NUTRITION, METABOLIC HORMONES, AND SEXUAL DEVELOPMENT IN BULLS

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By

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ABSTRACT

A series of experiments was conducted to evaluate the effects of nutrition during calfhood (defined as the period from 10 to 26-30 wk of age) and peripubertal period (defined as the period from 27-31 to 70-74 wk of age) on sexual development and reproductive function in beef bulls. The overall objective of these experiments was to evaluate the effects of nutrition on endogenous metabolic hormones (leptin, insulin, GH, and IGF-I), gonadotropins and testosterone concentrations, sexual development, sperm production, and semen quality in bulls. The results of these experiments demonstrated that nutrition affected GnRH secretion and sexual development in bulls. Increased nutrition during calfhood resulted in a more sustained increase in LH pulse frequency during the early gonadotropin rise and greater testicular development at maturity. On the other hand, low nutrition during calfhood suppressed LH secretion during the early gonadotropin rise and resulted in delayed puberty and reduced testicular development at maturity. When low nutrition was accomplished by restricted feed intake, hypothalamic and pituitary function were compromised and LH secretion was more severely affected. Temporal associations between LH secretion patterns and circulating IGF-I concentrations implied that IGF-I is a possible signal to the central "metabolic sensor" involved in translating body nutritional status to the GnRH pulse generator. Nutrition also affected testicular steroidogenesis (testosterone concentrations), indicating effects on the number or function of Leydig cells, or both. Age-related increases in physiological and GnRH-stimulated circulating testosterone concentrations were hastened in bulls receiving high nutrition and delayed in bulls receiving low nutrition;

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DEDICATION

I dedicate this thesis to my family, my wife, Daniela, and my son, Gabriel. Your love and support give me inspiration, motivation, and courage. I could not have done it without you!

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LIST OF ABBREVIATIONS

ADF	acid detergent fiber
ANOVA	analysis of variance
CP	crude protein
d	days
DM	dry matter
FSH	follicle-stimulating hormone
g	grams
GH	growth hormone
GnRH	gonadotrophin releasing hormone
h	hours
hCG	human chorionic gonadotrophin
IGFBP's	IGF-bindings proteins
IGF-I	insulin-like growth factor-I
IGF-IR	insulin-like growth factor-I receptors
im	intramuscular
kg	kilogram
LH	luteinizing hormone
Mcal	mega calories
mg	milligram
MHz	megahertz

ml	milliliter
mm	millimeter
min	minute
mo	months
NDF	neutral detergent fiber
NEg	net energy of gain
NEm	net energy of maintenance
ng	nanogram
Ob-R	leptin receptor
P450scc	cytochrome P450 side chain cleavage
±	plus/minus
SC	scrotal circumference
SEM	standard error of the mean
StAR	steroidogenic acute regulatory protein
TDN	total digestible nutrients
wk	week
µg	microgram

1. INTRODUCTION

Age at puberty is a major determinant of beef production efficiency. The ability to breed animals at younger ages reduces generation intervals and increases genetic gains. The use of yearling *Bos taurus* beef bulls as primary breeders is common throughout North America. However, reduced sperm production and poor semen quality due to immaturity are common causes of poor reproductive performance of young bulls and the inability to use yearling bulls represents a serious loss of superior genetic stock. The ability to collect and freeze semen from younger bulls is also desired to reduce the time required for progeny testing and to accelerate the process of artificial insemination and sire selection. Therefore, an understanding of pubertal changes and the factors that affect sexual development is required in order to promote the successful use of young bulls for reproductive purposes.

Puberty in bulls has been characterized by the production of an ejaculate containing more than 50 million sperm with at least 10% sperm motility (Wolf et al., 1965). *Bos taurus* bulls reach puberty at 8.5-12 mo of age (Almquist and Barber, 1974; Lunstra et al., 1978; Pruitt et al., 1986; Wolf et al., 1965), but sperm production and semen quality at puberty are not adequate to produce results that are considered satisfactory in the industry, whether breeding cows at pasture or producing semen for artificial

insemination. The efficiency of spermatogenesis and consequently sperm production and semen quality, must improve considerably after puberty before bulls can be used for breeding purposes, a process that requires a few months (Lunstra and Echternkamp, 1982). Therefore, the proportion of bulls with satisfactory semen quality (maturity) that are capable of passing a breeding soundness evaluation increases up to 12-16 months of age (Arteaga et al., 2001; Higdon et al., 2000; Kennedy et al., 2002). It is clear that bulls that attain puberty earlier will have a better chance to be selected as breeding bulls than bulls with delayed puberty. Better knowledge of the factors that affect sexual development would enable the cattle industry to optimize the management of bulls to reduce age at puberty, improve the ability to select early-maturing bulls, and perhaps develop treatments to hasten the onset of puberty.

For this thesis, a series of experiments were conducted to evaluate the effects of nutrition during calfhood (defined as the period from 10 to 26-30 wk of age) and during the peripubertal period (defined as the period from 27-31 to 70-74 wk of age) on sexual development and reproductive function in beef bulls. The overall objective of these experiments was to evaluate the effects of nutrition on endogenous metabolic hormones (leptin, insulin, growth hormone, and IGF-I), gonadotropins and testosterone concentrations, sexual development, sperm production, and semen quality in bulls. Specific objectives included the evaluation of the effects of different nutritional levels only during the peripubertal period (equivalent to the post-weaning period) versus during the entire calfhood-peripubertal period, the evaluation of the effects of nutrition supplementation during the peripubertal period after feed restriction during calfhood,

and the evaluation of nutrition supplementation only during the calfhood period. The hypothesis evaluated was that nutrition affects gonadotropins secretion and sexual development in bulls.

1.1. Sexual development in bulls

1.1.1. Postnatal testicular development and initiation of spermatogenesis

In bulls, the intertubular cell population is composed of mesenchymal-like cells, fibroblasts, Leydig cells, peritubular cells and mononuclear cells. From 4 to 8 wk of age, mesenchymal-like cells were the majority of the cells in the testicular interstitial tissue. These pluripotent cells proliferated by frequent mitoses and are the precursors of Leydig cells, contractile peritubular cells and fibroblasts. Approximately 20-30% of all intertubular cells at all ages are mononuclear cells, including lymphocytes, plasma cells, monocytes, macrophages, and light intercalated cells (monocyte-derived, Leydig cell-associated typical cells of the bovine testis). The differentiation of the two main components of the tubular lamina propria, i.e. the basal lamina and the peritubular cell sheath, does not occur concomitantly. The thickness of the basal lamina was approximately 3 μm at 16 wk of age, but decreased continuously to 1.2 μm at 20 wk of age. Mesenchymal-like cells transformed into peritubular cells with elongated nuclei around 16 wk of age; these transformed into contractile myofibroblasts around 24 wk of age (Sinowatz and Amselgruber, 1986; Wrobel et al., 1988).

Leydig cells were present in the testes from birth to adulthood. At 4 wk of age, most (70%) intertubular cells were mesenchymal-like cells, with a high mitotic rate. Typical Leydig cells constituted about 6% of all intertubular cells and a certain number of these cells were found in an advanced degenerative state (probably as remnants of the fetal Leydig cell population). Degenerating fetal and newly formed postnatal Leydig cells coexisted until 8 wk of age; thereafter, only Leydig cells formed postnatally were observed. At 8 wk of age, Leydig cells accounted for approximately 20% of all intertubular cells and comprised 10% of total testicular volume. At 16 wk of age, the mesenchymal-like cells cease proliferation and transformed into contractile peritubular cells or Leydig cells, thus decreasing the proportion of mesenchymal-like cells to approximately 20%. At 20 wk of age, undifferentiated mesenchymal-like cells were rare and the Leydig cell population increased by mitotic proliferation. From 16 to 20 wk of age, a considerable number of degenerating Leydig cells were present. The period during which newly differentiating, intact, and degenerating Leydig cells were in close proximity lasted until 30 wk. At 30 wk of age, the population that survived the phase of cell degeneration represented the Leydig cells found in the adult bull; thereafter, mitosis was rare (Wrobel, 1990).

In bulls, Leydig cell mass increased from 4 to 30-40 wk of age (0.15 to 5.8 g per testis, respectively). From 30-40 wk of age to young adulthood (75-100 wk), Leydig cell mass increased more slowly but continuously, starting with about 6 g (weight of testis, about 100 g) to reach about 10 g in adult testis (weight, about 300 g). Nuclear diameter, nuclear and Leydig cell volumes increased from 4 to 8-16 wk of age, but remained

basically unchanged up to 52 wk of age. However, from 52 wk to young adulthood, there was a considerable increase in nuclear diameter and volume, and Leydig cell volume. Leydig cell numbers per testis increased from 4 to 30 wk (0.42 to 6 x 10^9, respectively) and remained unchanged thereafter. Therefore, the increase in Leydig cell mass after 30 wk of age was attributed to hypertrophy and not hyperplasia. Leydig cell mitochondrial mass increased from 4 to 16 wk and remained relatively constant up to 40 wk of age; however, from 40 wk to young adulthood, mitochondrial mass more than doubled (Wrobel, 1990). Functional maturation of Leydig cells involved the expression of steroidogenic enzymes and production of testosterone. Age-dependent expression of steroidogenic enzymes did not necessarily parallel changes in the numbers of Leydig cells in the testis (Waites et al., 1985).

