRH injection. Mean serum LH, testosterone and FSH concentrations were also different ($P < 0.01$) between collection dates.

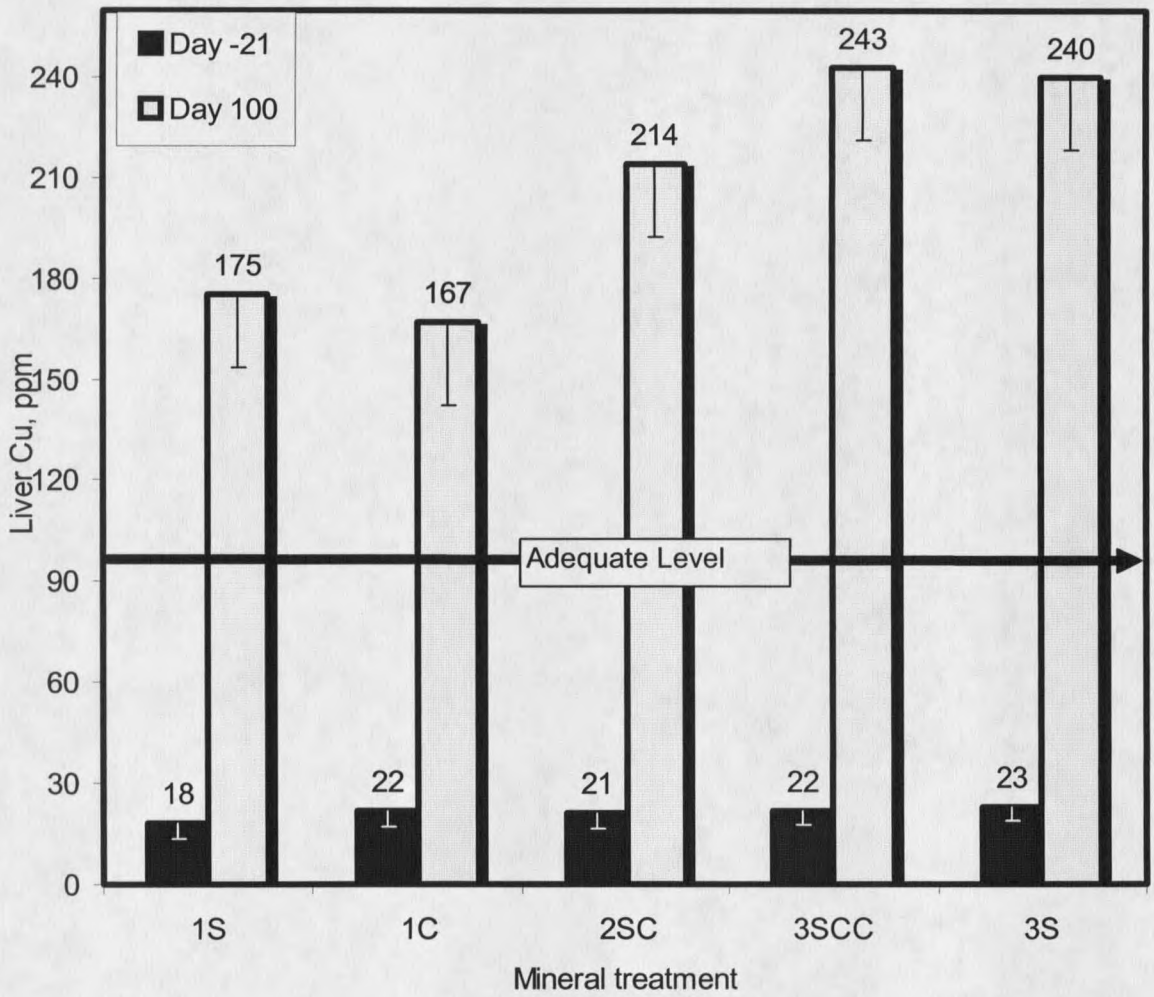


Figure 3.1. Liver concentrations of Cu (mean-SD) from bulls on d -21 and 100 of mineral supplementation. A difference was found between collection days ($P < 0.10$). The 3SCC and 3S treatment groups had a higher ($P < 0.10$) 100 d liver Cu concentration compared to 1C and 1S bulls, whereas 2SC bulls were intermediate. Mineral supplements were; 1x sulfate form (1S), 1x complexed form (1C), 1S + 1C (2SC), 1S + 2x1C (3SCC), and 3x1S (3S). Each 1x supplementation level contained 360 mg Zn, 125 mg Cu, 200 mg Mn and 12.5 mg Co.

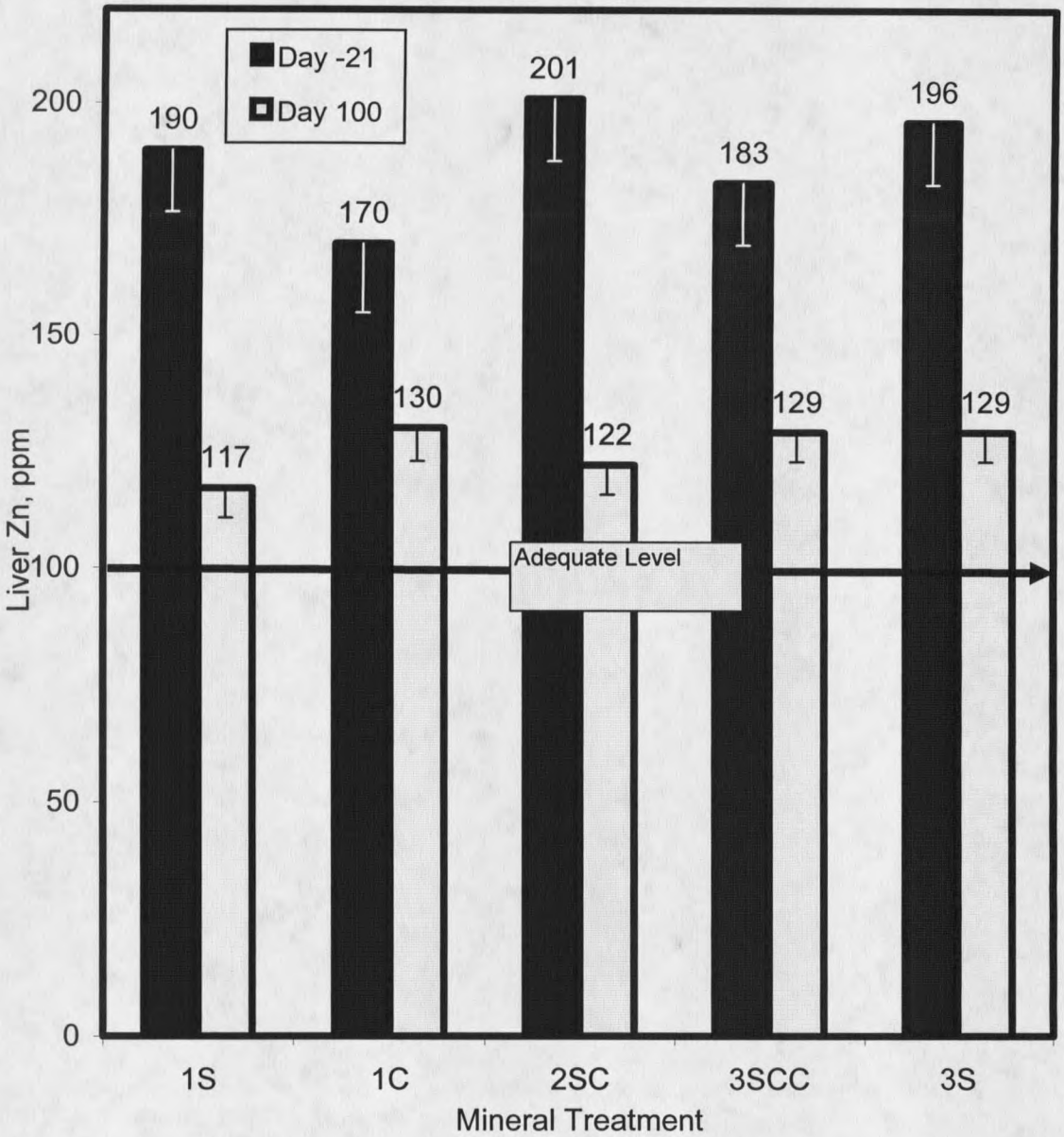


Figure 3.2. Liver concentrations of Zn (mean - SD) from bulls at d -21 and d 100 of mineral supplementation. Liver Zn concentrations were different ($P < 0.10$) between collection days however no treatment difference was detected ($P > 0.10$). Mineral supplements were; 1x sulfate form (1S), 1x complexed form (1C), 1S + 1C (2SC), 1S + 2x1C (3SCC), and 3x1S (3S). Each 1x supplementation level contained 360 mg Zn, 125 mg Cu, 200 mg Mn and 12.5 mg Co.

Table 3.6. Number of pubertal bulls in each treatment by collection day. Mineral supplements were; 1x sulfate form (1S), 1x complexed form (1C), 1S + 1C (2SC), 1S + 2x1C (3SCC), and 3x1S (3S). Each 1x supplementation level contained 360 mg Zn, 125 mg Cu, 200 mg Mn and 12.5 mg Co.

	Collection Day					
Treatments[a]	d -14	d 14	d 42	d 70	d 98	not pubertal within trial period
1S	1	2	3	2	1	1
1C	1	2	3	0	1	1
2SC	1	3	3	2	1	0
3SCC	1	1	7	0	1	0
3S	1	1	2	3	3	0
total	5	9	18	7	7	2

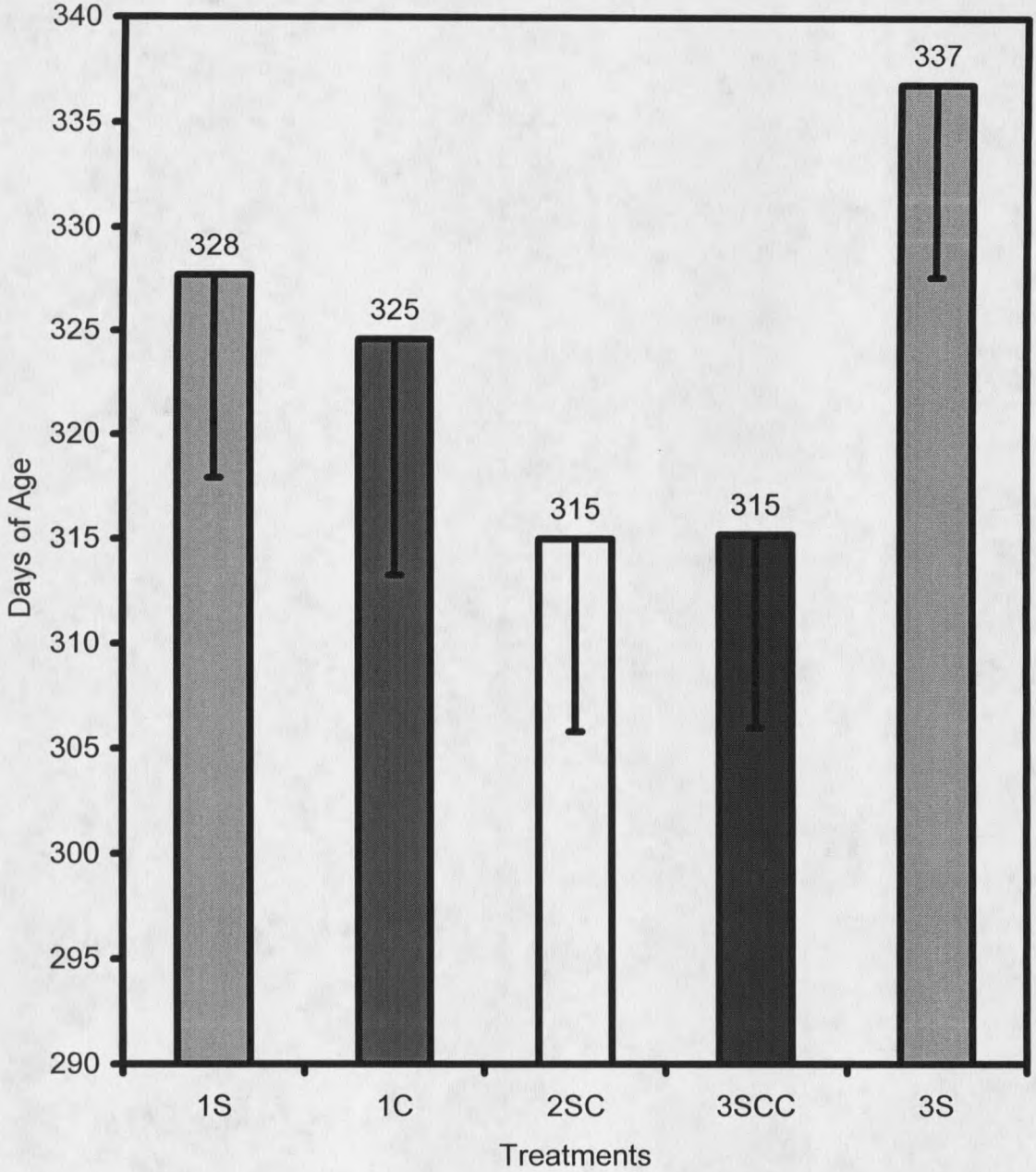


Figure 3.3. Age (d) of bulls at puberty (mean - SD) that received different mineral supplements ($P = 0.42$). Mineral supplements were; 1x sulfate form (1S), 1x complexed form (1C), 1S + 1C (2SC), 1S + 2x1C (3SCC), and 3x1S (3S). Each 1x supplementation level contained 360 mg Zn, 125 mg Cu, 200 mg Mn and 12.5 mg Co.

