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.

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
Bozeman, Montana

November, 2003

APPROVAL

of a thesis submitted by

Whisper Lynn Alexander

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style and consistency, and is ready for submission to the College of Graduate Studies.

Dr. Raymond P. Ansotegui Ray Ansotegui 11/26/03
(Signature) (Date)

Approved for the Department of Animal and Range Sciences

Dr. Michael W. Tess M. W. Tess 12/1/03
(Signature) (Date)

Approved for the College of Graduate Studies

Dr. Bruce R. McLeod Bruce R. McLeod 12-8-03
(Signature) (Date)

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Montana State University, I agree that the library shall make it available to borrowers under rules of the library.

If I have indicated my intention to copyright this thesis by including a copyright notice page, copying is allowable only for scholarly purposes, consistent with fair use as prescribed in the U.S. Copyright Law. Requests for permission for extended quotation from or reproduction of this thesis in whole or in parts may be granted only by the copyright holder.

Signature Whisper Alexander

Date 11/15/03

TABLE OF CONTENTS

TABLE OF CONTENTS-CONTINUED

LIST OF FIGURES

LIST OF FIGURES - CONTINUED

LIST OF TABLES

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

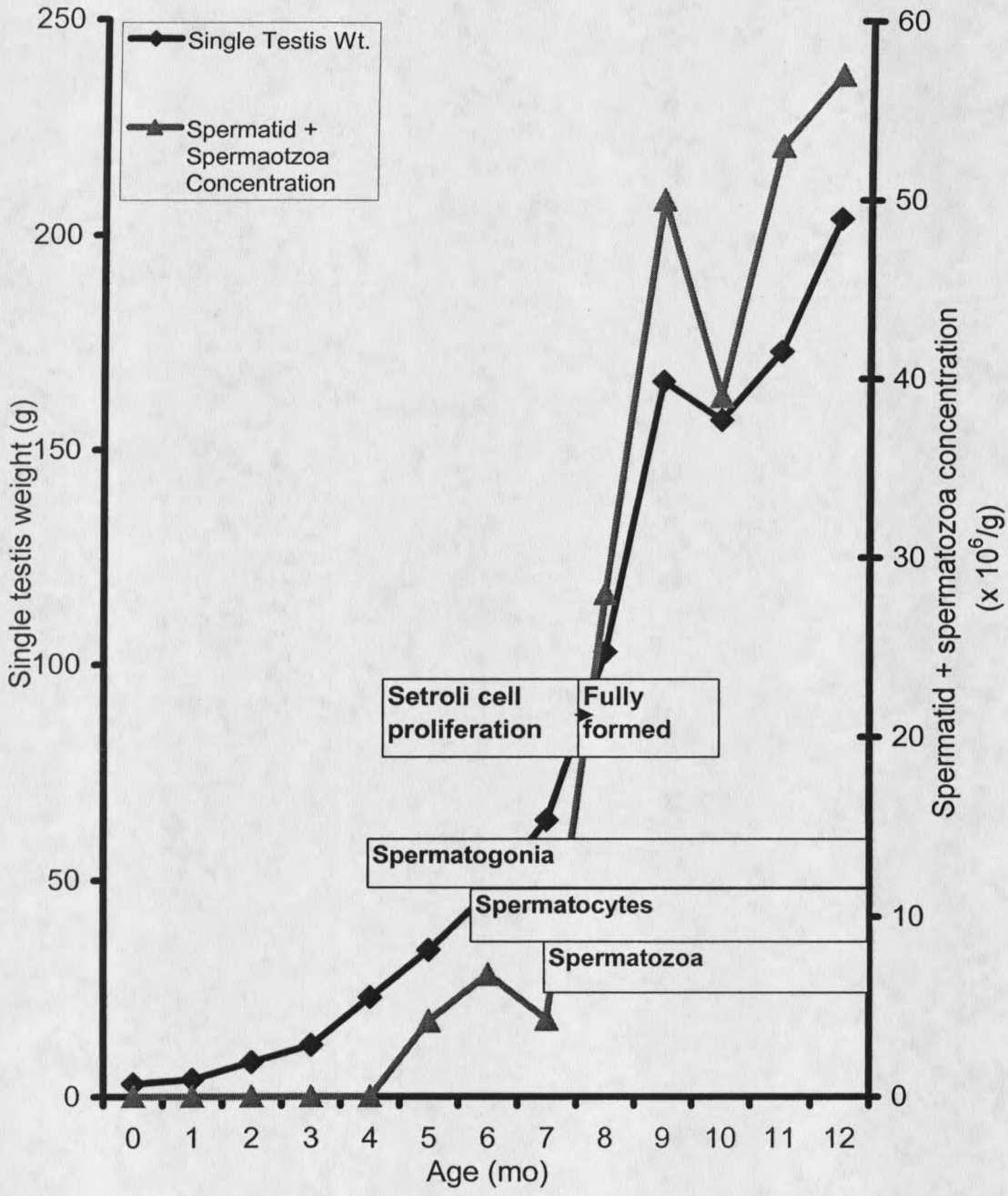
Measurements of Puberty Puberty is the maturational process of reproduction. There are several different ways in which puberty has been defined. A basic definition is the age at which an individual has the first capability to participate in reproduction. Brown (1994) defined puberty in the male as the 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 x 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).