Undifferentiated Sertoli cells (or indifferentiated supporting cells or pre-Sertoli cells) were present in the seminiferous tubules at birth and were the predominant intra-tubular cells from birth to 20 wk of age (Abdel-Raouf, 1960; Curtis and Amann, 1981). Basal (more numerous) and central undifferentiated Sertoli cells were also present, but the difference between these two types of cells is merely the position of the nuclei; they had no ultrastructural differences (Sinowatz and Amselgruber, 1986). Undifferentiated Sertoli cells had little mitotic activity until 4 wk of age, but cell proliferation increased thereafter. From 4 to 8 wk of age, undifferentiated Sertoli cell multiplication was maximal, but decreased from 8 to 16 wk; at the end of this period, undifferentiated Sertoli cells left the cell cycle and entered the G_0-phase for the rest of the animal's life. With the end of the proliferative phase, undifferentiated Sertoli cells began to transform

into adult-type Sertoli cells and their numbers increased rapidly between 20 and 40 wk of age; transformation into adult-type Sertoli cells occurred near the time that spermatogonia were formed (Abdel-Raouf, 1960; Curtis and Amann, 1981; Sinowatz and Amselgruber, 1986; Wrobel, 2000). In Holstein bulls, the number of Sertoli cells per testis increased tremendously from 20 to 24 wk age (202 to 3,520 x 10^6 cells, respectively) and subsequently increased until 32 wk of age (8,862 x 10^6 cells).

The differentiation process of Sertoli cells during sexual development includes distinct morphological changes in the cell shape, nucleus and cellular organelles, as well as an increase in surface specialization and subsequent interaction with other Sertoli cells and germ cells. Functional maturation of Sertoli cells included formation of the blood-testis barrier and acquisition of the ability to secrete several polypeptides, including androgen-binding protein (the secretion of which is under direct control of FSH), plasminogen activator factor, transferrin and ceruloplasmin (iron and copper transport proteins, respectively), and galactosyl transferase, an enzyme involved in the glycosylation of proteins. Sertoli cells also secrete lactate and pyruvate that serve as metabolic fuel to germ cells and protein secretion increase with establishment of spermatogenesis (Sinowatz and Amselgruber, 1986; Waites et al., 1985).

At 4 wk of age, undifferentiated Sertoli cells with round or oval nuclei constituted the largest population of cells observed in the seminiferous tubules (also called sex cords during this stage). Numerous flakes of heterochromatin were dispersed throughout the nucleoplasm and one or two larger aggregates of heterochromatin were often seen in

the center of the nucleus, sometimes associated with a small nucleolar-like structure. Lateral cell membranes of neighboring presumptive Sertoli cells contained few interdigitations and no special junctional complexes. The cytoplasm of presumptive Sertoli cells was characterized by a large amount of rough endoplasmic reticulum, abundant mitochondria, and a pronounced Golgi apparatus (in a supranuclear location). At 16 wk of age, above the spermatogonia, opposing cell membranes of adjacent undifferentiated Sertoli cells started to develop extended junctional complexes. The nuclear form was now more elongated and irregular and the amount of heterochromatin distinctly reduced. In many undifferentiated Sertoli cells, the development of the vacuolar nucleolus characteristic of the mature cell was in progress. At 20 wk, in the basal portion of the tubules, junctional complexes between neighboring Sertoli cells increased in extent. The nuclei of supporting cells were more elongated compared to previous stages and usually had a comparatively irregular outline. Most undifferentiated Sertoli cells were developing a typical Sertoli cell nucleolus and the clumps of heterochromatin within the nucleus (present at younger ages) had disappeared. The mitochondria had increased considerably in length and were oriented along the axis of the extending presumptive Sertoli cells. At 24 wk of age, the prevalence of junctional specialization between adjacent Sertoli cells was much greater and divided the tubular epithelium into a basal compartment containing spermatogonia and an adluminal compartment containing germ cells at later stages of spermatogenesis. A functional blood-testis barrier seemed present at this age. After 28 wk of age, most Sertoli cells have completed their morphological differentiation and attained adult structure, i.e. an irregularly-shaped nucleus containing a vacuolated nucleolus situated in the basal part

of the cell near the basal lamina. Bulky processes with whorls of smooth endoplasmic reticulum extended laterally into the basal region of the epithelium. Junctional complexes between interdigitating lateral cell membranes of adjacent Sertoli cells were extremely well developed in the basal part of the tubular epithelium. They consisted of many serially arranged points or lines of fusion involving neighboring Sertoli cell membranes, associated filaments and a varying amount of smooth endoplasmic reticulum situated close to the contact zone (Sinowatz and Amselgruber, 1986).

Intense germ cell proliferation occurred from 50 to 80 d post-conception; thereafter, germ cells entered a prolonged G_1- or G_0- phase and no mitotic activity was detected until after birth (Wrobel, 2000). At birth, the germ cell population was composed of only gonocytes (or prespermatogonia or prepubertal spermatogonia). Gonocytes were usually centrally located and had a large nucleus (~12 μm in diameter) with well-developed nucleolus. From 4 to 16 wk of age (seminiferous tubule diameter 50-80 μm) germ cell proliferation slowly resumed. Gonocytes were gradually displaced to a position close to the basal lamina and divided by mitosis giving rise to A-spermatogonia. Differentiation and degeneration resulted in the complete disappearance of gonocytes from seminiferous tubules around 20 wk of age. From 16 to 30 wk of age (tubule diameter 80-120 μm) germ cell proliferation reached a maximum level, resulting in up to 20 germ cells per tubular cross section; this represented the expansion of the spermatogonial stem cell line. In Holstein bulls, the total number of spermatogonia per testis increased from 181 x 10^6 at 16 wk to 3,773 x 10^6 at 32 wk of age; the number of

spermatogonia continue to increase, up to approximately 60 wk of age (Abdel-Raouf, 1960; Curtis and Amann, 1981; Sinowatz and Amselgruber, 1986; Wrobel, 2000).

A-spermatogonia divide mitotically to form In- and B-spermatogonia, that in turn enter meiosis around 20-24 wk of age, when primary spermatocytes were first observed. Primary spermatocytes numbers increased slowly until 32 wk of age, when the numbers exceed the number of spermatogonia. Secondary spermatocytes and round spermatids first appeared at approximately 28 wk of age, whereas elongated spermatids appeared around 30-32 wk of age; the number of spermatids increased rapidly and after 40 wk of age, spermatid numbers exceed the numbers of any other germ cell. Primary spermatocytes numbers continued to increase until approximately 60 wk of age. Mature sperm appeared in the seminiferous tubules at approximately 32-40 wk of age (Abdel-Raouf, 1960; Curtis and Amann, 1981; Evans et al., 1996). Testes weighing more than 100 g in Swedish Red-and-White bulls or more than 80 g in Holsteins bulls were likely to be producing sperm (Abdel-Raouf, 1960; Curtis and Amann, 1981).

During germ cell development, there was a gradual increase in the number of germ cells supported by each Sertoli cell (germ cells: Sertoli cell ratio) and an increase in the efficiency of the spermatogenesis, i.e. an increase in the number of more advanced germ cells resulting from the division of precursor cells (Aponte et al., 2005). The interval between first appearance of primary spermatocytes and first appearance of sperm in prepubertal bulls was approximately 90 d, longer than in mature bulls (41 d). This longer interval must result from degeneration of at least 3 to 5 cellular generations

developing from spermatogonia (13.5 d/generation). The yields of different germ cell divisions, low during the onset of spermatogenesis, increased progressively to the adult level. In Holstein bulls, the efficiency of sperm production increased from 4 x 10^6 sperm/g of testicular parenchyma at 32 wk to approximately 12 x 10^6 sperm/g in the mature bull (Curtis and Amann, 1981). Appearance of sperm in the ejaculate resulted from increasing sperm production efficiency after initiation of spermatogenesis.

Testicular cellular development was accompanied by a progressive increase in the proportion of the testicular parenchyma occupied by seminiferous tubules until approximately 32 wk of age. Seminiferous tubules diameter increased five-fold from birth until adulthood; diameter increased gradually from 8 to 20 wk and more rapidly from 24 to 40 wk of age. Around 24 wk of age, 'cracking' of the tubular cytoplasm was first detected, indicating formation of the tubular lumen. Formation of the tubular lumen is evidence of formed blood-testis barrier and preceded the appearance of primary spermatocytes and more advanced germ cells; lumen diameter increased from approximately 30 to 36 wk of age (Abdel-Raouf, 1960; Curtis and Amann, 1981; Evans et al., 1999). Seminiferous tubule length increased from 830 m/testis at 12 wk to 2,010 m at 32 wk of age in Holstein bulls. Therefore, increases in the proportion of parenchyma that is occupied by seminiferous tubules, as well as increases in the tubular diameter and the total length of seminiferous tubules per testis accounts for initial testicular growth up to approximately 8 mo of age; thereafter, testicular growth was primarily the result of increasing total seminiferous tubule length (Curtis and Amann, 1981). Testicular growth was rapid between 6 and 16 mo of age and SC increased

approximately 1.8 cm/mo. The rate of testicular growth diminished after 16 mo and the testes were approximately 90% of the maximum adult size by 24 mo of age (Barth and Ominski, 2000; Coulter, 1986).

1.1.2. Hormonal control of sexual development in bulls

The process of sexual development in bulls involves a complex maturation mechanism of the hypothalamus-pituitary-testes axis. Sexual development can be divided into three periods according to changes in gonadotropins and testosterone concentrations, namely the infantile, prepubertal, and pubertal periods. The infantile period is characterized by low gonadotropins and testosterone secretion and extends from birth to approximately 8-10 wk of age. A transient increase in gonadotropins secretion occurs from approximately 8-10 to 20-24 wk of age; this has been called the 'early gonadotropin rise' and characterizes the prepubertal period. Testosterone concentrations began to rise during the prepubertal period. The pubertal period corresponds to the period of accelerated reproductive development after 20-24 wk of age until puberty. During this period, gonadotropin secretions decreased, whereas testosterone secretion continued to increase (Amann, 1983; Amann and Walker, 1983; Amann et al., 1986; Evans et al., 1996; Lacroix and Pelletier, 1979; McCarthy et al., 1979a; McCarthy et al., 1979b). Although the timing of sexual development is determined primarily by the hypothalamus and GnRH secretion, the mechanisms regulating GnRH secretion during sexual development in bulls are poorly understood.