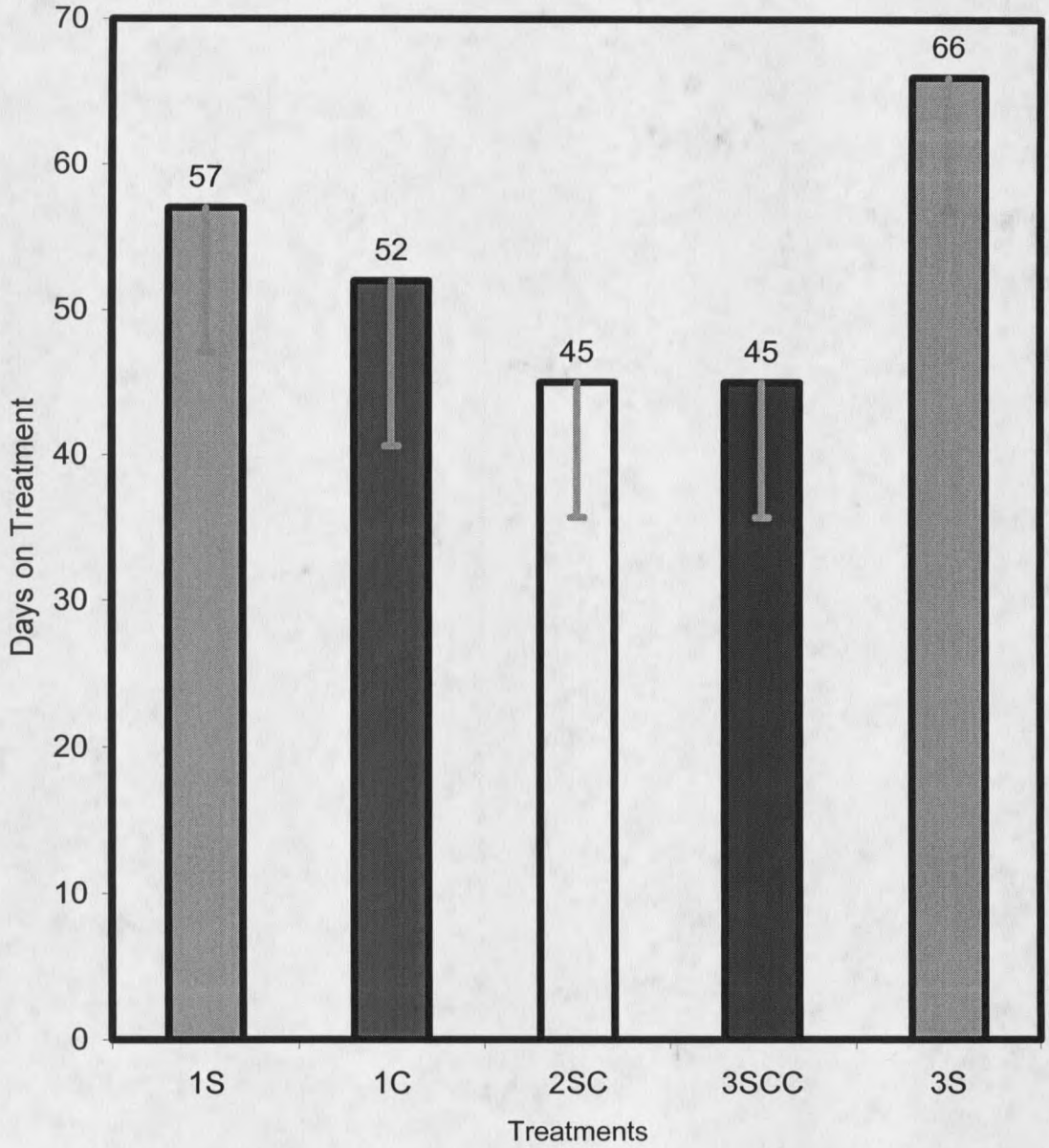


Figure 3.4. Number of days on mineral treatment for bulls (mean - SD) receiving one of 5 mineral supplements to reach puberty ($P = 0.35$). Mineral treatments were; 1x sulfate form (1S), 1x complexed form (1C), 1S + 1C (2SC), 1S + 2x1C (3SCC), and 3x1S (3S). Each 1x supplementation level contained 360 mg Zn, 125 mg Cu, 200 mg Mn and 12.5 mg Co.

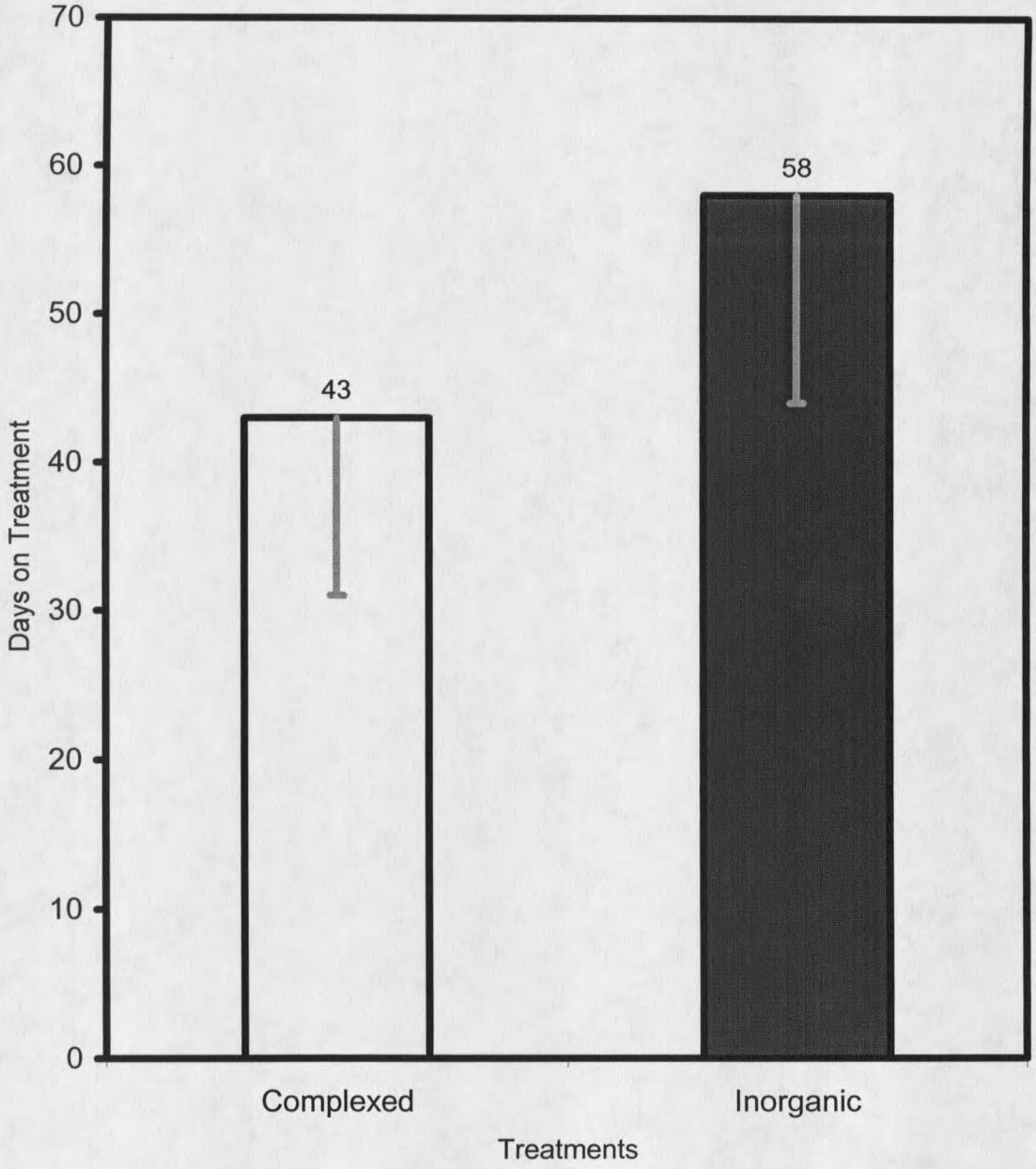


Figure 3.5. Number of days on treatment to puberty (P = 0.11, mean - SD)for bulls receiving one of two forms of mineral supplement. Bulls are grouped by form of mineral supplements: Complexed [1x complexed form (1C), 1S + 1C (2SC), and 1S + 2x1C (3SCC)] Inorganic [1x sulfate form (1S), and 3x1S (3S)] Each 1x supplementation level contained 360 mg Zn, 125 mg Cu, 200 mg Mn and 12.5 mg Co.