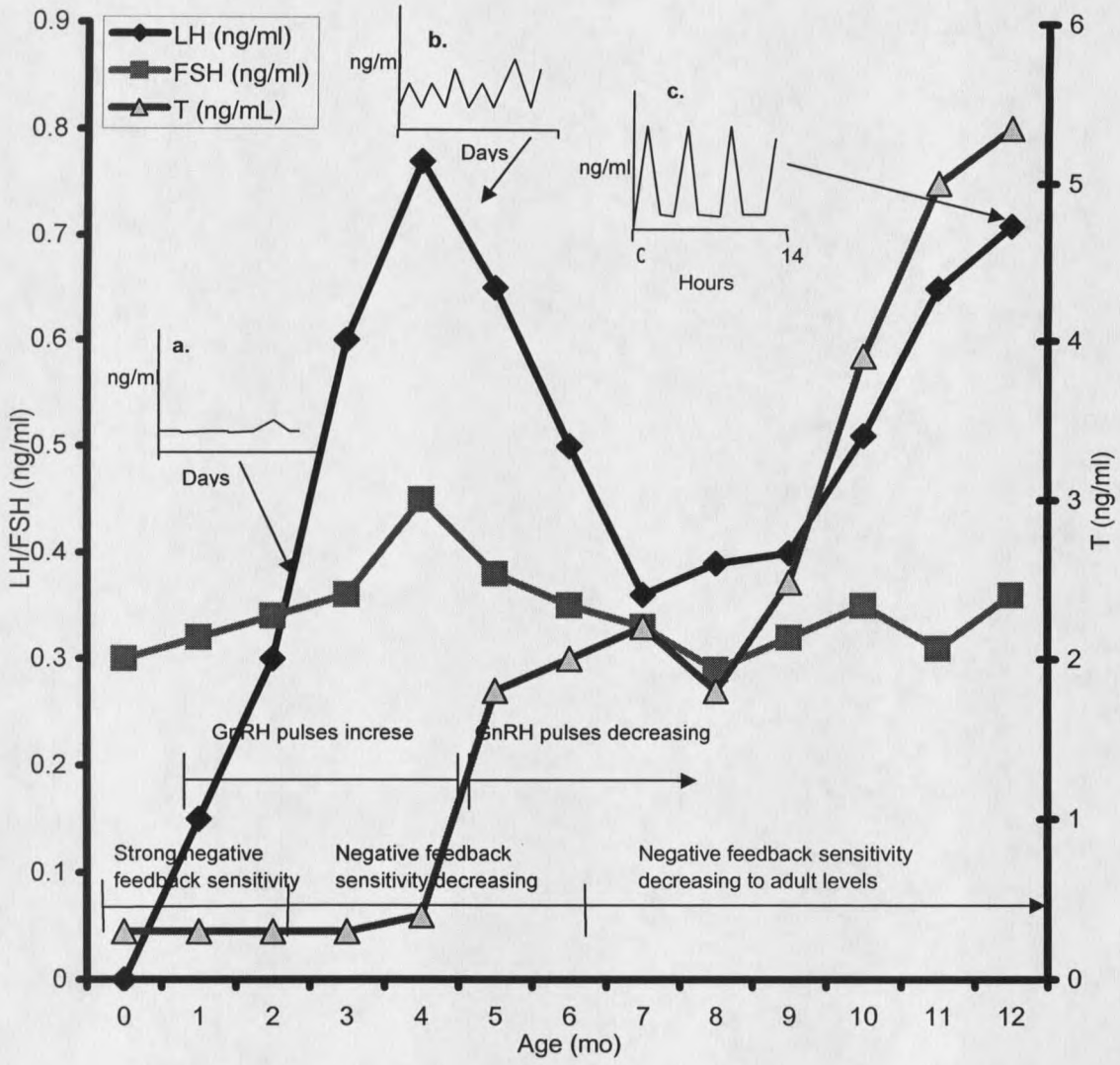
Histological examinations have provided evidence that the establishment of spermatogenesis in the developing bull calf is a progressive process that occurs over a number of weeks (Fig. 2.1; Abdel-Raouf, 1960; Curtis and Amann, 1981; van Den Dungen, 1990, Evans et al., 1996). Curtis and Amann (1981) evaluated 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.