Gonadotropin concentrations during the infantile period were low, due to reduced GnRH secretion, since it has been demonstrated that the pituitary was responsive to GnRH in the infantile period; although the response increased with age (Aravindakshan et al., 2000; Chandolia et al., 1997b; Miller and Amann, 1986; Mongkonpunya et al., 1975; Rodriguez and Wise, 1991). Maturation changes within the hypothalamus increased GnRH pulse secretion, driving the transition from the infantile to the prepubertal period of development by enhancing gonadotropins (especially LH) secretion. Increased GnRH secretion was dependent on either the development of central stimulatory inputs or removal of inhibitory inputs. Hypothalamus weight and GnRH content did not increase during the infantile period, but hypothalamic concentrations of estradiol receptors decreased after 6 wk of age, leading to suggestions that reduced sensitivity to sex steroids could be involved in the augmented GnRH secretion (Amann et al., 1986).

The hypothesis that gonadotropin secretion is low during infancy due to elevated sensitivity of the hypothalamus to the negative feedback of sex steroids (gonadostat hypothesis) has been questioned in bulls, since castration did not alter LH pulse frequency or mean concentrations before 10 wk of age (Wise et al., 1987). Nevertheless, since GnRH secretion into hypophysial portal blood is not necessarily accompanied by LH secretion during the infantile period in bulls (Rodriguez and Wise, 1989), experiments that use LH concentrations to infer GnRH secretion patterns during the infantile period in bull calves should be interpreted with caution. Another possibility is that removal of opioidodergic inhibition may be involved in triggering the increase in

GnRH secretion during the infantile period. Opioidergic inhibition of LH pulse frequency during the infantile period was demonstrated by increased LH pulse frequency after naxolone treatment in 4-wk-old bull calves. Moreover, naxolone treatment increased LH pulse amplitude and mean concentrations at 12 and 18 wk of age (Evans et al., 1993).

When samples from the hypophysial portal system were evaluated in bull calves, GnRH pulsatile secretion increased linearly from 2 wk (3.5 pulses/10 h) to 12 wk of age (8.9 pulses/10 h). Although GnRH secretion into hypophysial portal blood was detected at 2 wk, pulsatile LH secretion was not detected in the jugular blood samples before 8 wk of age. In addition, GnRH pulses were not necessarily accompanied by LH secretion until 8-12 wk of age, when all pulses of GnRH resulted in LH secretion. The increase of pulsatile GnRH release from 2 to 5 wk of age without a concomitant increase in LH secretion may represent a reduced ability of the pituitary gland to respond to GnRH stimulus (Rodriguez and Wise, 1989). The period in which GnRH pulses do not stimulate LH secretion corresponded to the period during which there is an increase in pituitary weight, GnRH receptor concentration, and LH content (Amann et al., 1986). Moreover, pulsatile GnRH infusion during the infantile period in bull calves increased pituitary LH-β mRNA, LH content, and GnRH receptors, with resulting increases in LH pulse frequency and mean concentrations (Rodriguez and Wise, 1991). Therefore, increased GnRH pulse frequency increased pituitary sensitivity to GnRH. Eventually, increased GnRH secretion resulted in the increased LH pulse frequency detected during the prepubertal period.

During the early gonadotropin rise characteristic of the prepubertal period, there was a characteristic increase in mean LH concentrations from 8 wk to 16-18 wk of age. Mean LH concentrations decreased thereafter and by 20-24 wk of age, reached levels compared to those observed during the infantile period. The main factor responsible for increased LH mean concentrations was a dramatic increase in LH pulse frequency. The number of LH pulses increased from less than 1 per day at 4 wk to approximately 12 per day (≥ 1 pulse/2 h) at 12-16 wk of age. Changes in pulse amplitude during this period were not consistent among reports; amplitude may be reduced, unchanged, or augmented. Mean FSH concentrations reportedly also increased during the early gonadotropin rise, although some experiments have failed to show any significant changes in FSH concentrations. Pulsatile discharges of FSH have been observed in bulls, but are much less evident than that of LH (Amann and Walker, 1983; Amann et al., 1986; Aravindakshan et al., 2000; Evans et al., 1995; Evans et al., 1996; Lacroix and Pelletier, 1979; McCarthy et al., 1979a; McCarthy et al., 1979b; Schams et al., 1981).

Luteinizing hormone binding sites in testicular interstitial tissue have been demonstrated in bulls at birth and at 16 wk of age (Schanbacher, 1979); pulsatile LH secretion was an essential requirement for inducing Leydig cell proliferation and differentiation and for maintenance of fully differentiated structure and function. Hypophysectomy, suppression of gonadotropins by steroid administration, or neutralization of GnRH/LH by specific antibodies caused Leydig cell atrophy and loss of cellular volume, reduction of the number of LH receptors and steroidogenic enzyme activity, and a decrease in the ability to secrete testosterone in response to LH. The

characteristic pulsatile nature of LH secretion was important for testosterone production, since continuous exposure of Leydig cells to LH resulted in reduced steroidogenic responsiveness due to downregulation of LH receptors (Saez, 1994).

Initiation of Leydig cell steroidogenesis was characterized by increased androstenedione secretion, which decreased as the cells completed maturation and begin secreting testosterone, at approximately 16 wk of age (Aravindakshan et al., 2000; Evans et al., 1995; Evans et al., 1996; Lacroix and Pelletier, 1979; Rawlings et al., 1978; Rawlings et al., 1972; Schams et al., 1981). During the first 16 wk of age, testosterone concentrations were low and secretion did not necessarily accompany LH pulses. After 16 wk of age, LH pulses were followed by testosterone pulses and mean testosterone concentrations began to increase. The number of testosterone pulses increased from approximately 0.3 to 2.3 pulses/24 h at 4 to 16 wk to 9 to 7.5 pulse/24 h at 20 wk of age; testosterone pulse frequency does not increase thereafter and remain at approximately 4.5 to 6.8 pulses/24 h from 24 to 40 wk of age (McCarthy et al., 1979a; McCarthy et al., 1979b).

Follicle-stimulating hormone binding sites in seminiferous tubules were detected in bulls at birth and 16 wk of age (Schanbacher, 1979) and increased FSH concentrations stimulated the proliferation of undifferentiated Sertoli cells and gonocytes. Although there is considerable evidence that FSH is essential for normal Sertoli cell function, the period of Sertoli cell differentiation coincided with the initiation of testosterone secretion by the Leydig cells, indicating that testosterone may be involved in promoting

maturation of undifferentiated Sertoli cells. Maturation of Sertoli cells and increased testosterone secretion are probably also involved in the differentiation of gonocytes into spermatogonias. The end of the prepubertal period was marked by completion of Sertoli cell differentiation, with the establishment of the blood-testis barrier and formation of tubular lumen, and initiation of germ cell meiosis (Amann, 1983).

The crucial role of the early gonadotropin rise (especially LH secretion pattern) in regulating sexual development in bulls has been recently demonstrated. Differences in LH pulse frequency and mean LH concentrations during the prepubertal period were associated with age at puberty in early- and late-maturing Hereford bulls (age at puberty 41-42 and 48 wk, respectively). Luteinizing hormone pulse frequency was greater around 10 to 20 wk of age, and mean LH concentrations increased approximately 10 wk earlier and reached greater maximum levels in early- than in late-maturing bulls (Aravindakshan et al., 2000; Evans et al., 1995). Prolonged treatment with a GnRH agonist in Hereford bulls from 6 to 14 wk of age decreased LH and FSH pulse frequency, pulse amplitude, and mean concentrations at 12 wk, delayed the peak mean LH concentration from 20 to 24 wk, and reduced FSH and testosterone concentrations from 14 to 18 wk of age. These hormonal alterations were associated with delayed puberty (47 and 42 wk of age in treated and control bulls, respectively), and reduced paired testes weight and number of germ cells in tubular cross-sections at 50 wk of age (Chandolia et al., 1997a). On the other hand, treatment with GnRH every 2 h from 4 to 6 wk of age in Hereford bulls increased LH pulse frequency and mean concentration, mean testosterone concentration, scrotal circumference, paired testes weight,

seminiferous tubules diameter, and number of germ and Sertoli cells in tubular cross-sections at 54 wk of age (Chandolia et al., 1997b).

Increasing testosterone secretion and possibly increased hypothalamic sensitivity to negative feedback from androgens were probably responsible for the decrease in LH pulse frequency and mean concentrations during the pubertal period (Rawlings and Evans, 1995). Inhibin produced by Sertoli cells may act on the gonadotrophs to limit FSH secretion, since immunization with inhibin anti-serum resulted in a marked increase in FSH concentrations in prepubertal bulls; LH and testosterone concentrations were not affected (Kaneko et al., 1993). During the pubertal period, testosterone pulse frequency remained unchanged, but pulse amplitude increased with age, with consequent increase in testosterone mean concentrations until approximately 1 yr of age (McCarthy et al., 1979a; McCarthy et al., 1979b; Rawlings et al., 1978; Rawlings et al., 1972; Schams et al., 1981). Elevated testosterone secretion is essential for increasing the efficiency of spermatogenesis that leads to the appearance of sperm in the ejaculate (puberty).

1.1.3. Effects of nutrition on sexual development and function in bulls

There is very little information regarding the effects of nutrition from birth to maturity on sexual development, sperm production, and semen quality. The sole study (Bratton et al., 1959) that evaluated different nutrition from 1 to 80 wk of age in

Holstein bulls indicated that bulls receiving low nutrition (approximately 60% of TDN requirements) had smaller testes and delayed puberty, whereas bulls receiving high nutrition (approximately 160% of TDN requirements) had larger testes and earlier puberty when compared to bulls receiving control nutrition (100% of TDN requirements). Nearly all studies reported in the literature evaluated the effects of nutrition after weaning, i.e. after the initial hormonal changes that regulate sexual development have occurred. In general, these studies demonstrated that low nutrition had adverse effects on growth and sexual development.

In one study, bulls received control and low nutrition (1/3 of the amount supplied to the control group). Body and vesicular gland weights, vesicular gland fructose and citric acid content, and circulating and testicular testosterone concentrations were lower in the low nutrition group, whereas circulating androstenedione concentrations were increased. Not only there was less testicular testosterone in the low nutrition group, but the physiological decline in androstenedione: testosterone ratio was also arrested; puberty was probably delayed in the low-nutrition group (Mann et al., 1967).