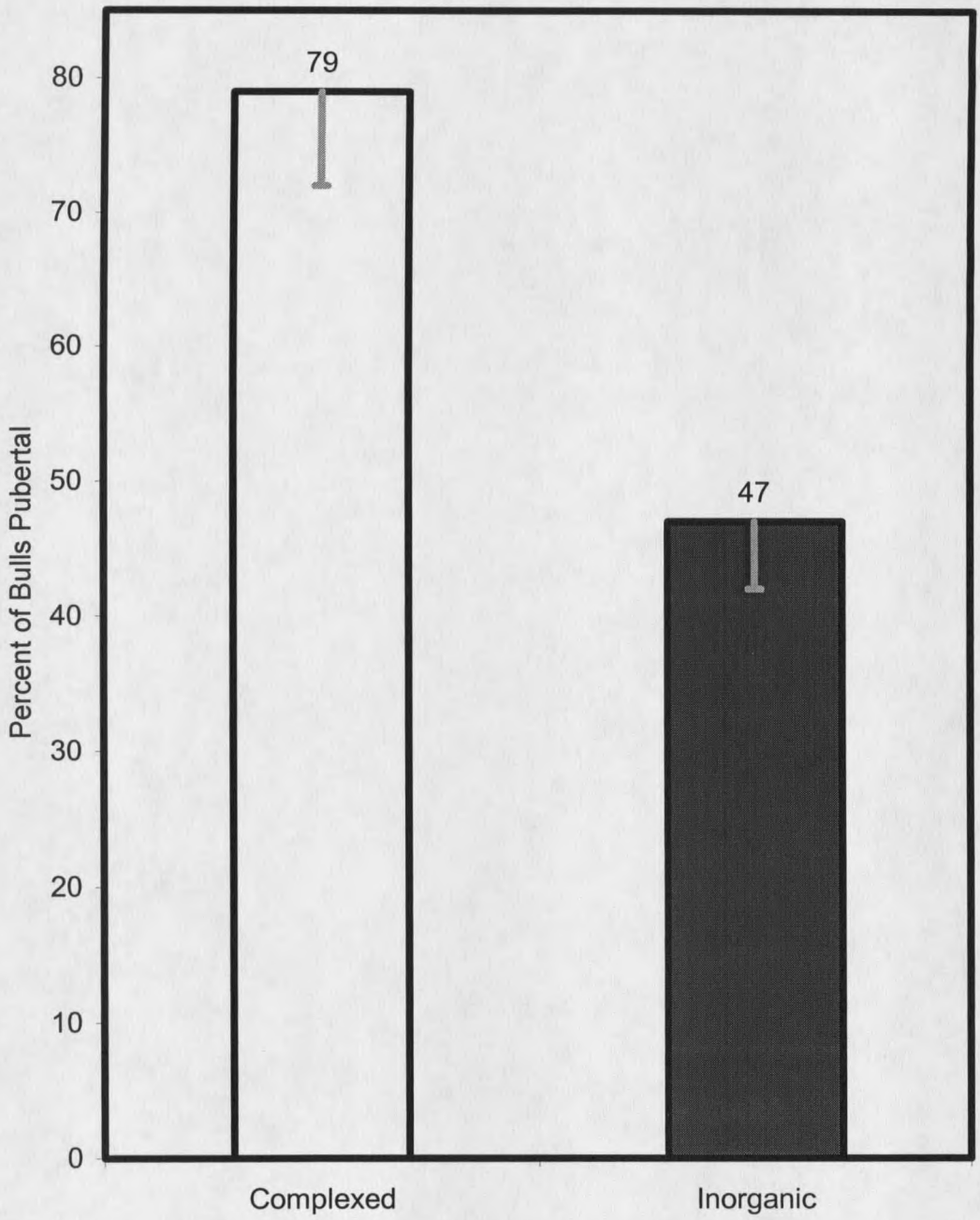
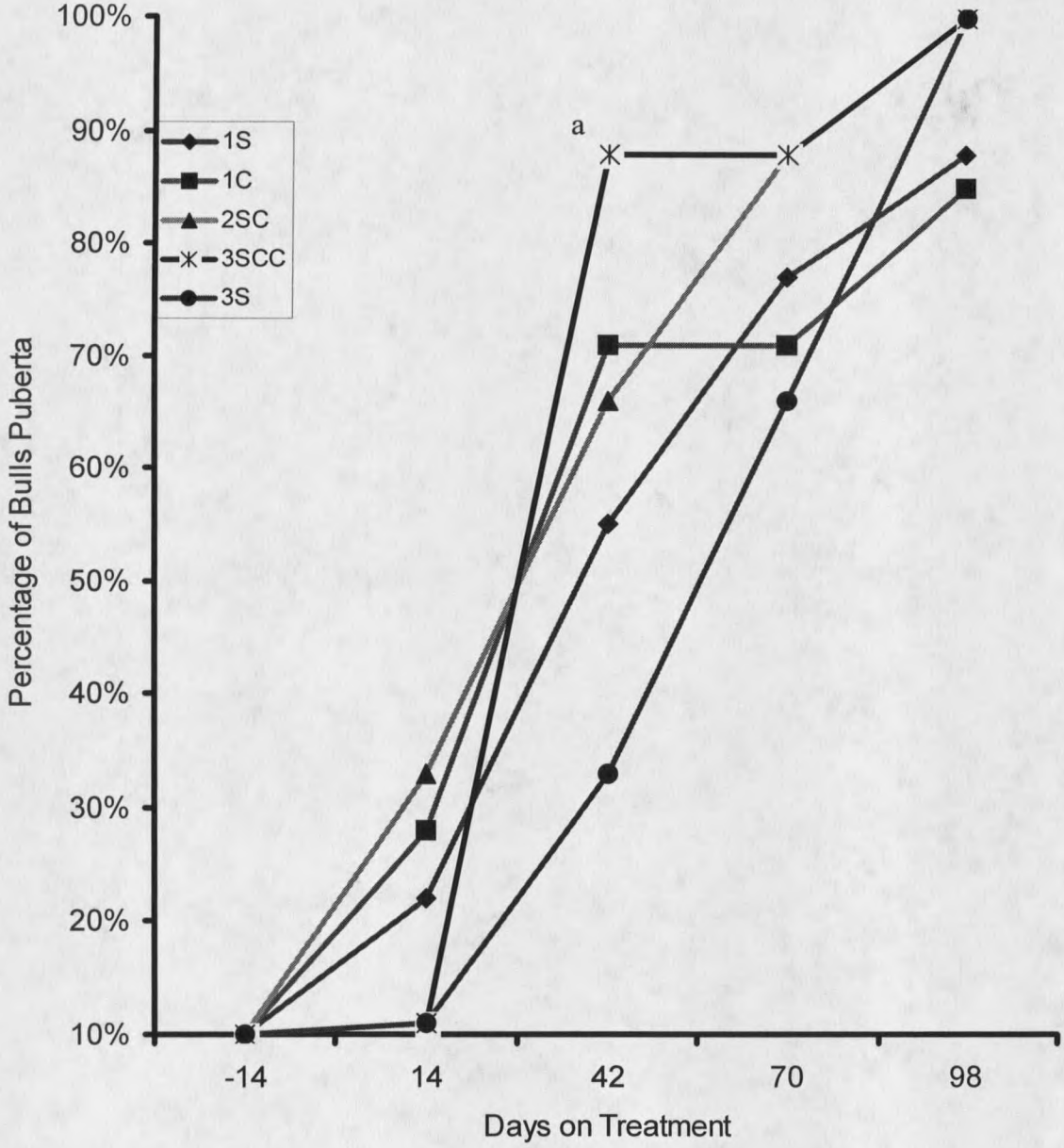


Figure 3.6. Percentage of pubertal bulls by d 42 of supplement by mineral form (P = 0.03). Bulls were grouped by form of mineral supplement: Complexed [1x complexed form (1C), 1S + 1C (2SC), and 1S + 2x1C (3SCC)] Inorganic [1x sulfate form (1S), and 3x1S (3S)] Each 1x supplementation level contained 360 mg Zn, 125 mg Cu, 200 mg Mn and 12.5 mg Co.



a. Day 42 ($P = 0.12$)

Figure 3.7. Cumulative percentage of pubertal bulls in each treatment by collection day. No differences were found ($P > 0.10$) except for a trend on collection day 42. Mineral supplements were; 1x sulfate form (1S), 1x complexed form (1C), 1S + 1C (2SC), 1S + 2x1C (3SCC), and 3x1S (3S). Each 1x supplementation level contained 360 mg Zn, 125 mg Cu, 200 mg Mn and 12.5 mg Co.

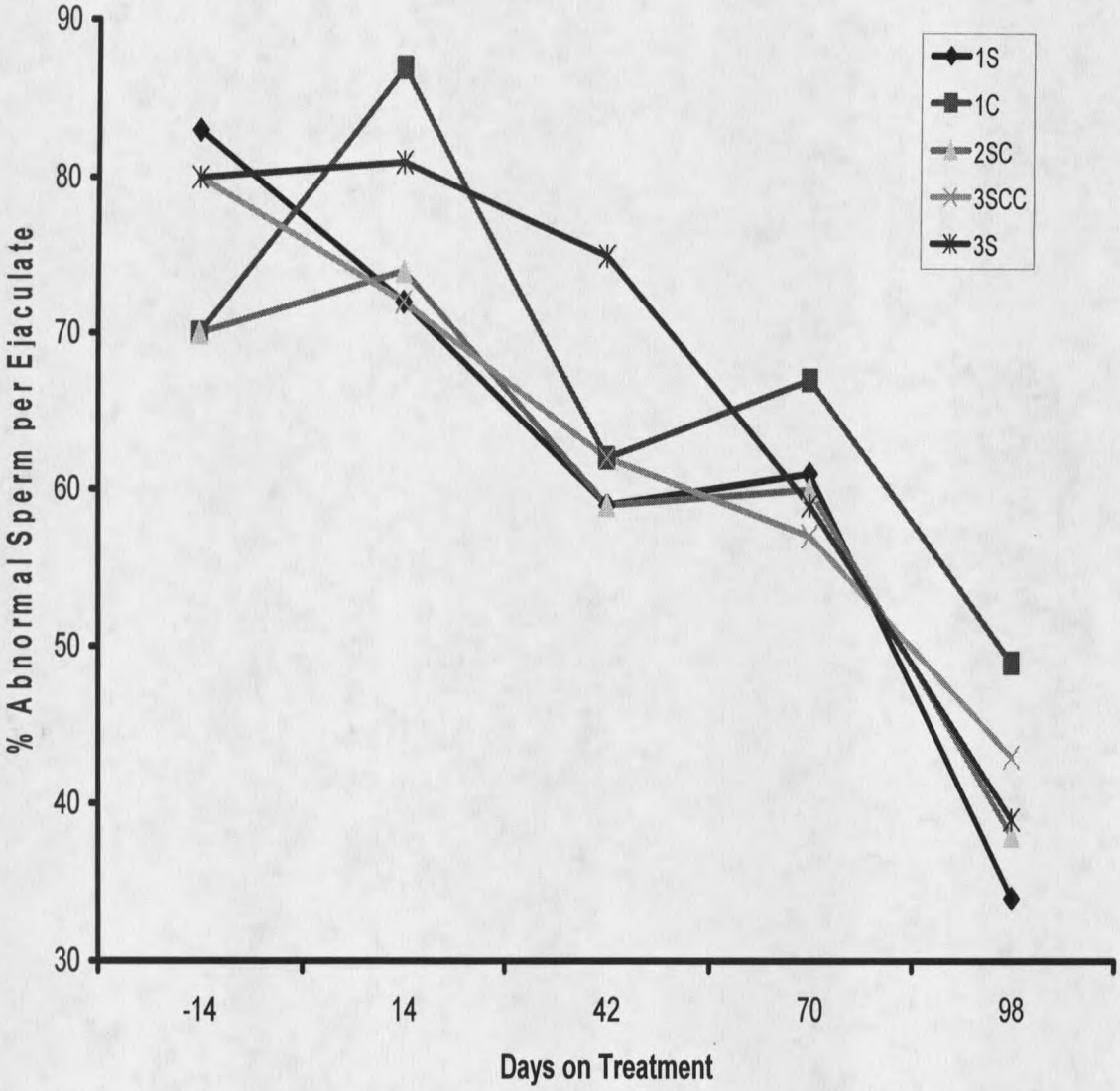


Figure 3.8. Percentage (mean) of abnormal spermatozoa per ejaculate ($P > 0.10$)on each collection day. Mineral supplements were 1x sulfate form (1S), 1x complexed form (1C), 1S + 1C (2SC), 1S + 2x1C (3SCC), and 3x1S (3S). Each 1x supplementation level contained 360 mg Zn, 125 mg Cu, 200 mg Mn and 12.5 mg Co.

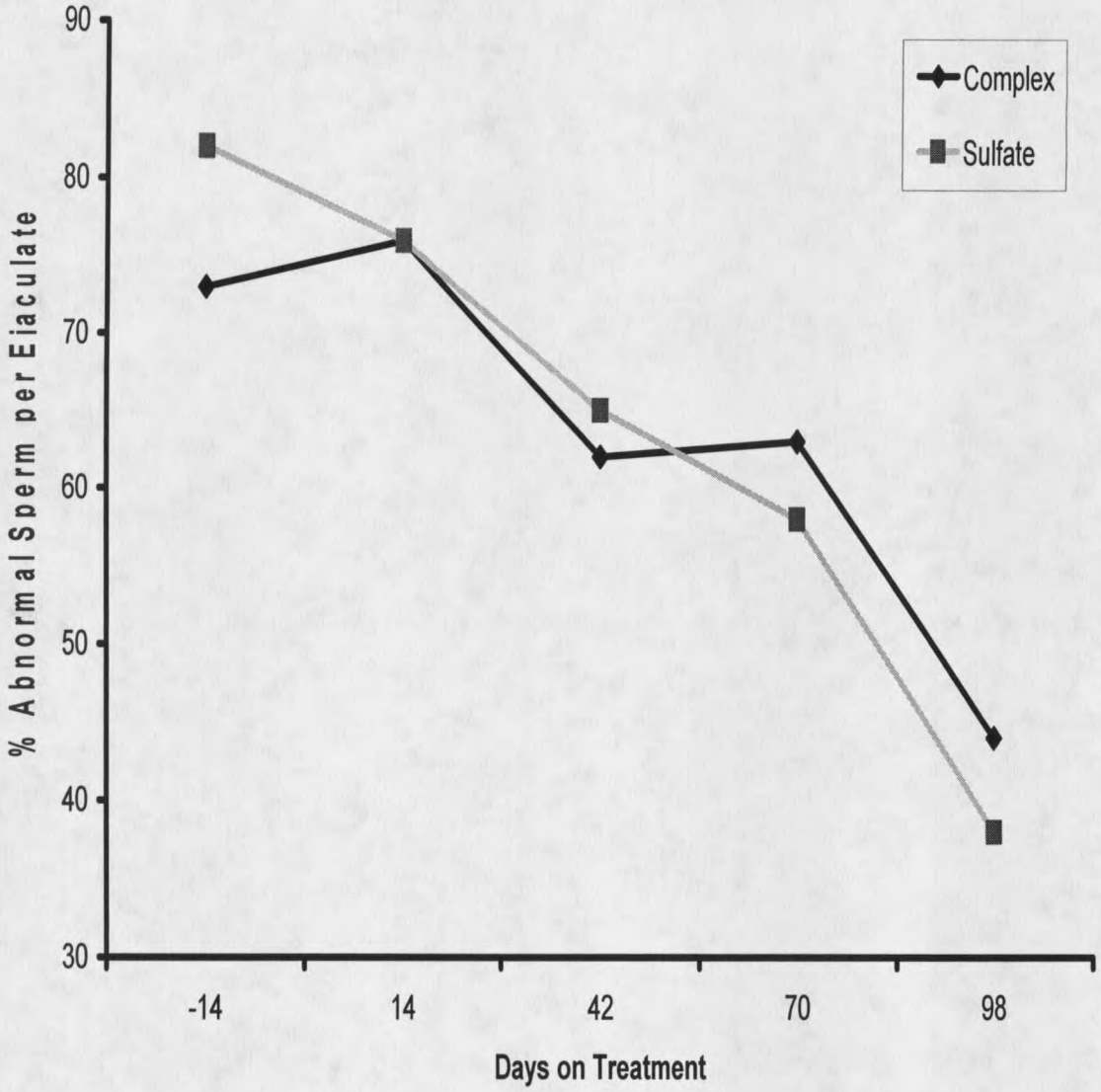


Figure 3.9. Percentage (mean) of abnormal spermatozoa per ejaculate ($P > 0.10$) on each collection day. Bulls were grouped by form of mineral supplement: Complexed [1x complexed form (1C), 1S + 1C (2SC), and 1S + 2x1C (3SCC)], Inorganic [1x sulfate form (1S), and 3x1S (3S)]. Each 1x supplementation level contained 360 mg Zn, 125 mg Cu, 200 mg Mn and 12.5 mg Co.