Restricted protein in the diet has deleterious effects on sexual function. In a series of experiments, beef bulls were fed various amounts of crude protein (CP) starting at 8, 10 or 12 mo. Bulls in the control group received diets containing approximately 14% CP, whereas those in the treatment groups received decreasing levels of protein (8, 5, and approximately 1.5% CP) for periods of 84 to 170 d. Testes, epididymis, and seminal glands weights were markedly reduced in bulls fed protein-deficient rations. Moreover,

seminiferous tubule diameter and seminiferous epithelium thickness were smaller in bulls with a restricted protein intake. Semen characteristics were not affected until CP was reduced to 1.35%; semen volume and total sperm in the ejaculate were decreased, but sperm morphology and motility were not affected. Increasing dietary protein after protein restriction increased semen volume, but sperm numbers in the ejaculate did not increase. It is worth noting that protein restriction was so severe in these studies that some bulls died or were slaughtered before eminent death; these bulls had lost approximately 40% of their initial body weight (Meacham et al., 1963; Meacham et al., 1964). Similarly, body weight, body condition score, SC, semen volume, sperm concentration, total sperm in the ejaculate, and sperm motility were greater after 12-14 mo of age in bulls receiving high protein in the diet (14.5%) than in bulls receiving low protein (8.5%) from 6 to 21 mo of age (Rekwot et al., 1988).

Differences in yearling SC due to age of the dam could also be interpreted as an indication that nutrition during calfhood affects sexual development in bulls, although possible in-utero effects could not be disregarded. Scrotal circumference increased with increasing age of the dam, up to 5 to 9 yr of age. The effects on SC are especially marked for bulls raised by 2-yr-old dams; they had a 0.7 to 1.3 cm smaller SC than bulls raised by 5- to 9-yr-old dams. On the other hand, SC decreased with increasing age of the dam after 10 yr of age (Bourdon and Brinks, 1986; Crews Jr. and Porteous, 2003; Evans et al., 1999; Kriese et al., 1991; Lunstra et al., 1988). In models that included weight as a covariate, effects of age-of-dam on SC decreased. Therefore, the significance of age-of-dam effects on testicular size seemed to be primarily the result of

age-of-dam effects on bull's body weight (Bourdon and Brinks, 1986; Crews Jr. and Porteous, 2003; Lunstra et al., 1988), likely related to differences in milk production.

The adverse effects of low nutrition on sexual development and function can also be inferred from studies where bulls received feed supplementation only during the dry-season. This is a common practice in tropical countries where bulls are raised on pasture during their entire lives and supplemented to minimize weight loss when pasture quality is low. Protected protein supplementation for 60 days in crossbred Brahman bulls (3.5 yr old) resulted in maintenance of SC, whereas SC decreased in non-supplemented bulls after 30 d. Paired testes, epididymides, and vesicular glands weights were greater in supplemented than in non-supplemented bulls at the end of the experiment. Changes in SC paralleled changes in body weight in non-supplemented bulls; each 10 kg loss of body weight was associated with a decline of 21.3 g of testes weight, which represented a decrease in the production of 268 x 10^6 sperm/day (Ndama et al., 1983). Similarly, Bos indicus and Bos indicus x Bos taurus crossbred bulls weaned at 6 mo and supplemented or during two dry seasons (6 mo each) until 21 mo of age had greater body weight and greater testes and epididymal weights than bulls that were not supplemented. Supplemented bulls also had greater seminiferous tubule diameter, total daily sperm production, and epididymal sperm reserves (Tegegne et al., 1992).

The potential beneficial effects of high nutrition after weaning on sexual development and function are not very clear. Effects of energy on sexual development were not consistent in a study with Simmental and Hereford bulls fed diets with a low,

medium, or high energy content (approximately 14, 18, and 23 Mcal/bull/d, respectively) from 212 to 422 d of age and pastured for 38 d. Dietary energy affected sexual development in Simmental bulls, but not in Hereford bulls. Simmental bulls in the high-energy group were heavier and had greater SC and testosterone concentrations than bulls in the low-energy group (in general, the medium energy group was intermediate). However, increased dietary energy did not hasten age at puberty. The only semen trait affected by dietary energy was semen volume, which was depressed in Simmental bulls in the medium-energy group. Serving capacity was greater for Hereford bulls in the high-energy diet, but medium- and high-energy diets were associated with a decrease in the number of services between two testing periods in Simmental bulls. There was a trend for lower sperm motility and proportion of normal sperm in Simmental bulls fed the low-energy diet (Pruitt and Corah, 1985; Pruitt et al., 1986).

Supplementation of Brahman bulls, 6-9 mo of age, with lasalocid (200 g/head/d) for 130 d resulted in greater body weight, SC, and testes volume, and hastened puberty (Neuendorff et al., 1985). However, in Brahman bulls (8-9 mo of age) fed to gain 0.1-0.25 kg/d or 0.75-1.0 kg/d until first appearance of sperm in the ejaculate (defined as puberty), diet did not affect the weight of the median eminence and pituitary, median eminence GnRH content or release, pituitary LH content, or age at puberty, but circulating testosterone (mean and basal concentrations, and pulse frequency and amplitude), testicular testosterone, and Leydig cell size were greater in bulls in the high-

gain diet group. Bulls in the high-gain diet group seemed heavier at puberty and had greater paired testes, epididymides, and vesicular glands weights (Nolan et al., 1990).

Under field conditions, high-energy post-weaning diets are frequently associated with impaired reproductive function in bulls due to altered testicular thermoregulation. "Not infrequently, show ring champions have proven to be temporarily or permanently infertile. It is possible that the lowered fertility of these bulls results from excessive fat deposition above and around the testes in the scrotum, preventing heat exchange mechanisms from functioning efficiently" (Skinner, 1981). Sperm motility decreased and the proportion of sperm defects increased with age in Hereford bulls fed to gain > 1.75 kg/d; significantly different from bulls fed to gain approximately 1 kg/d (control) after 76 wk of age. Even after the high nutrition diet was changed to a control diet, bulls previously receiving high nutrition continued to have lower semen quality. There was greater deposition of fat around the testicular vascular cone in the scrotal neck in bulls in the high nutrition group; the difference between body and testes temperature was reduced in this group compared to bulls in the control group. This difference was still present after the diets were changed (Skinner, 1981).

In a series of experiments, Angus, Hereford, and Simmental bulls were fed high nutrition (80% grain and 20% forage) or medium nutrition (forage only) from weaning (6-7 mo) to 12-24 mo of age. In general, bulls receiving high nutrition had greater body weight and backfat, but paired testes weight was not affected by diet. Moreover, bulls receiving high nutrition had lower daily sperm production and epididymal sperm

reserves, and greater proportion of sperm abnormalities. The authors indicated that increased dietary energy may adversely affect sperm production and semen quality due to fat deposition in the scrotum, that reduced the amount of heat that can be radiated from the scrotal skin, thereby increasing the temperature of the testes and scrotum (Coulter and Bailey, 1988; Coulter et al., 1987; Coulter et al., 1997; Coulter and Kozub, 1984). Another interesting observation from a different study was that bulls fed high-nutrition diets had greater SC than bulls fed medium-nutrition diets, but paired testes weight was the same. In this study, scrotal weight was greater in bulls fed high nutrition, suggesting that fat deposition in the scrotum may have been responsible for the greater SC in these bulls (Seidel et al., 1980).

High-energy diets may also result in abnormal foot growth due to laminitis, as well as abnormal bone and cartilage growth, and may increase the risk of rumen inflammation, liver abscess, and vesicular adenitis (Dargatz et al., 1987; Greenough et al., 1990).

1.2. Metabolic hormones and male reproductive function

The mechanisms controlling reproduction and energy balance are intrinsically related and have evolved to confer reproductive advantages and guarantee the survival of species. The neural apparatus designed to gauge metabolic rate and energy balance has been denominated the body “metabolic sensor”. This sensor translates signals

provided by circulating (peripheral) concentrations of specific hormones into neuronal signals that ultimately regulate the GnRH pulse generator and control the reproductive process. Metabolic indicator hormones, e.g. leptin, insulin, growth hormone (GH), and insulin-like growth factor-I (IGF-I), may signal nutritional status to the hypothalamus-pituitary-gonads axis and affect sexual development (Blache et al., 2003; I'Anson et al., 1991; Martin and Walkden-Brown, 1995; Schneider, 2004; Williams, 1998). Full appreciation of pubertal progression will require understanding of how peripheral signals relating information about energy metabolism are sensed by the brain and how that information is routed through pathways controlling GnRH secretion.

Leptin is a recently discovered hormone that is produced by adipose tissue and regulates feed intake and energy balance. The crucial roles of leptin on reproduction have been demonstrated by observation of the *ob*/*ob* mouse, which lacks a functional leptin gene; these mice have impaired gonadotropin secretion and are infertile, but treatment with exogenous leptin restored fertility (Barb and Kraeling, 2004; Zieba et al., 2005). In female cattle, acute fasting decreased circulating leptin and LH concentrations, but LH secretion was restored by leptin treatments (Amstalden et al., 2002; Maciel et al., 2004b). In vitro studies in cattle demonstrated that leptin may increase hypothalamic GnRH secretion and pituitary LH secretion, although these effects were dependent on nutritional status (Amstalden et al., 2003).

Insulin receptors were present in the hypothalamus in mice; those with disrupted insulin receptors had reduced circulating LH concentrations (60% reduction in males and 90% reduction in females) and impaired spermatogenesis and ovarian follicle

maturation (Bruning et al., 2000). Insulin receptors were also observed in the hypothalamus in rams; increased GnRH/LH secretion promoted by improved nutrition was accompanied by increased circulating and cerebrospinal fluid insulin concentrations (Blache et al., 2000). In a diabetic sheep model, pulsatile LH secretion was decreased by insulin withdrawal and restored by insulin infusion into the lateral ventricle (Tanaka et al., 2000). Moreover, infusion of insulin into the third ventricle of normal rams also increased LH secretion and the response was not enhanced by glucose, suggesting a direct role of insulin on GnRH secretion (Martin and Walkden-Brown, 1995).

Growth hormone and IGF-I concentrations increased during sexual development in humans and have been suggested to be involved in regulating GnRH secretion (Wilson, 2001). Receptors for IGF-I (IGF-IR) have been identified in immortalized GnRH-secreting cells (GT-17 cells) and in the hypothalamus in rodents (Bohannon et al., 1988; Olson et al., 1995). Expression of IGF-IR in the hypothalamus increased with age in both male and female mice and expression of IGF-IR in the preoptic, septal, and anterior hypothalamic regions was abundant. Dual-label immunohistochemistry indicated that GnRH-secreting neurons expressed IGF-IR, indicating that IGF-I can have a direct effect on GnRH perikarya or neuroterminals (Miller and Gore, 2001). Furthermore, GnRH neurons expressed mRNA for IGF-I, IGF-II, IGF-IR, and IGFBP's in an age-dependent manner, indicating the existence of an autocrine regulatory mechanism of the IGF system in neuronal GnRH secretion (Anderson et al., 1999). Moreover, treatment with IGF-I increased GnRH secretion by GT1-7 cells in vitro

(Anderson et al., 1999) and increased LH secretion in castrated rams in vivo (Adam et al., 1998).

Metabolic hormones may also have direct effects on the testes and be involved in regulating cell proliferation and maturation, steroidogenesis, and secretion of local regulatory factors. The great difference in expression of testicular leptin receptors (Ob-R) between two rodent species indicated that leptin effects in the testes were strictly species-specific. In mouse testis, leptin receptors (Ob-R) were observed mainly in the seminiferous tubules and localized to germ cells; Ob-R expression varied among germ cell types and was affected by age (El-Hefnawy et al., 2000). In rat testis, Ob-R were observed in Leydig and Sertoli cells and there was an overall increase in the expression of testicular Ob-R during the peripubertal period; the expression of each Ob-R isoform was independently regulated (Caprio et al., 2003; Tena-Sempere et al., 2001b). Leptin decreases in vitro testosterone secretion in adult rats testis, an effect related to decreased expression of steroidogenic enzymes including StAR and P450 scc, but does not affect testosterone secretion by the prepubertal rat testes, demonstrating that the effects of leptin on steroidogenesis are age-dependent (Tena-Sempere et al., 2001a; Tena-Sempere et al., 1999).

Growth hormone receptors have been identified in Leydig, Sertoli, and germ cells in rats (immunoreactivity was greater in Leydig and Sertoli cells), demonstrating the possibility of GH-regulated testicular effects (Lobie et al., 1990). Treatment with GH or IGF-I in dwarf mice (GH deficient) produced similar effects on testicular development, suggesting that the effects of GH were mediated through IGF-I (Chatelain et al., 1991).

However, GH was also able to directly induce the expression of several steroidogenic enzymes. In Leydig cells, GH stimulated the production of StAR and 3α-hydroxysteroid dehydrogenase (Hull and Harvey, 2000). Therefore, GH is likely to modulate testicular steroidogenesis by IGF-I-independent and possibly by IGF-I-dependent mechanisms.

Insulin and IGF-I receptors have been identified in Leydig cells in several species (Abele et al., 1986; Lin, 1995; Rouiller-Fabre et al., 1998) and IGF-I receptors have also been identified in rat Sertoli cells (Borland et al., 1984). Insulin-like growth factor-I increased the proliferation of Leydig cell precursors from immature animals and increased the differentiation of mesenchymal precursors into Leydig cells when combined with LH. Expression of Leydig cell-specific steroidogenic enzymes was decreased in IGF-I-null mice and circulating testosterone concentration was only 30% of that observed in adult controls (Wang et al., 2003). Leydig cell numbers increased by 200% with IGF-I replacement in IGF-I-null mice and additive effects on testicular cell numbers were observed in response to IGF-I plus LH treatments, indicating that the two hormones used separate signaling pathways (Wang and Hardy, 2004). Adding IGF-I to Leydig cells in vitro increased hCG (LH) binding without any changes in receptor affinity; therefore, IGF-I may regulate Leydig cell steroidogenesis by regulating the number of LH receptors. Insulin and IGF-I increased in vitro basal and stimulated testosterone secretion in a dose-dependent manner (Bernier et al., 1986; Lin, 1995; Spiteri-Grech and Nieschlag, 1992). In rat Sertoli cells, IGF-I stimulated DNA synthesis, up-regulated metabolism, and increased the secretion of plasminogen activator (Borland et al., 1984; Oonk et al., 1989).

2. MATERIALS AND METHODS

2.1. Bulls and treatments

Angus and Angus X Charolais bull calves (from Angus and Charolais heifers) were weaned at 8 wk of age (± 12 d; range) and were used in four experiments. The experiments were designed to investigate the effects of nutrition during different stages of growth on sexual development. The effects of nutrition during the peripubertal period (27-31 to 70 wk of age) were investigated in Experiment I, whereas the effects of nutrition during calfhood (10 to 26-30 wk of age) and the peripubertal period were investigated in Experiment II. The effects of feed restriction or supplementation during calfhood were investigated in Experiments III and IV, respectively.

In Experiment I, 40 bull calves were weaned and received the same diet until 26 wk of age. After 26 wk, the bulls were block-randomized according to genotype and age into three groups to receive low (n = 14), medium (n = 13), or high (n = 13) nutrition until 70 wk of age. Bulls were fed *ad libitum* and diets composition and analysis are shown in Tables 2.1 and 2.2.

In Experiment II, 37 bull calves were weaned and block-randomized according to genotype and age into three groups to receive low (n = 13), medium (n = 12), or high (n = 12) nutrition from 10 to 70 wk of age. Bulls were fed *ad libitum* and diets composition and analysis are shown in Table 2.3 and 2.4.

In Experiment III, 44 bull calves were weaned and block-randomized according to genotype and age into two groups to receive low (n = 29) or medium (n = 15) nutrition from 10 to 26 wk of age. Bulls in the medium nutrition group were fed *ad libitum* and bulls in the low nutrition group were fed 75% of the amount consumed by the bulls in the medium nutrition group. From 27 to 70 wk of age, bulls in the medium nutrition group continued to receive the same diet (medium/medium), while the diet was changed to the same as that fed to the medium nutrition group (low/medium; n = 15) or to high nutrition (low/high; n = 14) for bulls previously receiving low nutrition. Bulls were fed *ad libitum* after 27 wk of age and composition and analysis are shown in Table 2.5 and 2.6.

In Experiment IV, 33 bull calves were weaned and block-randomized according to genotype and age into two groups to receive medium (n = 16) or high (n = 17) nutrition from 10 to 30 wk of age. From 31 to 74 wk of age, all bulls received the same medium nutrition. Bulls were fed *ad libitum* and diets composition and analysis are shown in Table 2.7 and 2.8.

Table 2.1.
Diets in Experiment I.

Diet components	26 to 70 wk		
(% as fed)	Low	Medium	High
Barley silage	99	85	62
Rolled barley	-	8.5	22
Canola meal	-	5.5	15
Molasses	0.5	0.5	0.5
Mineral-vitamin mix	0.5	0.5	0.5

Table 2.2.
Analysis of the diets in Experiment I.

Diet analysis	26 to 70 wk		
	Low	Medium	High
DM (%)	36.8	40.8	55.6
CP (% DM)	13.1	15.0	22.7
ADF (% DM)	23.8	22.6	16.4
NDF (% DM)	49.5	49.7	50.2
Ash (% DM)	7.1	7.2	5.9
TDN (% DM)	69.0	70.3	76.6
NEm (Mcal/kg DM)	1.60	1.64	1.84
NEg (Mcal/kg DM)	1.00	1.03	1.20

DM: dry matter; CP: crude protein; ADF: acid detergent fiber; NDF: neutral detergent fiber; TDN: total digestible nutrients; NEm: net energy of maintenance; NEg: net energy of gain.

Table 2.3.
Average daily dry matter intake (kg) by bulls receiving low, medium, and high nutrition in Experiment I.

Age	Low	Medium	High
26-30 wk	4.9	6.7	7.7
30-34 wk	5.5	6.7	7.1
34-38 wk	5.6	7.1	7.5
38-42 wk	6.2	7.4	7.3
42-46 wk	7.1	9.3	8.8
46-50 wk	8.2	9.5	10.7
50-54 wk	9.0	8.2	11.7
54-58 wk	10.0	9.2	11.9
58-62 wk	9.9	10.6	10.7
62-66 wk	9.5	12.0	10.2
66-70 wk	10.1	12.7	11.2

Table 2.4.
Average crude protein (CP), net energy of maintenance (NEm), and net energy of gain (NEg) intake by bulls receiving low, medium, and high nutrition in Experiment I.

Age	CP (kg/d)			NEm (Mcal/d)			NEg (Mcal/d)		
	Low	Medium	High	Low	Medium	High	Low	Medium	High
26-30 wk	0.64	1.00	1.76	7.77	10.93	14.21	4.84	6.88	9.32
30-34 wk	0.72	1.01	1.62	8.78	11.02	13.07	5.47	6.93	8.58
34-38 wk	0.73	1.06	1.70	8.94	11.60	13.74	5.57	7.30	9.02
38-42 wk	0.81	1.11	1.65	9.83	12.11	13.37	6.12	7.62	8.77
42-46 wk	0.92	1.40	1.99	11.28	15.30	16.06	7.03	9.62	10.54
46-50 wk	1.07	1.43	2.43	13.03	15.56	19.66	8.12	9.79	12.90
50-54 wk	1.17	1.23	2.66	14.31	13.37	21.47	8.92	8.42	14.09
54-58 wk	1.30	1.38	2.71	15.90	15.09	21.87	9.91	9.50	14.35
58-62 wk	1.30	1.60	2.44	15.81	17.41	19.72	9.85	10.95	12.94
62-66 wk	1.24	1.80	2.31	15.18	19.60	18.69	9.46	12.33	12.26
66-70 wk	1.33	1.91	2.54	16.21	20.82	20.56	10.10	13.10	13.49

Table 2.5.
Diets in Experiment II.