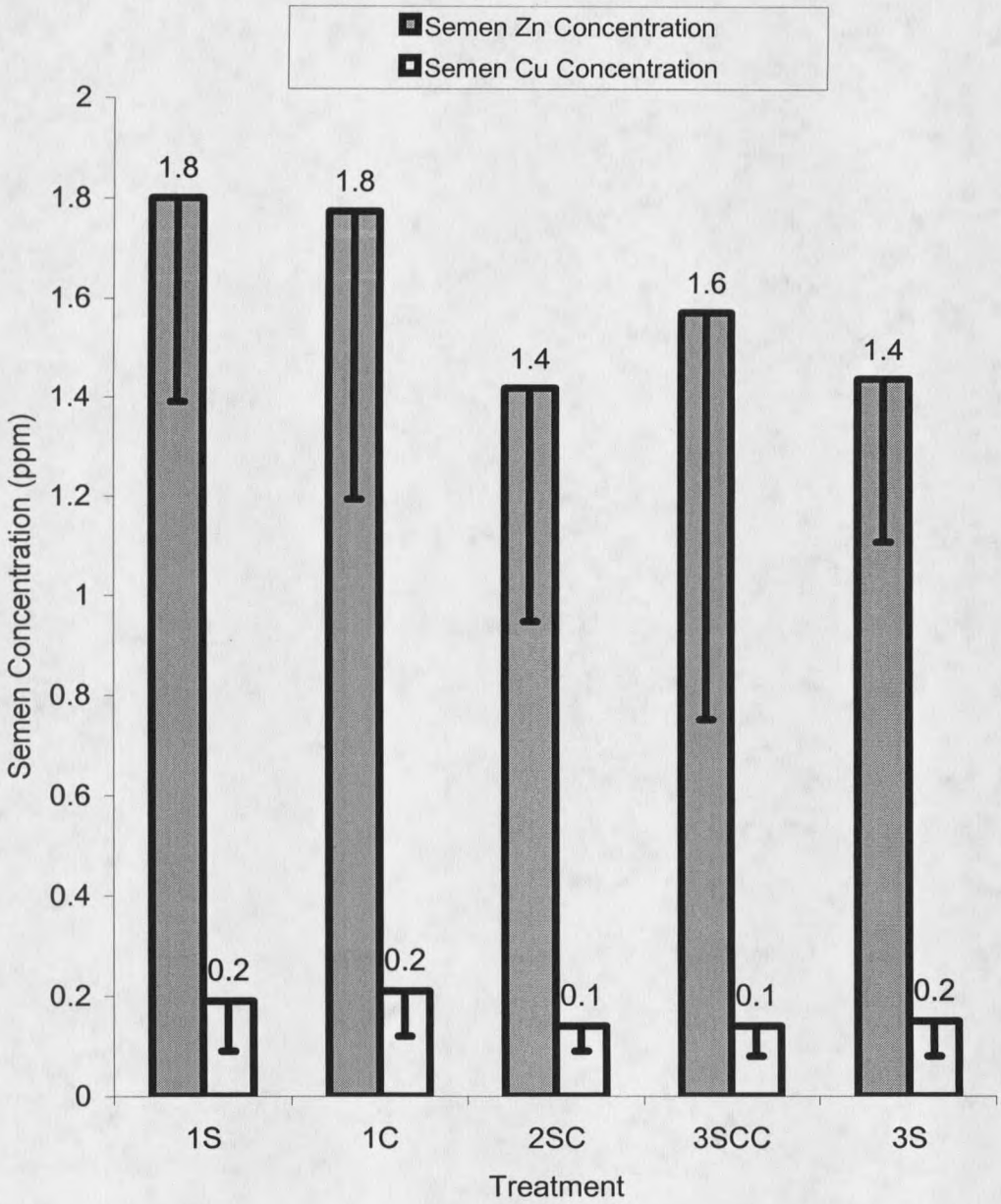


Figure 3.10. Zinc and Cu concentration (mean, SD) in semen of bulls ($P > 0.10$) on d 42 of a 100 d mineral supplement study. Mineral supplements were 1x sulfate form (1S), 1x complexed form (1C), 1S + 1C (2SC), 1S + 2x1C (3SCC), and 3x1S (3S). Each 1x supplementation level contained 360 mg Zn, 125 mg Cu, 200 mg Mn and 12.5 mg Co.

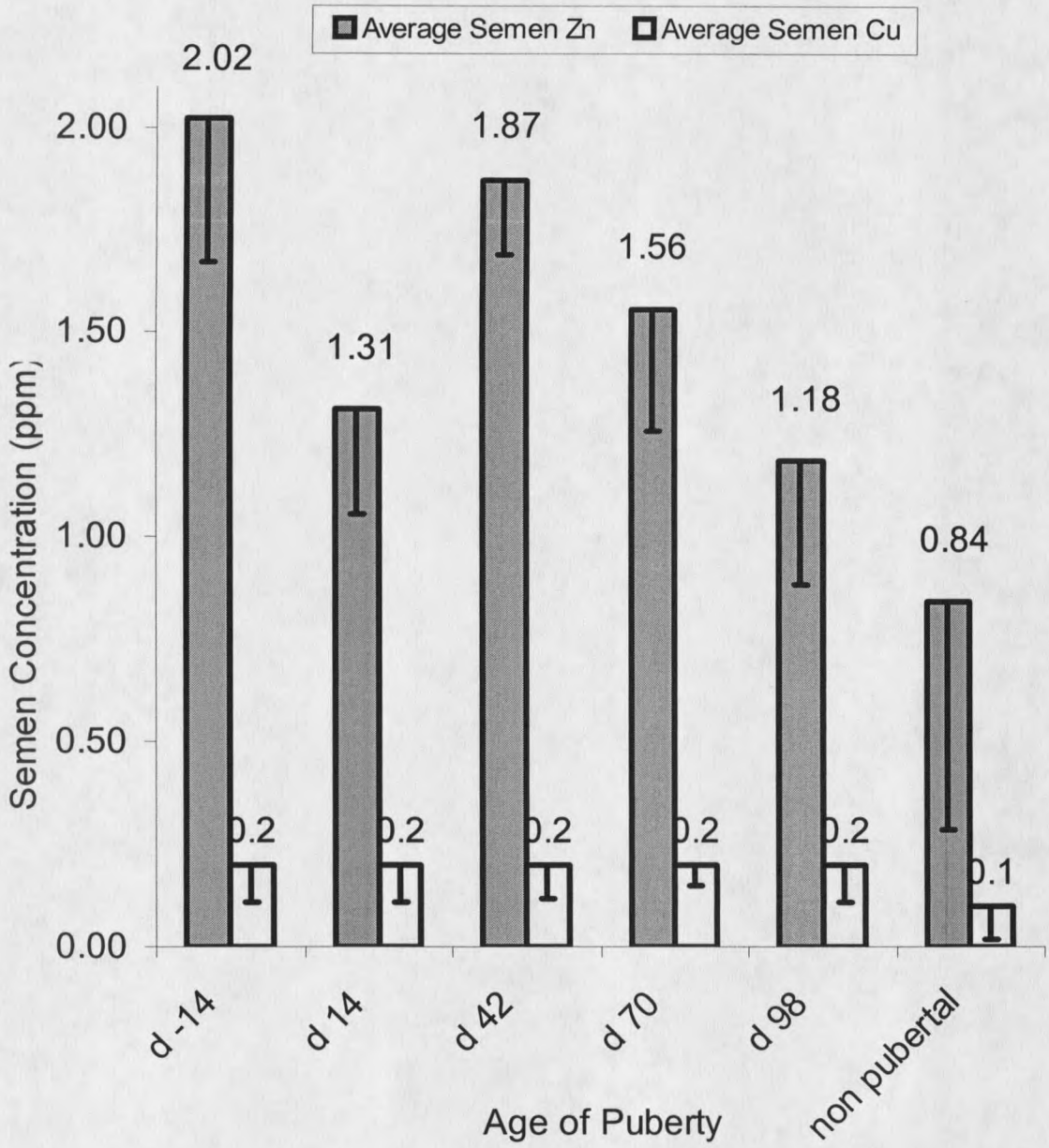


Figure 3.11. Zinc and Cu concentration (mean, SD) in semen, from bulls by pubertal day ($P > 0.10$) on d 42 of a 100 d mineral supplement study. Bulls were grouped by the collection date they reached puberty (n = 5, 9, 18, 7, 7, and 2 for d -14, 14, 42, 70, and 98 respectively). Two bulls did not reach puberty by February thus were compared in a separate group.

Table 3.7 Zinc and Cu concentration (mean) in semen from bulls ($P > 0.10$) on d 42 of a 100 d mineral supplement study. Bulls are grouped by puberty status. No differences were detected at the $P > 0.10$ level.

Trace Mineral	Pubertal Bulls	Non-Pubertal Bulls
Zn	1.7 ppm	1.3 ppm
Cu	.17 ppm	.16 ppm

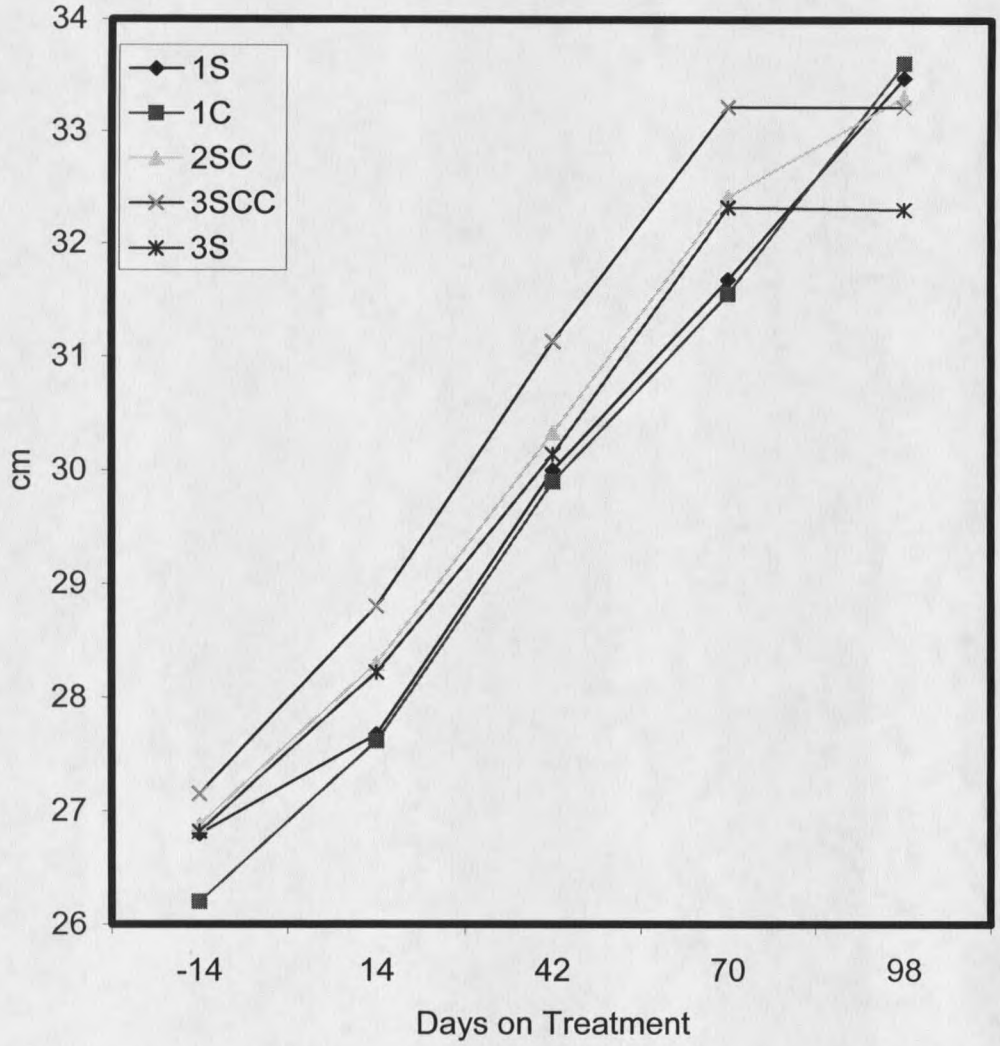


Figure 3.12. Scrotal circumference of bulls ($P > 0.10$, mean) receiving one of five mineral supplements on each semen collection day. Mineral supplements were 1x sulfate form (1S), 1x complexed form (1C), 1S + 1C (2SC), 1S + 2x1C (3SCC), and 3x1S (3S). Each 1x supplementation level contained 360 mg Zn, 125 mg Cu, 200 mg Mn and 12.5 mg Co.