Diet components	10 to 30 wk			31 to 45 wk			46 to 54 wk			55 to 70 wk		
(% as fed)	Low	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High
Barley silage	99	85	62	99	90	62	99	94.5	62	98	93.5	61
Rolled barley	-	8.5	22	-	6	22	-	3	22	-	3	22
Canola meal	-	5.5	15	-	3	15	-	1.5	15	-	1.5	15
Molasses	0.5	0.5	0.5	-	-	-	-	-	-	1	1	1
Mineral-vitamin mix	0.5	0.5	0.5	1	1	1	1	1	1	1	1	1

Table 2.6.
Analysis of the diets in Experiment II.

Diet analysis	10 to 30 wk			31 to 45 wk			46 to 70 wk		
	Low	Medium	High	Low	Medium	High	Low	Medium	High
DM (%)	43.1	49.2	60.0	41.2	45.0	57.1	41.2	43.7	58.4
CP (% DM)	12.1	16.3	20.2	12.5	14.7	21.0	12.3	13.1	19.9
ADF (% DM)	27.5	24.4	16.9	26.4	23.3	18.3	24.0	22.6	16.0
NDF (% DM)	55.9	61.8	56.5	62.2	60.7	53.4	53.7	49.8	49.1
Ash (% DM)	7.0	7.0	5.6	7.2	6.6	6.5	7.7	7.3	5.8
TDN (% DM)	65.2	68.4	76.1	66.3	69.5	74.7	68.8	70.3	77.1
NEm (Mcal/kg DM)	1.48	1.58	1.82	1.51	1.61	1.77	1.59	1.64	1.85
NEg (Mcal/kg DM)	0.89	0.98	1.19	0.92	1.01	1.15	0.99	1.03	1.21

DM: dry matter; CP: crude protein; ADF: acid detergent fiber; NDF: neutral detergent fiber; TDN: total digestible nutrients; NEm: net energy of maintenance; NEg: net energy of gain.

Table 2.7.
Average daily dry matter intake (kg) by bulls receiving low, medium, and high nutrition in Experiment II.

Age	Low	Medium	High
10-14 wk	2.7	3.1	2.9
14-18 wk	3.7	4.5	4.5
18-22 wk	4.2	5.5	6.4
22-26 wk	4.8	6.2	7.5
26-30 wk	5.1	6.9	8.1
30-34 wk	5.8	7.4	8.0
34-38 wk	6.1	7.5	8.2
38-42 wk	6.6	8.0	8.1
42-46 wk	7.3	9.6	9.1
46-50 wk	8.4	10.2	11.3
50-54 wk	8.7	9.3	11.7
54-58 wk	10.4	10.2	12.6
58-62 wk	10.3	11.1	11.4
62-66 wk	10.5	13.0	11.5
66-70 wk	9.3	11.2	11.0

Table 2.8.
Average crude protein (CP), net energy of maintenance (NEm), and net energy of gain (NEg) intake by bulls receiving low, medium, and high nutrition in Experiment II.

Age	CP (kg/d)			NEm (Mcal/d)			NEg (Mcal/d)		
	Low	Medium	High	Low	Medium	High	Low	Medium	High
10-14 wk	0.33	0.51	0.59	4.01	4.91	5.34	2.41	3.04	3.50
14-18 wk	0.44	0.74	0.91	5.42	7.12	8.21	3.26	4.41	5.37
18-22 wk	0.50	0.89	1.30	6.16	8.66	11.72	3.70	5.36	7.67
22-26 wk	0.58	1.01	1.51	7.07	9.79	13.62	4.25	6.07	8.91
26-30 wk	0.62	1.12	1.63	7.57	10.89	14.72	4.55	6.75	9.63
30-34 wk	0.73	1.09	1.69	8.78	11.92	14.27	5.33	7.45	9.26
34-38 wk	0.76	1.10	1.72	9.24	12.12	14.55	5.62	7.58	9.44
38-42 wk	0.82	1.18	1.70	9.94	12.93	14.36	6.04	8.09	9.32
42-46 wk	0.91	1.41	1.91	11.05	15.50	16.10	6.71	9.70	10.45
46-50 wk	1.04	1.34	2.36	13.44	16.72	20.81	8.36	10.52	13.68
50-54 wk	1.07	1.22	2.45	13.91	15.25	21.54	8.65	9.60	14.17
54-58 wk	1.28	1.34	2.64	16.58	16.71	23.22	10.31	10.51	15.27
58-62 wk	1.26	1.46	2.39	16.31	18.20	21.04	10.14	11.46	13.84
62-66 wk	1.29	1.70	2.41	16.68	21.30	21.19	10.37	13.41	13.93
66-70 wk	1.14	1.47	2.31	14.80	18.36	20.33	9.21	11.55	13.37

Table 2.9.
Diets in Experiment III.

Diet components	10 to 26 wk		27 to 70 wk	
(% as fed)	Low*	Medium	High	Medium
Barley silage	91	91	85	91
Rolled barley	8	8	12	8
Canola meal	0.5	0.5	2.5	0.5
Mineral-vitamin mix	0.5	0.5	0.5	0.5

* Feed supplied to the low nutrition group was restricted to 75% of that consumed by bulls in the medium nutrition group.

Table 2.10.
Analysis of the diets in Experiment III.

Diet analysis	Medium	High
DM (%)	42.8	47.6
CP (% DM)	13.2	14.4
ADF (% DM)	21.9	19.1
NDF (% DM)	52.1	51.6
Ash (% DM)	7.0	6.4
TDN (% DM)	71	73.9
NEm (Mcal/kg DM)	1.66	1.75
NEg (Mcal/kg DM)	1.05	1.13

DM: dry matter; CP: crude protein; ADF: acid detergent fiber; NDF: neutral detergent fiber; TDN: total digestible nutrients; NEm: net energy of maintenance; NEg: net energy of gain.

Table 2.11.
Average daily dry matter intake (kg) by bulls receiving low-high, low-medium, and medium-medium nutrition in Experiment III.

Age	Low-High	Low-Medium	Medium-Medium
10-14 wk	2.5	2.3	3.1
14-18 wk	3.4	3.1	4.2
18-22 wk	3.8	3.8	5.1
22-26 wk	4.5	4.5	6.0
26-30 wk	6.2	5.2	5.7
30-34 wk	6.5	7.0	7.0
34-38 wk	7.9	7.2	7.7
38-42 wk	8.5	7.8	7.9
42-46 wk	9.5	8.7	8.7
46-50 wk	10.7	10.1	10.3
50-54 wk	11.9	10.9	10.8
54-58 wk	13.0	12.1	11.8
58-62 wk	13.9	12.8	12.9
62-66 wk	13.7	12.8	13.1
66-70 wk	13.8	12.4	13.5

Table 2.12.
Average crude protein (CP), net energy of maintenance (NEm), and net energy of gain (NEg) intake by bulls receiving medium-medium, low-high, and low-medium nutrition in Experiment III.

Age	CP (kg/d)			NEm (Mcal/d)			NEg (Mcal/d)		
	Med-Med	Low-High	Low-Med	Med-Med	Low-High	Low-Med	Med-Med	Low-High	Low-Med
10-14 wk	0.41	0.33	0.31	5.13	4.10	3.85	3.24	2.59	2.44
14-18 wk	0.56	0.44	0.41	7.04	5.56	5.19	4.45	3.52	3.29
18-22 wk	0.68	0.50	0.50	8.50	6.34	6.24	5.38	4.01	3.95
22-26 wk	0.80	0.60	0.60	10.02	7.53	7.53	6.34	4.77	4.77
26-30 wk	0.76	0.90	0.69	9.51	10.88	8.61	6.02	7.02	5.45
30-34 wk	0.93	0.93	0.92	11.63	11.30	11.62	7.36	7.29	7.35
34-38 wk	1.02	1.14	0.95	12.83	13.86	11.88	8.12	8.94	7.52
38-42 wk	1.04	1.22	1.03	13.13	14.78	12.91	8.31	9.54	8.17
42-46 wk	1.15	1.37	1.15	14.50	16.60	14.41	9.18	10.72	9.11
46-50 wk	1.36	1.54	1.33	17.06	18.65	16.76	10.80	12.04	10.61
50-54 wk	1.43	1.72	1.43	17.99	20.87	18.02	11.38	13.47	11.40
54-58 wk	1.56	1.87	1.60	19.63	22.71	20.08	12.42	14.66	12.71
58-62 wk	1.70	2.01	1.68	21.34	24.37	21.16	13.50	15.73	13.39
62-66 wk	1.73	1.98	1.69	21.72	24.00	21.26	13.75	15.49	13.45
66-70 wk	1.78	1.98	1.64	22.37	24.09	20.65	14.16	15.55	13.06

Table 2.13.
Diets in Experiment IV.

Diet components	10 to 18 wk		19 to 30 wk		31 to 74 wk
(% as fed)	Medium	High	Medium	High	Medium
Barley silage	91	78	91	62	91
Rolled barley	8	18	8	22	8
Canola meal	0.5	0.5	0.5	15	0.5
Canola oil	-	3	-	-	-
Molasses	-	-	-	0.5	-
Mineral-vitamin mix	0.5	0.5	0.5	0.5	0.5

Table 2.14.
Analysis of the diets in Experiment IV.