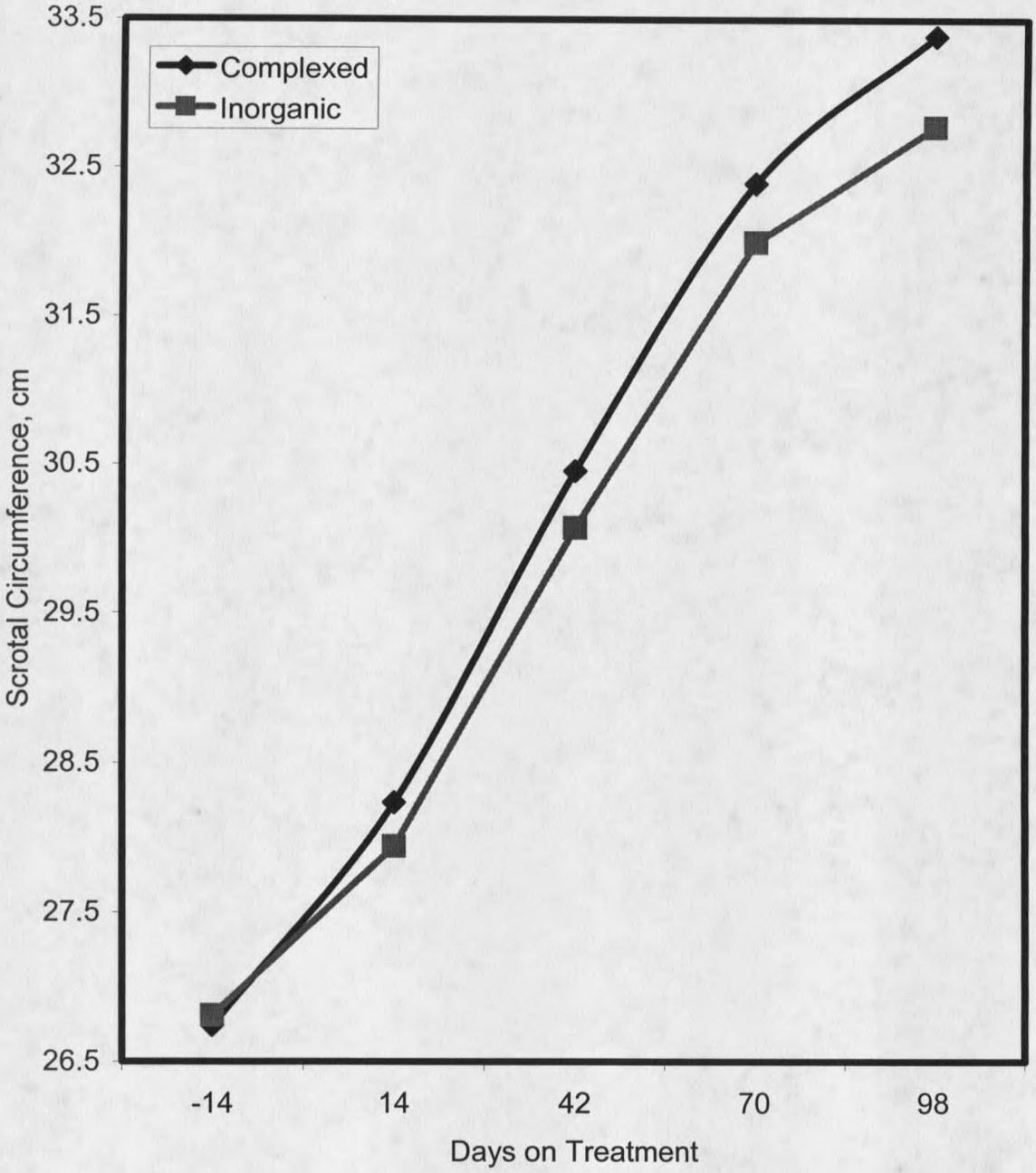


Figure 3.13. Scrotal circumference of bulls ($P > 0.10$, mean) on each collection day. Bulls were grouped by form of mineral supplement 1.) Complexed [1x complexed form (1C); 1S + 1C (2SC); and 1S + 2x1C (3SCC)] 2.) Inorganic [1x sulfate form (1S), and 3x1S (3S)]. Each 1x supplementation level contained 360 mg Zn, 125 mg Cu, 200 mg Mn and 12.5 mg Co.

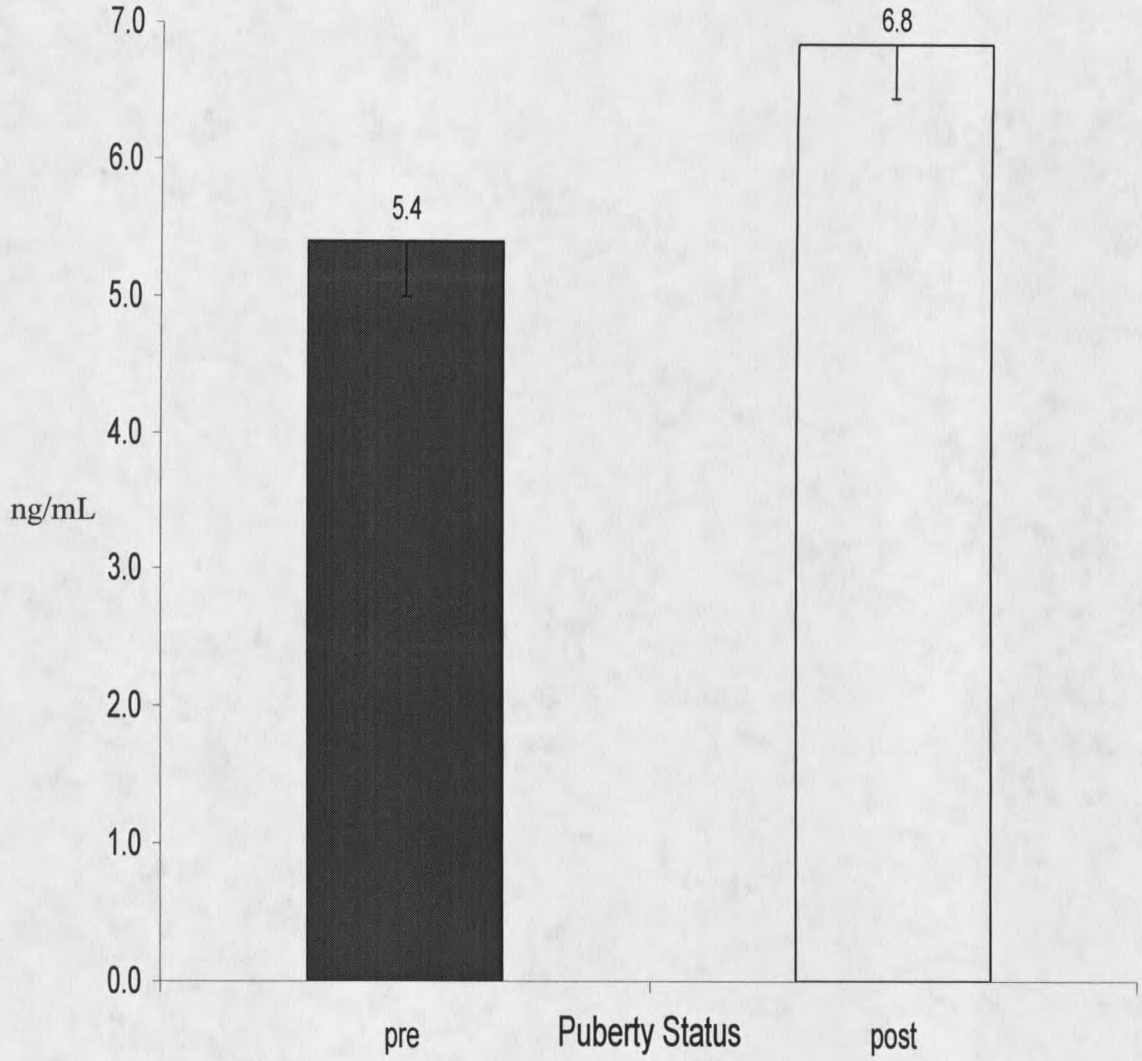


Figure 3.14. Testosterone levels ($P < 0.01$, mean, SD) of bulls on the collection day before puberty (pre) compared to the collection day in which they were considered pubertal (post).

Discussion

Very little information is available in the literature regarding the role of trace minerals on reproductive health or on puberty establishment in bulls. Even less is known about the specific role, if any, Cu plays in the male reproductive system. Reduced libido

and spermatogenesis have been attributed to a Mo antagonist induced Cu deficiency in bulls (Thomas et al., 1951). However, no other literature directly linking Cu to specific processes in bovine male reproduction has been reported. In the current study, the role of Cu in establishment of puberty is unclear due to the lack of adequate controls. However, bulls were deficient in liver Cu concentrations at the initiation of supplementation and all bulls demonstrated an increase in liver Cu concentrations by the end of supplementation (Figure 3.1). Thus, inadequate Cu was not an obvious factor in any traits measured. We do know, however, that Cu is vitally important for overall health, growth and trace mineral balance and thus, an important aspect in any bovine diet.

Normal liver Zn concentrations (DM) ranged from 83 to 300 ppm (mean, 111 ppm) for an aged cow (Mertz, 1986). Swenson (1999) reported a mean liver Zn concentration among pregnant, first calf heifers before supplementation at approximately 100 ppm. Trace mineral supplements were removed from bulls in the present study 81 days before treatment, yet liver Zn concentrations were all above the adequate liver Zn concentrations as proposed by Mertz (1996). The range forage and subsequent weaning ration of bulls in the current study may have provided adequate amounts of Zn without supplementation, resulting in higher than expected Zn liver concentrations. It is also very possible that the bull calves and their dams were able to compensate for the lack of Zn supplementation by lowering excretion and recycling. It is theorized that the body has very strong homeostatic mechanisms involved in Zn absorption and metabolism (Kendall et al., 2000). Thus, when rations are deficient in Zn, the body may become more efficient in maintaining and recycling Zn stores within the body. As an example, many intestinal brush border carrier membrane proteins are influenced by nutritional and physiological

status and these proteins quickly increase in number during Zn depletion in the diet (Cousins, 1985) allowing for more Zn to be absorbed by the mediated diffusion mechanism. Increased absorption of Zn during a fast (Mertz, 1986) and increased storage during late gestation of cows (Graham, 1991; Swenson, 1999) have been reported. Swenson (1999) suggested that in preparation for high requirements of Zn during parturition and lactation, the body absorbed and stored Zn more efficiently during late gestation. Absorption of Zn increased in other species in preparation for high Zn-requiring physiological stages (Davis and Williams, 1977; Underwood, 1976; Mertz, 1986). Zinc absorption increased in rats during the latter stages of pregnancy (Davis and Williams, 1977). The body content of Zn increases by 1.5-1.7% in hens before the high Zn requiring laying period (Underwood, 1976). The initial Zn liver concentrations from this study following 60 d of no mineral supplementation (Figure 3.2) suggest that the peripubertal bull calf may increase absorption and storage in response to a possible high Zn requiring physiological stage.

All liver Zn concentrations of bulls, except one, on d 100 of treatment were decreased relative to d -21 (Figure 3.2), suggesting an increased use of Zn during the peripubertal stage. Even the 20 bulls fed the high levels (either complexed or inorganic forms) had decreased ($P < 0.01$) liver Zn content on d 100 relative to d -21. Although no previous studies have investigated Zn liver concentrations of bulls during this age period, Swenson (1999) reported that liver Zn concentrations decreased in heifers during their first lactation regardless of form or level of Zn supplementation. Davies and Williams (1977) reported absorption of the duodenum during different stages of pregnancy and lactation in the rat. By d 21 of pregnancy the amount of Zn absorbed had doubled and

continued to rise until d 14 of lactation. By d 28 post-partum, Zn absorption by the duodenum had declined to the values of non-pregnant rats. This research supports the possibility that there are physiological times in the life cycle in which Zn requirements are higher. Supporting this further, are several studies investigating other male species during the peripubertal time of life. Parizek et al. (1966) reported that testis Zn concentrations decreased to normal adult rat concentration levels after a peripubertal increase. Crichton et al. (1982) reported that low Zn intake by young males of several species interfered with normal sexual development. Several authors (Parizek et al., 1966; Mason et al., 1982; Salem et al. 1984, Mertz, 1986) have reported altered testis growth and only immature spermatozoa among male rats that were deficient in Zn. Taken together, we suggest that current NRC (1996) requirements for Zn may be too low and result in subclinical deficiencies in the peripubertal bull.