Diet analysis	High		Medium
	10 to 18 wk	19 to 30 wk	
DM (%)	52.2	58.0	43.6
CP (% DM)	15.1	21.3	13.5
ADF (% DM)	16.8	19.5	24.5
NDF (% DM)	52.3	54.7	57.4
Ash (% DM)	5.7	6.6	7.3
TDN (% DM)	76.3	73.9	68.3
NEm (Mcal/kg DM)	1.82	1.74	1.57
NEg (Mcal/kg DM)	1.19	1.12	0.98

DM: dry matter; CP: crude protein; ADF: acid detergent fiber; NDF: neutral detergent fiber; TDN: total digestible nutrients; NEm: net energy of maintenance; NEg: net energy of gain.

Table 2.15.
Average daily dry matter intake (kg) by bulls receiving medium and high nutrition in Experiment IV.

Age	Medium	High
10-14 wk	3.4	2.9
14-18 wk	4.2	3.9
18-22 wk	5.1	6.1
22-26 wk	4.7	7.1
26-30 wk	4.7	7.8
30-34 wk	5.7	9.3
34-38 wk	6.4	11.1
38-42 wk	6.5	11.3
42-46 wk	6.6	10.5
46-50 wk	6.8	10.6
50-54 wk	7.9	11.4
54-58 wk	7.9	11.7
58-62 wk	10.7	15.1
62-66 wk	12.4	16.3
66-70 wk	11.9	15.6
70-74 wk	11.9	16.3

Table 2.16.
Average crude protein (CP), net energy of maintenance (Nem), and net energy of gain (NEg) intake by bulls receiving medium and high nutrition in Experiment IV.

Age	CP (kg/d)		NEm (Mcal/d)		NEg (Mcal/d)	
	Medium	High	Medium	High	Medium	High
10-14 wk	0.46	0.44	5.42	5.30	3.35	3.47
14-18 wk	0.56	0.58	6.58	7.03	4.08	4.61
18-22 wk	0.68	1.30	7.95	10.63	4.92	6.84
22-26 wk	0.64	1.52	7.45	12.38	4.61	7.97
26-30 wk	0.64	1.65	7.45	13.48	4.61	8.68
30-34 wk	0.77	1.25	8.98	14.57	5.56	9.02
34-38 wk	0.86	1.50	10.06	17.45	6.23	10.80
38-42 wk	0.88	1.53	10.24	17.84	6.34	11.05
42-46 wk	0.88	1.42	10.32	16.60	6.39	10.28
46-50 wk	0.92	1.43	10.69	16.69	6.62	10.34
50-54 wk	1.07	1.53	12.44	17.89	7.70	11.08
54-58 wk	1.07	1.58	12.44	18.44	7.70	11.42
58-62 wk	1.44	2.03	16.77	23.74	10.38	14.69
62-66 wk	1.67	2.20	19.53	25.61	12.09	15.85
66-70 wk	1.61	2.10	18.76	24.49	11.61	15.16
70-74 wk	1.61	2.20	18.73	25.72	11.59	15.92

2.2. Sexual development

The bulls were examined every 4 wk during the experimental period. Body weight was recorded and backfat was measured between the 12th and 13th ribs over the longissimus muscle, using an A-mode ultrasound machine (Krautkramer USK 7; Krautkramer Inc., Lewistown, PA, USA). Scrotal circumference (SC) was determined with a Coulter Scrotal Tape (Trueman Manufacturing; Edmonton, AB, Canada). Testes width and length were measured with calipers and paired testes volume was calculated using a formula [volume = 0.5236 x length x width^2; (Bailey et al., 1998)].

A B-mode ultrasound scanner equipped with a 7.5 MHz linear array transducer (Aloka 500; Aloka Co., Tokyo, Japan) was used to image the testicular vascular cones and the testes. Lubricant gel was used as a coupling material between the transducer and the scrotum and minimum pressure was applied to obtain the images. Ultrasonograms were frozen, downloaded to a computer, and analyzed with image analysis software (Image 1.58; National Institutes of Health, Bethesda, MD, USA). For imaging the testicular vascular cones, the transducer was held horizontally on the caudal surface of the scrotal neck and a cross-sectional image of each cone was recorded. The diameter of the testicular vascular cones and the distance from the inner surface of the skin to the proximal edge of the vascular cone (defined as fat) were determined. In Experiment III, the testicular vascular cones were examined only at 70 wk of age. For imaging the testes, the transducer was held vertically (parallel to the long axis of the testes) on the caudal surface of the scrotum and aligned so that the mediastinum was readily apparent before an image of each testis was recorded. Testicular pixel-intensity was determined on a

selected area of the parenchyma on a scale of 1 (white) to 255 (black); therefore, reduced pixel-intensity corresponded to increased tissue echodensity (brightness). The area for analysis was selected by drawing a rectangle 0.5 to 1 cm deep into the parenchyma, where it appeared homogeneous; the area was selected above the mediastinum and the edges of the image were avoided (Gabor et al., 1998).

To determine age at puberty, semen was collected by electroejaculation at 2-wk intervals once SC reached 26 cm. Sperm concentration was determined with a hemocytometer and the percentage of progressively motile sperm was estimated with phase-contrast microscopy at 400X magnification. Puberty was defined as the first time that an ejaculate contained ≥ 50 million spermatozoa with ≥ 10% motile spermatozoa (Wolf et al., 1965). Ejaculates were collected every 4 wk after puberty and sperm morphology was determined in eosin/nigrosin-stained smears. A total of 100 sperm were examined under 1000X magnification according to the criteria previously described (Barth and Oko, 1989). Sexual maturity was characterized by an ejaculate containing ≥70% morphologically normal spermatozoa (Brito et al., 2004).

Scrotal surface temperature of the caudal aspect of the scrotum was determined with an infrared thermography camera (Inframetrics Model 760; Inframetrics Ltd., North Billerica, MA, USA) at the end of the experimental period. The camera was held at the level of the scrotum, approximately 1.0 m behind the bull. Thermographic images were frozen, captured on a flash card, and subsequently downloaded to a computer for image analysis (Image 1.58, National Institutes of Health). Top scrotal surface temperature was

measured at the top of the testes (ventral to the scrotal neck) and bottom scrotal surface temperature was measured about 1.5 cm from the bottom of the scrotum (Cook et al., 1994; Kastelic et al., 1995).

Bulls were sent to slaughter at the end of the experimental period and the testes were recovered and weighed. In Experiments I and II, one testis was sectioned in the midsagital plane and 1-cm thick samples of parenchyma were collected from the dorsal and ventral portions of the testis. These samples were fixed in Bouin's solution for 24 h, stored in 70% ethanol, embedded in paraffin, sectioned (7-µm thick), and stained with Period Acid Schiff and hematoxylin-eosin. Histology samples were evaluated with the aid of image analysis software (Northern Eclipse 6.0; Empix Imaging Inc., Cheektowaga, NY, USA). The percentage of the testicular volume occupied by seminiferous tubules was determined by recording the structure in focus in 600 points ("hits") in 120 randomly selected fields under 100 X magnification (Chalkley, 1943). Seminiferous tubule diameter and area, and area of the seminiferous epithelium were determined from 40 randomly selected tubular cross sections with a round and visible lumen under 200X magnification. Tubule diameters were measured at right angles and the average was used for analysis. Tubule area was determined by overlaying the entire tubule, whereas the seminiferous epithelium area was determined by subtracting the area of the tubule lumen from the tubule area.

Daily sperm production per gram of the testicular parenchyma (DSP/g) and epididymal sperm reserves were estimated from one testis and one epididymis.

Testicular parenchyma samples (approximately 15 g) and the entire epididymis were thoroughly homogenized and diluted with 0.05% Triton X-100 solution (Sigma Chemical Co., St. Louis, MO, USA). Cells were counted using a hemocytometer and phase-contrast microscopy under 400 X magnification. Calculations were based on dilutions; DSP/g also used a correction factor of 5.32 d (Amann et al., 1974). Total daily sperm production was calculated by multiplying DSP/g by paired testes weight.

2.3. Blood sampling and radioimmunoassays (RIA)

Blood samples were collected from a subgroup of bulls (n = 7 or 8) from each group in each experiment. In Experiment I, single blood samples were collected every 4 wk from 26 to 70 wk of age. In Experiment II, intensive blood samplings (sampling every 15 min for 10 h) were performed every 4 wk from 10 to 26 wk of age and at 44 and 48 wk of age. Gonadotropin-releasing hormone (Fertagyl, Intervet, Whitby, ON, Canada) was administered (0.04 μg/kg, IV) after the first 10 h and blood samples were collected every 15 min for another 90 min. Additionally, single blood samples were collected every 2 or 4 wk during the period in which intensive samplings were not performed. In Experiment III, intensive blood samplings followed by GnRH challenge were performed every 4 wk from 14 to 34 wk of age, and single blood samples were collected every 4 wk during the period in which intensive samplings were not performed. In Experiment IV, intensive blood samplings followed by GnRH challenge were performed every 4 wk

from 14 to 30 wk of age and single blood samples were collected every 4 wk during the period in which intensive samplings were not performed.

Blood samples were allowed to clot at room temperature overnight and were centrifuged to separate serum, which was stored at -20^{o}C before analysis. In Experiments II, III, and IV, LH concentrations were determined in serum samples obtained during the entire intensive sampling period, including after GnRH treatment. Leptin, insulin, GH, IGF-I, FSH, and testosterone concentrations were determined in single serum samples and pooled samples from intensive samplings. Serum GH concentrations were determined only until 33 wk of age in Experiment III and leptin and GH concentrations were not evaluated in Experiment IV. Testosterone concentrations were also determined in serum samples obtained after GnRH challenge in Experiments II, III, and IV and FSH concentrations were determined in serum samples obtained after GnRH challenge in Experiments II and III. Serum concentrations of leptin, insulin, GH, IGF-I, LH, and FSH were determined by double-antibody RIA, whereas serum concentrations of testosterone were determined by solid-phase RIA. Intra- and inter-assay CV's were < 10% for all hormones.