In the current study, more bulls receiving the complexed mineral supplement (1C, 2SC, 3SCC) were pubertal at 42 d ($P = 0.03$) compared to bulls receiving sulfate mineral supplements (1S and 3S). The tendency ($P= 0.12$) for more bulls receiving the 3SCC treatment to be pubertal on d 42, suggests additional complexed mineral may decrease age of puberty in bulls regardless of deficiency. Initially, the 42 d pubertal response to supplement appeared to have occurred too early to be related to treatment, as the total spermatogenic cycle in the bull takes 61 days (Senger, 1999). However, much of the influence Zn has on spermatogenesis occurs during the later stages. Parizek et al. (1966) reported a rapid increase in the Zn concentration of testis (120 μg/g to 200 μg/g at 35 and 58 d of age, respectively) in rats during the stage of sexual development that coincides with the first completion of spermiogenesis (first spermatids transforming into

spermatozoa). Elevated testis Zn concentration in rats during this stage of development was thought to be due to the high content of Zn bound to protein in the mature sperm cell. Additionally, testosterone contributes a larger role in the later stages of spermatogenesis, by quantitatively maintaining meiosis and spermiogenesis (Courot and Ortavant, 1981). During spermiogenesis, there is an increase in the activity of lactate dehydrogenase, a Zn dependent enzyme used in the mitochondrial sheath of the spermatozoa for ATP production in anaerobic conditions (Parizek et al., 1966). Development of spermatids into spermatozoa in the bull requires approximately 17.2 days with transportation through the epididymis requiring approximately 14 days (Senger, 1999; Fitzpatrick et al., 2002). This makes the 42-day response to supplementation more reasonable.

This is the first study to demonstrate a possible role of mineral form on age of puberty in bulls. Further investigation into this area, may improve our understanding of the effect mineral level and form may have on days to puberty. Such an effect could be significant to the bull industry, as increased production demands are expected of bulls. In addition, various authors have reported spermatozoa quantity and quality continue to improve after puberty (Almquist and Aman, 1975; Almquist et al., 1976, Lunstra and Echternkamp, 1982; Evans et al., 1994). As full reproductive capacity is attained, spermatozoa motility and cell numbers increase greatly, allowing for potentially greater fertility in each ejaculate (Abdel-Raouf, 1960). This is further supported by Arthington et al. (2002) who compared yearling bull response to different forms of mineral, Zn sulfate (ZnS) or Zn proteinate (ZnP), and levels (40 or 60 ppm) of Zn supplementation. Bulls fed 60 ppm of ZnS and bulls fed 40 ppm ZnP had a higher ($P < 0.05$) percentage of normal spermatozoa in ejaculates. Arthington et al. (2002) suggests that when Zn is

supplemented above NRC (1996) recommendations, there may be beneficial effects to ejaculates after bulls reach one year of age. Bulls from the current study were removed from treatment at approximately one year of age. It would have been interesting to have continued feeding mineral treatments to the bulls for a longer period of time.

Another aspect deserving attention is the possible negative effect from the high sulfate treatment (3S) on the pubertal process of bulls. There was a 22 d difference in days to puberty between bulls fed elevated sulfate only minerals (3S) compared to bulls fed a similar amount of complexed mineral (3SCC; Figure 3.3). Swenson (1999) reported a similar negative response in first calf heifers, where more heifers fed either complexed mineral or no mineral were pregnant to their first service (AI) compared to heifers fed sulfate trace minerals. Smart et al. (1985) reported that a diet with 0.3% sulfates decreased plasma Cu yet had no effect on plasma Zn. The presence of high sulfur reduced the bioavailability of selenium and interfered with copper metabolism (Spears, 2003), but any effect high sulfur might have on Zn utilization was not reported. Further investigations may be warranted to study the effects high sulfates may have on bull reproduction.

Mineral level or form in the current study did not affect spermatozoa motility, concentration, head or tail abnormalities. The increased percentage of cytoplasmic droplets on d 42 among 3S bulls was not surprising because at 42 d, the 3S treatment group had the fewest number of pubertal bulls. Lunstra and Echternkamp (1982) reported a rapid decrease in ($P < 0.01$) the percentage of spermatozoa with proximal cytoplasmic droplets in bulls that reached puberty. Barth and Oko (1989) stated that in

many cases, an ejaculate with a high percentage of cytoplasmic droplets indicates lack of maturity within the male reproductive system.

Several studies in other species have compared Zn supplementation to semen characteristics. A decrease in sperm count was reported in men consuming Zn deficient diets (10 mg/day). Total sperm per ejaculate was less than 40 million in four out of five men. After the Zn repletion diets were consumed, total sperm count per ejaculate increased to 416.3 ± 102.9 million per ejaculate (Abbasi et al., 1980). All negative responses were reversed after repletion of adequate Zn in their diet. Root et al. (1979) reported that induced zinc deficiency in rats reduced serum testosterone. In both studies, subjects were severely Zn deficient, possibly resulting in contrasting results to the current study. The optimum concentration of Zn required to influence spermatogenesis may be species-specific, which may help explain the differences observed in the present and other studies (Saito et al., 1967; Mason et al., 1982).

From 2 to 21 weeks of age, Holstein bull calves were fed Zn deficient diets (4 ppm/d) and compared to both bull calves fed a Zn adequate ad libitum diet and bull calves fed a Zn adequate intake restricted diet. At 21 weeks, all calves were fed Zn sufficient diets. When calves were 48 wks of age, semen was collected. At this latter age, there were no effects of treatment on volume or concentration of semen produced, percentage of motile spermatozoa or percentage of abnormal spermatozoa (Pitts et al., 1966). The Pitts et al. (1966) study established that no irreversible effects on testis function occurred when dairy bulls were fed Zn deficient diets early in life. Thus, early trace mineral status of the bulls in the present study should not have negatively influenced the results achieved.

Kendall et al. (2000) utilized thirty-eight, eight month old lambs (mid to late breeding season) that were not considered to be deficient in any element. One treatment consisting of bolused (33g, 15.2% Zn, .5% Co, .15% Se) animals was compared to non-bolused animals. On d 73 and 79 after bolusing, viable spermatozoa in the ejaculate was increased compared to rams not bolused. Bolused rams also had increased spermatozoa motility on d 44 and d 73. As with the current study, there were no differences between bolused and control rams for any spermatozoa abnormalities. Kendall et al. (2000) suggested that the increase in sperm motility and spermatozoa numbers may have been due to the increased activity by glutathione peroxidases, however it is difficult to evaluate which specific mineral was responsible for this effect.

Due to the variation in the quality of ejaculates obtained in the current study and the difficulties in obtaining ejaculates repeatedly, it was difficult to truly assess differences in traits such as spermatozoa numbers, motility, and morphology. Also, even though bulls in the current study reached puberty, they may not have reached full reproductive capacity (Abdel-Raouf, 1960). Additional stress imposed on bulls in the current study caused by several trips through the chute on collection days may have affected our ability to obtain a representative sample from every bull. Some bulls that had reached puberty failed to ejaculate during subsequent collection attempts. This presented a major problem in the current study and thus, prevented extensive evaluation of spermatozoa characteristics. The use of an artificial vagina may have allowed for collection of a more representative sample.

Scrotal circumference was also measured and analyzed to monitor reproductive response to mineral supplementation. There are conflicting results on scrotal

circumference and Zn deficiencies in the literature. Several authors have reported inadequate scrotal growth when animals were severely deficient in Zn (Pitts et al, 1966; Underwood and Somers, 1969; Abbasi et al., 1980; Taneja and Nirmal, 1980; Hesketh, 1982; Griffin and Ojeda, 1988; Martin and White, 1992). The bulls in the current study were fed at least the NRC recommended levels and were not deficient in Zn at the beginning of treatment, while bulls in the above studies were fed little or no Zn. Arthington et al. (2002) reported no change in scrotal circumference between yearling bulls supplemented with levels at or above NRC (1996) recommendations. Among severely Zn deficient males, other clinical signs such as decreased appetite and suboptimal growth might compound scrotal growth (Martin and White, 1992). Apgar (1985) stated that while major Zn deficiencies decreased testicular weight, there was no effect on testicular weight among mildly Zn deficient rats. Because bulls in the current study were fed adequate Zn levels, under NRC (1996) standards, it is not surprising that scrotal growth was unaffected.

Due to the large temperature variation between collection dates, the repeatability of scrotal circumference may have been compromised. On days when the ambient temperature was lower, bulls tend to carry the scrotum closer to the body for temperature control. It must be recognized that this is a possible factor contributing to the consistency of measurements between collection dates.

Semen Zn concentration on d 42 (Figure 3.11) appears to be negatively associated with interval to puberty. It seems plausible that as bulls reach puberty and continue to mature, they will have a higher Zn concentration in their ejaculates due to the higher concentration of mature spermatozoa. This concept is supported at least, in the testis, by

Bedwal and Bahuguna (1994) who reported Zn content to be higher in the adult testis compared to the immature testis. We did recognize that bulls reaching puberty on the 11/19/02 collection date broke the linear trend. This may be due to inconsistencies of semen collections from bulls on this date. It would have been beneficial to compare this data to liver and testis biopsies taken also on d 42. Additionally, it would have been interesting to have the semen and seminal fluid analyzed on all collection days. However, the pattern from this limited data suggests Zn utilization during and after puberty is shifting compared to prepubertal development.

Implications

Minimum Zn requirements vary with the age and physiological state of the animal, with the composition of the diet, and with the form in which Zn is supplemented. Ideally, mineral intakes must meet the requirements of the body for optimal maintenance and production. However, over-consumption will increase cost margins and possibly cause toxic symptoms in the animal. This research demonstrated the importance of Zn supplementation (level and form) to peripubertal bulls. Zinc utilization by the peripubertal bull is high and the National Research Council recommendations may be too low for bulls during this stage. Bulls in the present study still had adequate liver Zn storage after 60 d of no trace mineral supplementation, illustrating the homeostatic ability of the body to adjust to a low Zn diet in preparation for an increase in Zn requirements. After 100 d of trace mineral supplementation, liver Zn concentrations were lower than d -21 even when Zn was supplemented at 3x recommended levels.

Feeding bulls at least some complexed trace mineral decrease the number of days to puberty compared to feeding sulfate trace minerals only. While trace mineral form affected the age at puberty in bulls, no differences in semen characteristics existed among bulls at one year of age.

LITERATURE CITED

Abbasi, A.A., A.S. Prasad, P. Rabbani, E. DuMouchelle. 1980. Experimental zinc deficiency in man: Effect on testicular function. J. Lab. Clin. Med. 96:544-50.

Abdel-Raouf, M. 1960. The postnatal development of the reproductive organs in bulls with special reference to puberty. Acta. Endocrinol. 34(49):1-28.

Abdel-Raouf, M. 1960. The postnatal development of the reproductive organs in bulls with special reference to puberty. Acta Endocrinol. 49:1-109.

Aggett, P.J. 1985. Physiology and metabolism of essential trace elements: an outline. Clinics in Endocrinology and Metabolism. 14:513-43.

Aire, T.A. and J.U. Akpokodje. 1975. Development of puberty in the White Fulani bull calf. Br. Vet. J. 131:146-150.

Allenby, G., P.M.D. Foster, and R.M. Sharpe. 1991. Evidence that secretion of immunoactive inhibin by seminiferous tubules from the adult rat testis is regulated by specific germ cell types: Correlation between in vivo and in vitro studies. Endocrinology. 128:467-76.

Almquist, J.O. and R.P. Aman. 1975. Reproductive capacity of dairy bulls. XI. Pubertal characteristics and postpubertal changes in production of semen and sexual activity of Holstein bulls ejaculated frequently. J. Dairy Sci. 59:986-990.

Almquist, J.O., R.J. Branas, and K.A. Barber. 1976. Postpuberal changes in semen production of Charolais bulls ejaculated at high frequency and the relation between testicular measurements and sperm output. J. Anim. Sci. 42:670-676.

Amann, R.P. 1983. Endocrine changes associated with onset of spermatogenesis in Holstein bulls. J. Dairy Sci. 66:2606-22.

Amann, R.P., and B.D. Schanbacher. 1983. Physiology of male reproduction. J. Anim. Sci. 57:380-401.

Amann, R.P., M.E. Wise, J.D. Glass, and T.M. Nett. 1985. Prepubertal changes in the hypothalamic-pituitary axis of Holstein bulls. Biol. Reprod. 34:71-80.

Amann, R.P., M.E. Wise, J.D. Glass, and T.M. Nett. 1986. Prepubertal changes in the hypothalamic pituitary axis of Holstein bulls. Biol. Reprod. 1986. 34(1):71-80.

Apgar, J. 1985. Zinc and reproduction. Ann Rev Nutr. 5:43-68.

Aravindakshan, J. P., A. Honaromooz, P.M. Bartlewski, A.P. Beard, R.A. Pierson, and N.C. Rawlings. 2000. Pattern of gonadotropin secretion and ultrasonographic evaluation of developmental changes in the testis of early and late maturing bull calves. Theriogenology. 54:339-354.

Arthington, J.D., L.R. Corah, and D.A. Hill. 2002. The effects of dietary zinc concentration and source on yearling bull growth and fertility. J. Anim. Sci. 18:282-285.

Barth, A.D. and R.J. Oko. 1989. Abnormal Morphology of Bovine Spermatozoa. Iowa State University Press, Ames, IA.

Bedwal. R.S. and A. Bahuguna. 1994. Zinc, copper and selenium in reproduction. Experientia. 50(7):626-40.

Berardinelli, J.G. 2003. Personal Communication.

Berndston, W.E., and C. Desjardins. 1974. The cycle of the seminiferous epithelium and spermatogenesis in the bovine testis. Am. J. Anat. 140:167-178.

Berndtson, W.E., G. Igboeli, and B.W. Pickett. 1987. Relationship of absolute numbers of sertoli cells to testicular size and spermatogenesis in young beef bulls. J. Anim. Sci. 64:241-246.

Bloom, E., and C. Wolstrup. 1976. Zinc as a possible causal factor in the sterilizing sperm tail defect, the 'dag-defect,' in Jersey bulls. Nord. Vet. Med. 28(10):515-8.

Braselton, W.E., K.J. Stuart, T.P. Mullaney, and T.H. Herdt. 1997. Biopsy mineral analysis by inductively coupled plasma-atomic emission spectroscopy with ultrasonic nebulization. J. Vet. Diagn. Invest. 9:395-400.

Brito, L.F.C., A.E.D.F. Silva, L.H. Rodrigues, F.V. Vieira, L.A. Deragon, and J.P. Kastelic. 2001. Effect of age and genetic group on characteristics of the scrotum, testes and testicular vascular cones, and on sperm production and semen quality in AI bulls in Brazil. Theriogenology. 58:1175-1186.

Brown, B.W. 1994. A review of nutritional influences on reproduction in boars, bulls and rams. Reprod. Nutr. Dev. 34:89-114.

Carson, R.L., and J.G.W. Wenzel. 1997. Observations using the new bull-breeding soundness evaluation form in adult and young bulls. Veterinary Clinics of North America: Food Animal Practice. 13(2):305-11.

Chada, M., R. Prusa, J. Bronsky, K. Kotaska, K. Sidlova, M. Pechova, and L. Lisa. 2003. Inhibin B, follicle stimulating hormone, luteinizing hormone and testosterone

during childhood and puberty in males: Changes in serum concentrations in relation to age and stage of puberty. Physiol. Res. 52:45-51.

Crichton, E.G., P.H. Drutzsch, and M. Chvapil. 1982. Studies on prolonged spermatozoa survival in chiroptera-II. The role of zinc in the spermatozoa storage phenomenon. Comp. Biochem. Physiol. A71:71-77.

Corah, L.R. and S. Ives. 1991. The effects of essential trace minerals on reproduction in beef cattle. Vet. Clinics of North America. 7:41-57.

Corah, L.R., and J.D. Arthington. 1994. Determining the trace mineral status in cattle. Publication and Video Distribution. Kansas State University Cooperative Extension Service, Manhattan, KS.

Corah, L.R. 1996. Trace mineral requirements of grazing cattle. Anim. Feed Sci. Tech. 59:61-70.

Courot, M., and R. Ortaveant. 1981. Endocrine control of spermatogenesis in the ram. J Reprod Fertil Suppl. 30:47-60.

Coulter, G.H., R.B. Cook, and J.P. Kastelic. 1997. Effects of dietary energy on scrotal surface temperature, seminal quality, and sperm production in young beef bulls. J. Anim. Sci. 75:1048-52.

Cousins, R.J. 1985. Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplamin. Physiol Rev. 65(2):238-309.

Curtis, S.K., and R.P. Amann. 1981. Testicular development and establishment of spermatogenesis in Holstein bulls. J. Anim. Sci. 53:1645 -57.

Dargartz D. 1993. Beef Cow/Calf Health and Productivity Audit. Fort Collins, Co. USDA:APHIS:VS Centers for Epidemiology and Animal Health.

Davies, N.T., and R.B. Williams. 1977. The effect of pregnancy and lactation on the absorption of zinc and lysine by the rat duodenum *in situ.* Br. J. Nutr. 38:417.

Davies, S. 1984-85. Yearbook of nutritional medicine. Keats Publishing, New Canaan, Conn.

DeRobertis, E.D.P., and E.M.F. DeRobertis. 1980. Cell and Molecular Biology. Saunders College, Philadelphia, PA.

Eckhert, C.D. and L.S. Hurley. 1977. Reduced DNA synthesis in zinc deficiency: regional differences in embryonic rats. J. Nutr. 107: 855-61.

Elmore, R.G., C.J. Bierschwal, C.E. Martin, and R. S. Youngquist. 1975. A summary of 1127 breeding soundness examinations on beef bulls. Theriogenology. 3(6):209-18.

Evans, A.C.O., F.J. Davies, L.F. Nasser, P. Bowman, and N.C. Rawlings. 1994. Differences in early patterns of gonadotrophin secretion between early and late maturing bulls, and changes in semen characteristics at puberty. Theriogenology. 43:569-78.

Evans, A.C.O., R.A. Pierson, A. Garcia, L.M. McDougall, F. Hudka, and N.C. Rawlings. 1986. Changes in circulating hormone concentrations, testes histology and testes ultrasonography during sexual maturation in beef bulls. Theriogenology. 46:345-357.

Fitzpatrick, L.A., G. Fordyce, M.R. McGowan, J.D. Bertram, V.J. Doogan, J. DeFaveri, R.G. Miller, and R.G. Holroyd. 2002. Bull selection and use in northern Australia. Animal Repro. Sci. 71:39-49.

Flipse, R.J. and J.O. Almquist. 1961. Effect of total digestible nutrient intake from birth to four years of age on growth and reproductive development and performance of dairy bulls. J. Dairy Sci. 905:905-914.

Foote, R.H., G.E. Seidel, J. Hahn, W.E. Berndtson, and G.H. Coulter. 1976. Seminal quality, spermatozoal output, and testicular changes in growing Holstein bulls. J. Dairy Sci. 60:85-88.

Georgievskii, V.I., B.N. Annenkov, and V.I. Amokhin. 1981. Mineral nutrition of animals. Mansells Bookbinders Ltd, Witham, Essex.

Gosey, J.A. 1983. Cow-Calf Corner. Department of Animal Science, Oklahoma State University. www.ansi.okstate.edu/exten/cc-corner/archbullsale.html.

Graham, T.W. 1991. Element deficiencies in cattle. Clin of North Amer. Food Anim. Pract. 7:153-215.

Griffin, J.E. and S.R. Ojeda. 1988. Textbook of Endocrine Physiology. Oxford University Press, New York, NY.

Grossman, M., W.J. Koops, and J.G. Den Daas. 1995. Multiphasic analysis of reproductive efficiency of dairy bulls. J. Dairy Sci. 78:2871-2876.

Guyton, A.C. 1986. Textbook of medical physiology. W.B. Sauders Co. Philadelphia, PA.

Heder, G., W.E. Siems, H. Nehring, K. Muiller, H. Hilse and K.D. Jentzsch. 1989. Occurrence and potential importance of selected peptidases in bull ejaculates. Andrologia 21(3):247-55.

Hafez, E.S.E. 1993. Reproduction in farm animals. Lea and Febiger, Malvern, Pennsylvania.

Hammerstedt, R.H. 1996. Evaluation of sperm quality: Identification of the subfertile male and courses of action. Anim. Repro. Sci. 42:77-87.

Hatfield, P.G., C.K. Swenson, R.W. Kott, R.P. Ansotegui, N.J. Roth, and B.L. Robinson. 2001. Zinc and copper status in ewes supplemented with sulfate- and amino acid-complexed forms of zinc and copper. J. Anim. Sci. 79:261-266.

Henkel, R., J. Bittner, R. Weber, F. Huther, and W. Miska. 1999. Relevance of zinc in human sperm flagella and its relation to motility. Fertil. Steril. 71:1138-43.

Henkel, R., C. Baldauf, and W.B. Schill. 2003. Reabsorption of the element zinc from spermatozoa by the epididymal epithelium. Reprod. Dom. Anim. 38:97-101.

Hesketh, J.E. 1982. Effects of dietary zinc deficiency on Leydig cell ultrastructure in the boar. Comp. Pathol. 92:239-47.

Hochereau-de Reviers, M.T., C. Monet-Kuntz, and M. Courot. 1987. Spermatogenesis and sertoli cell numbers and function in rams and bulls. J. Reprod. Fert. 34:101-114.

Humphrey, J.D. and P.W. Ladds. 1975. A quantitative histological study of changes in the bovine testis and epididymis associated with age. Res. Vet. Sci. 19:135-141.

Hutchenson, D.P. and N.A. Cole. 1986. Management of transit- stress syndrome in cattle: nutritional and environmental effects. J. Anim. Sci. 62:555-60.

Irvine, D.S. 1996. Glutathione as a treatment for male fertility. Rev. Reprod. 1:6-12.

Ishizuka, M., H. Ohshima, N. Tamura, T. Nakada, A. Inoue, S. Hirose, and H. Hagiwara. 2003. Molecular cloning and characteristics of a novel zinc finger protein and its splice variant whose transcripts are expressed during spermatogenesis. Biochemical and Biophysical Research Communications. 301(4):1079-1085.

Jimenez-Severiano, H. 2002. Sexual development of dairy bulls in the Mexican tropics. Theriogenology. 58:921-932.

Kasari, T.H., S.E. Wikse, and R. Jones. 1996. Use of yearling bulls in beef cattle operations. Compend. Contin. Educ. Pract. Vet. 18: 1244-53.

Kendall, N.R., S. McMullen, A. Green, and R.G. Rodway. 2000. The effect of a zinc, cobalt, and selenium soluble glass bolus on trace element status and semen quality on ram lambs. Animal Reproduction Science. 62:277-83.

Kennedy, S.P., J.C. Spitzer, F.M. Hopkins, H.L. Higdon, and W.C. Bridges. 2002. Breeding soundness evaluations of 3,348 yearling beef bulls using the 1993 Society for Theriogenology guidelines. Theriogenology. 58(5):947-61.

Killian, G.J. and R.P. Amann. 1972. Reproductive capacity of dairy bulls. IX Changes in reproductive organ weights and semen characteristics of Holstein bulls during the first thirty weeks after puberty. J. Dairy Sci. 55:1631-1634.

Knobil, E. and J.D. Neill. 1994. The physiology of reproduction. Raven Press, New York, NY.

Krebs, N.F. 2000. Overview of zinc absorption and excretion in the human gastrointestinal tract. J Nutr. 130(5S Suppl):1374S-7S.

Lacroix, A. and J. Pelletier. 1979. LH and testosterone release in developing bulls following LH-RH treatment. Acta. Endocrinol. 91(4):719-29.

Latimer, F.G., L.L. Wilson, and M.F. Cain. 1982. Scrotal measurements in beef bulls: heritability estimates, breed and test station effects. J. Anim. Sci. 54:473-479.

Leissner, K.H., B. Fjelkegard, and L.E. Tisell. 1980. Concentration and content of zinc in the human prostate. Invest. Urol. 18:32-35.

Lunstra, D.D., J.J. Ford, and S.E. Echternkamp. 1978. Puberty in beef bulls: Hormone concentrations, growth, testicular development, sperm production and sexual aggressiveness in bulls of different breeds. J. Anim. Sci. 46:1054-1061.

Lunstra, D.D., and S.E. Echternkamp. 1982. Puberty in beef bulls: acrosome morphology and semen quality in bulls of different breeds. J. Anim. Sci. 55:638-648.

Maas, M.S. 1987. Relationship between nutrition and reproduction in beef cattle. Vet. Clinics of North America: Food Animal Practice. 3(3):633-46

Maddocks, S. and R.M. Sharpe. 1990. The effects of sexual maturation and altered steroid synthesis on the production and route of secretion of inhibin-α from the rat testis. 126:1541-50.

Macmillan, K.L. and H.D. Hafs. 1968. Gonadal and extra gonadal sperm numbers during reproductive development of Holstein bulls. J. Anim. Sci. 27:697-700.

Martin, G.B. and C.L. White. 1992. Effects of dietary zinc deficiency on gonadotrophin secretion and testicular growth in young male sheep. J. Reprod. Fert. 96:497-07.

Mason, K.E., W.A. Burns, and J.C. Smith. 1982. Testicular damage associated with zinc deficiency in pre and post pubertal rats. Response to zinc repletion. J. Nutr. 112:1019-28.

Mason, K.E., W.A. Burns, and J. Cecil Smith. 1982. Testicular damage associated with zinc deficiency in pre and postpubertal rats: response to zinc repletion. J. Nutr. 112:1019-28.

Mathews, C.K., and K.E. Holde. 1991. Biochemestry. Benjamin/Cummings Publishing Company. Redwood City, California.

McCarthy, M.S., H.D. Hafs, and E.M. Convey. 1979. Serum hormone patterns associated with growth and sexual development in bulls. J. Anim. Sci. 49:1012-1020.

McLachlan, R.I., L. O'Donnel, S.J. Meachem, P.G. Stanton, K. Pratis, and D.M. Robertson. 2002. Hormonal regulation of spermatogenesis in primates and man: insights for development of the male hormonal contraceptive. J. Androl. 23:149:-62.

Mertz, W. 1986. Trace elements in human and animal nutrition. Academic Press Inc. Orlando, Florida.

Miller, J.K., and R.G. Cragle. 1965. Gastrointestinal sites of absorption and endogenous secretion of zinc in dairy cattle. J. Dairy Science. 48:370-373.

Minetti, C., M.M. Oshiro, M.F. Santos, J.H. Romaldini, N.M. Moura, L.Valle, R.M. Oliveira-Filho. 1992. Ann Nutr. Metab. 36:167-74.

Moura, A. A. and B. H. Erickson. 1997. Age-related changes in peripheral hormone concentrations and their relationships with testis size and number of sertoli and germ cells in yearling beef bulls. J. Reprod. Fertil. 111:183-190.

Moura, A.A. and B.H. Erickson. 2001. Testicular development, histology, and hormone profiles in three yearling Angus bulls with spermatogenic arrest. Theriogenology. 55:1469-1488.

Mwansa, P.B., R.A. Kemp, D.H. Crews, J.P. Kastelic, D.C. Baily, and G.H. Coulter. 1999. Comparison of models for genetic evaluation of scrotal circumference in crossbred bulls. J. Anim. Sci. 78:275-282.

Neathery, N.W., S. Rachmat, W.J. Miller, R.P. Gentry, and D.M. Blackmon. 1972. Effect of chemical form of orally administered Zn on absorption and metabolism in cattle. Proc. Soc. Exp. Biol. Med. 139:953-67.

Neathery, M.W., W.J. Miller, D.M. Blackmon, F.M. Pate and R.P. Gentry. 1972. Effects of long term zinc deficiency on feed utilization, reproductive characteristics, and hair growth in the sexually mature male goat. J. Dairy Sci. 56:98-105.

Neathery, M.W., W.J. Miller, D.M. Blackmon, and R.P. Gentry. 1974. Zn tissue distribution in lactation Holstein cows fed normal and low-zinc practical diets. J. Anim. Sci. 38:854-859.

Niswender, G.D., L.E. Reichert, A.R. Midgley, and A.V. Nalbandov. 1969. Radioimmunoassay for bovine and ovine luteinizing hormone. Endocrinology. 84(5): 1166-73.

National Research Council. 2000. Nutrient requirements of beef cattle. National Academy Press, Washington, DC.

Oestreicher, P., and R.J. Cousins. 1982. Influence of intraluminal constituents on zinc absorption by isolated, vascularly perfused rat intestine. J. Nutr. 112(10):1978-82.

Om A.S., and K.W. Chung. 1996. Dietary zinc deficiency alters 5 alpha reduction and aromatization of testosterone and androgen and estrogen receptors in rat liver. J. Nutr. 126:842-8.

Parizek, J., J.C. Boursnell, M.F. Hay, A. Babicky, and D.M. Taylor. 1966. Zinc in the maturing rat testis. J. Rerpod. Fert. 12:501-07.

Parks, J.E., D.R. Lee, S. Huang, and M.T. Kaproth. 2002. Prospects for spermatogenesis in vitro. Theriogenology. 59:73-86.

Pelletier, J., S. Carrez-Camous, and J.C. Thiero. 1981. Basic neuroendocrine events before puberty in cattle, sheep and pigs. J. Reprod. Fert., 30:91-102.

Pitts, W.J., W.J. Miller, O.T. Fosgate, J.D. Morton, and C.M. Clifton. 1966. Effect of zinc deficiency and restricted feeding from two to five months of age on reproduction in Holstein bulls. J. Dairy Sci. 49:995-00.

Puls, R. 1995. Mineral levels in animal health. Sherpa International. Clearbrook, British Columbia, Canada.

Qui, W., S. Zhang, C. Xiao, W. Xu, Y. Ma, Y. Liu and Q. Wu. 2003. Molecular cloning and characterization of a mouse spermatogenesis-related ring finger gene znf230. Biochemical and Biophysical Research Communications. 306 (2):347-53.

Rice, L.E. 1991. The effects of nutrition on reproductive performance of beef cattle. North American: Food Animal Practice. 7(1):1-26.

Rojas, L.X., L.R. McDowell, R.J. Cousins, F.G. Martin, N.S. Wilkinson, A.B. Johnson, and J.B. Velasquez. 1995. Relative bioavailability of two organic and two inorganic zinc sources fed to sheep. J. Anim. Sci. 73:1202-07.

Root, A.W., G. Duckett, M. Sweetland, and E.O. Reiter. 1979. Effects of zinc deficiency upon pituitary function in sexually mature and immature male rats. J. Nutr. 109:958-64.

Ruckebusch Y. and P. Thivend. 1980. Digestive physiology and metabolism in ruminants. AVI Publishing Co. Westport, Connecticut

Saito S., I.M. Bush and W.F. Whitmore. 1967a. The effects of certain metals and chelating agents on rat and dog epididymal spermatozoan motility. Fert. Steril. 18:517-29.

Salem, S.I., W.A. Coward, P.G. Lunn, and G.J. Hudson. 1984. Response of the reproductive system of male rats to protein and zinc deficiency during puberty. Ann. Nutr. Metab. 28:44-51.

Sanford, L.M., C. Moore, J.K. Voglmayr, and M.H. Fahmy. 2000. Sexual maturational changes in circulatory inhibin concentration in relation to FSH concentration and testicular size in Suffolk and DLS rams. Theriogenology 54:719-30.

SAS. 1994. SAS User's Guide: Statistics. SAS inst. Inc., Cary, NC.

Schanbacher, B.D. 1982. Hormonal interrelationships between hypothalamus, pituitary and testis of rams and bulls. J. Anim. Sci. 55(2):56-67.

Senger, P.L. 1999. Pathways to pregnancy and parturition. Current Conceptions, inc., Pullman, WA.

Short, R.E. and D.C. Adams. 1988. Nutritional and hormonal interrelationships I beef cattle reproduction. Can. J. Anim. Sci. 68:29-39.

Shyu, H., S. Hsu, H. Hsieh-Li, and H. Li. 2003. Forced expression of RNF36 induces cell apoptosis. Experimental Cell Research. 287:301-13.

Smart, M.E., R. Cohen, D.A. Christensen, and C.M. Williams. 1985. The effects of sulphate removal from the drinking water on the plasma and liver copper and zinc concentrations of beef cows and their calves. J. Anim. Sci. 66:669-80.

Spears, J.W. 1996. Organic trace minerals in ruminant nutrition. Animal Feed Science Tech. 58. 151-63.

Spears, J.W. 2003. Trace mineral bioavailability in ruminants. J. Nutr. 133:1506S-9S.

Spitzer, J.C., F.M. Hopkins, H.W. Webster, F.D. Kirkpatrick, and H.S. Hill. 1988. Breeding soundness examination of yearling beef bulls. J. Am. Vet. Med. Assoc. 193(9):1075-9.

Srivastava, A., A.R. Chowdhury, and B.S. Setty. 1983. Zinc content of maturing spermatozoa in oestrogen treated rats. Int. J. Androl. 6(1):103-8.

Steel, L. and R.J. Cousins. 1985. Kinetics of zinc absorption by luminally and vascularly perfused rat intestine. Am. J. Physiol. 248:G46-53.

Stevenson, J.W., and I.P. Earle. 1956. Studies on parakeratosis in swine. J. Anim. Sci. 15:1036-46.

Stowe, H.D., W.E. Braselton, J.B. Kaneene, and M. Slanker. 1985. Multielement assays of bovine tissue specimens by inductively coupled argon plamsma emission spectroscopy. American J. Vet. Research. 46:561-565.

Suttle, N.F., 1991, The interactions between copper, molybdenum, and sulphur in ruminant nutrition. Annu. Rev. Nutr. 11:121-40.

Suttle, N.F., C.F. Mills. 1966. Studies of the toxicity of copper to pigs. Br J Nutr. 20(2):149-61.

Swenson, C.K. 1999. Effect of trace mineral supplementation on cow/calf performance. Ph.D. Dissertation. New Mexico Sate University, Las Cruces, New Mexico.

Taneja, S. and K. Nirmal. 1980. Histopathology of testes of mice fed on zinc-deficient diet. Indian J. of Experimental Biology. 18:1411-14.

Thomas, J.W. and S. Moss. 1951. The effect of orally administered molybdenum on growth, spermatogenesis and testes histology in young dairy bulls. J. Dairy Sci. 34:929-33.

Underwood, E.J. and M. Somers. 1969. Studies of zinc nutrition in sheep. I The relation of zinc to growth, testicular development and spermatogenesis in young rams. Australian Journal of Agricultural Research. 20:889-897.

Underwood, E.J. 1976. Mineral imbalances in farm animals and their study and diagnosis with isotopic tracers. Atomic Energy Review. 14:591-19.

Underwood, E.J. and N.F. Suttle. 1999. The mineral nutrition of livestock. CABI Publishing, New York, NY.

Vallee, B.L. 1983. In "Zinc Enzymes" (T.G. Spiro, ed). p. 1-20 Wiley, New York.

van Baren, M.J., H.C. van der Linde, G.J. Breedveld, W.M. Baarends. P. Rizzu, E. de Graaff, B. Oostra, and P. Heutink. 2002. A double ring-H2 domain in RNF32, a gene expressed during sperm formation. Biochemical and Biophysical Research Communications. 292:58-65.

van Den Dungen, H.M., J. van Dieten, G.P. van Rees, and J. Shoemaker. 1990. Testicular weight, tubular diameter and number of Sertoli cells in rats are decreased after early prepubertal administration of an GnRH antagonist: the quality of spermatozoa is not impaired. Life Sci. 46:1081-1089.

Van Dissel-Emilian F.M., A.J. Grootenhuis, F.H. DeJong, and D.G. DeRooij. 1989. Inhibin reduces spermatogonial numbers in testes of adult mice and Chinese hamsters. Endocrinology. 125:1899-1903.

Wallace, E., H.I. Calvin, M.P. Salgo, J.E. Dennis, and K. Ploetz. 1984. Normal levels of zinc and sulfhydryls in morphologically abnormal populations of spermatozoa from moderately zinc-deficient rats. Gamete Research. 9:375-86.

Weissig, H., S. Narisawa, C. Sikstrom, P.G. Olsson, J.R. McCarrey, P.A. Tsonis, K. Del Rio-Tsonis, and J.L. Millan. 2003. Three novel spermatogenesis-specific zinc finger genes. Federation of European Biochemical Societies. 547:61-68.

Wellington, B.K., J.A. Paterson, C.K. Swenson, R.P. Ansotegui, P.G. Hatfield and A.B. Johnson. 1 998. T he i nfluence o f s upplemental c opper a nd z inc o n b eef h eifers performance and changes in liver copper. Proc. West. Sec. Amer. Soc. of Anim. Sci. 49:323-326.

Willett, W.L. and J.I. Ohms. 1957. Measurement of testicular size and its relation to production of spermatozoa by bulls. J. Dairy Science. 50:1559-69.

Wood, R.I., F.J.P. Ebling, H. I'Anson, S.M. Yellon, and D.L. Foster. 1991. Prenatal androgens time neuroendocrine sexual maturation. Endocrinology, 128: 2457-2468.

Xing, W. and M.R. Sairam. 2002. Cross talk of two Krupple transcription factors regulates expression of the ovine FSH receptor gene. Biochemical and Biophysical Research Communications. 295(5):1096-1101.

Yan, W., S.J. Hirvonen-Santti, J. J. P alvimo, J. T oppari, and O.A. Janne. 2002. Mech. Dev. 118(1-2):247-53.

Yong-Xin, M., Z. Si-Zhong, H. Yi-Ping, H. Xin-Li, W. Qia-Qing, and S. Yan. 2002. Identification of a novel human zinc finger protein gene ZNF313. Acta. Bioch. 35(3):230-37.

Zirkin, B.R., L.L. Ewing, N. Kroman, and R.C. Cochran. 1980. Testosterone secretion by rat, rabbit, guinea pig and hamster testis perfused in vitro: correlation with Leydig cell ultrastructure. Endocrinology. 107:1867-1874.