Leptin concentrations were determined using a multi-species leptin RIA kit (Linco Research, St. Charles, MO, USA) in Experiments I and II; the sensitivity of the assay was 0.1 ng/ml. In Experiment III, purified recombinant bovine leptin (Ehrhardt et al., 2000) was used for preparation of standards and for tracer labeling. Tracer leptin was labeled using Iodo-gen (Ehrhardt et al., 2000). Leptin concentrations were determined

using rabbit bovine-leptin antiserum as previously described in cattle (Ehrhardt et al., 2000) and the sensitivity of the assay was 0.1 ng/ml.

Tracer insulin, GH, and IGF-I were labeled with ^{125}I by the Chloramine T method (Greenwood et al., 1963). Insulin, GH, and IGF-I concentrations were determined as previously described in ruminants (Van Kessel et al., 1990). Purified recombinant bovine insulin (Lilly Research Laboratories, Indianapolis, IN, USA) was used for preparation of standards and for tracer labeling. Insulin concentrations were determined using guinea-pig bovine-insulin antiserum (Rutter and Manns, 1987) and the sensitivity of the assay was 0.1 ng/ml.

Purified recombinant bovine GH AFP-11182B (National Hormone & Pituitary Program [NHPP]; Torrance, CA, USA) was used for preparation of standards and for tracer labeling. Growth hormone concentrations were determined using monkey bovine-GH antiserum (Anti-bGH AFPB55; NHPP) and the sensitivity of the assay was 0.05 ng/ml.

Purified recombinant human IGF-I (Ciba Geigy Animal Health, St. Aubin, Switzerland) was used for preparation of standards and for tracer labeling. Insulin-like growth factor-I concentrations were determined using mouse human-IGF-I monoclonal antibody (Kerr et al., 1990). Acid-ethanol extraction was performed prior to analysis. Briefly, serum samples were incubated with acid-ethanol (87.5% ethanol, 12.5% 2 mol HCl; v/v) for 30 min and centrifuged for 30 min. The supernatant was neutralized with

0.855 Tris and diluted with buffer before analysis. The sensitivity of the assay was 1.0 ng/ml.

Gonadotropins and testosterone concentrations were determined as previously described in bulls (Evans et al., 1995). Luteinizing hormone NIH-bLH-B4 (NHPP) was used for preparation of standards and USDA-bLH-I-1 (NHPP) was used for tracer labeling. Luteinizing hormone concentrations were determined using rabbit bovine-LH antiserum (Rawlings et al., 1984) and the sensitivity of the assay was 0.05 ng/ml.

Follicle-stimulating hormone USDA-bFSH-I-1 (NHPP) was used for preparation of standards and AFP-5332B (NHPP) was used for tracer labeling. Follicle-stimulating hormone concentrations were determined using rabbit ovine-FSH antiserum (NIDDK-anti-oFSH-1; NHPP) and the sensitivity of the assay was 0.1 ng/ml.

Testosterone concentrations were determined using a total testosterone RIA kit (Diagnostic Products Corporation, Los Angeles, CA, USA), but testosterone standards were prepared with purified hormone (Sigma Chemical Co., St. Louis, MO, USA); the sensitivity of the assay was 0.04 ng/ml.

2.4. Statistical analyses

Statistical analyses were conducted using the Statistical Analysis System (SAS Institute; Cary, NC, USA) and Statistix (Analytical Software; Tallahassee, FL, USA). Sperm morphology data were arcsine transformed prior to analysis.

2.4.1. Experiment I

In order to describe the changes that occurred before and after puberty, data were normalized to the date of puberty prior to analysis. Mixed-model analysis with Tukey's test was used to determine and locate nutrition (low, medium, and high nutrition), age, and nutrition-by-age interaction effects on body weight, backfat, SC, paired testes volume, testicular pixel-intensity, testicular vascular cone diameter and fat thickness, proportion of normal sperm, and serum hormones concentrations. The covariate structure that best fitted each end point was used (Littell et al., 1998). One-way ANOVA with LSD test was used to determine and locate nutrition effects on ages at puberty and maturity, paired testes weight, proportion of testicular parenchyma occupied by seminiferous tubules, seminiferous tubule diameter and area, seminiferous epithelium area, daily sperm production, epididymal sperm reserves, and proportion of normal sperm at 70 wk of age. Mixed-model analysis with Tukey's test was used to determine and locate the effects of nutrition and position (top and bottom) on scrotal surface temperature. Correlations among variables were calculated and stepwise regression models for body weight, backfat, SC, paired testes volume were evaluated, using

monthly hormone concentrations as independent variables. Stepwise regression models for ages at puberty and maturity, paired testes weight, daily sperm production, epididymal sperm reserves, seminiferous tubule diameter and area, seminiferous epithelium area, proportion of testicular parenchyma occupied by tubules, and proportion of normal sperm at 70 wk of age were evaluated using monthly body weight, backfat, SC, paired testes volume, and hormone concentrations as independent variables.

2.4.2. Experiment II

Characteristics of LH secretion were determined with the PC-pulsar software (J. Gitzen and V. Ramirez, University of Illinois, Chicago, IL, USA) and included pulse frequency, pulse amplitude, total secretion (area under the curve), basal, mean, and peak concentrations during the period of intensive blood sampling. Mean and total secretion (area under the curve) of LH, FSH, and testosterone after GnRH challenge were also evaluated. Mixed-model analysis with Tukey's test was used to determine and locate nutrition (low, medium, and high nutrition), age, and nutrition-by-age interaction effects on body weight, backfat, SC, paired testes volume, testicular pixel-intensity, testicular vascular cone diameter and fat thickness, proportion of normal sperm, and serum hormone concentrations. The covariate structure that best fitted each end point was used (Littell et al., 1998). One-way ANOVA with LSD test was used to determine and locate the effects of nutrition on ages at puberty and maturity, paired testes weight, proportion of testicular parenchyma occupied by seminiferous tubules, seminiferous tubule diameter and area, seminiferous epithelium area, daily sperm production, epididymal

sperm reserves, and proportion of normal sperm at 70 wk of age. Mixed-model analysis with Tukey's test was used to determine and locate the effects of nutrition and position (top and bottom) on scrotal surface temperature. Hormone concentrations were averaged around 4-wk intervals for calculation of correlations and evaluation of regression models. Correlations among variables were calculated and stepwise regression models for body weight, backfat, SC, and paired testes volume were evaluated using monthly hormone concentrations as independent variables.

2.4.3. Experiment III

Characteristics of LH secretion were determined with the PC-pulsar software and included pulse frequency, pulse amplitude, total secretion (area under the curve), basal, mean, and peak concentrations. Mean, peak, peak amplitude, time to peak, and total secretion (area under the curve) of LH, FSH, and testosterone after GnRH challenges were also evaluated. Analysis was divided in two periods; Period 1 was the period of feed restriction (10 to 26 wk of age) and Period 2 was the period after feed restriction (30 to 70 wk of age). Mixed-model analysis with Tukey's test was used to determine and locate the effects of nutrition (low and medium nutrition during Period 1; medium/medium, low/medium, and low/high nutrition during Period 2), age, and the nutrition-by-age interaction on body weight, backfat, SC, paired testes volume, testicular pixel-intensity, proportion of normal sperm, and serum hormone concentrations. The covariate structure that best fitted each end point was used (Littell et al., 1998). One-way ANOVA with LSD test was used to determine and locate nutrition effects on ages at

puberty and maturity, testicular vascular cone diameter and fat thickness, paired testes weight, daily sperm production, and epididymal sperm reserves. Mixed-model analysis with Tukey's test was used to determine and locate the effects of nutrition and position (top and bottom) on scrotal surface temperature.

2.4.4. Experiment IV

Characteristics of LH secretion were determined with the PC-pulsar software and included pulse frequency, pulse amplitude, total secretion (area under the curve), mean, and peak concentrations during the period of intensive blood sampling. Mean and peak concentrations and total secretion (area under the curve) of LH and testosterone after GnRH challenge were also evaluated. Mixed-model analysis with Tukey's test was used to determine and locate the effects of nutrition (medium/medium and high/medium), age, and the nutrition-by-age interaction on body weight, backfat, SC, paired testes volume, testicular pixel-intensity, testicular vascular cone diameter and fat thickness, proportion of normal sperm, and serum hormone concentrations. The covariate structure that best fitted each end point was used (Littell et al., 1998). A two-sample *t*-test was used to determine the effects of nutrition on ages at puberty and maturity, paired testes weight, daily sperm production, and epididymal sperm reserves. Mixed-model analysis with Tukey's test was used to determine and locate the effects of nutrition and position (top and bottom) on scrotal surface temperature.

3. EXPERIMENT I - CIRCULATING METABOLIC HORMONES DURING THE PERIPUBERTAL PERIOD AND THEIR ASSOCIATION WITH SEXUAL DEVELOPMENT, SPERM PRODUCTION, AND SEMEN QUALITY IN BULLS

3.1. Results

There were age effects ($P < 0.0001$) on body weight, backfat, SC, paired testes volume, testes pixel-intensity, and testicular vascular cone diameter and fat thickness. Overall, body weight, backfat, SC, and paired testes volume increased ($P < 0.05$) continuously from 20 wk before puberty to 20 wk after puberty (Figures 3.1 and 3.2), whereas testicular vascular cone diameter and fat thickness increased ($P < 0.05$) from 8 wk before puberty to 4 and 8 wk after puberty, respectively (Figure 3.3). Testes pixel-intensity decreased ($P < 0.05$), i.e. ultrasonograms became more echodense, from 12 wk before puberty to 16 wk after puberty (Figure 3.2). There was a tendency for a group effect ($P = 0.06$) on body weight; overall, bulls in the medium nutrition group tended to be heavier than bulls in the low nutrition group.

There were age effects ($P < 0.001$) on serum leptin, insulin, GH, IGF-I, and testosterone concentrations (Figure 3.4). Leptin concentrations increased ($P < 0.0001$) from 16 wk before puberty to 8 wk after puberty and insulin concentrations increased (*P*

< 0.01) from puberty to 8 wk after puberty. Growth hormone concentrations decreased ($P < 0.05$) 4 wk after puberty, whereas IGF-I concentrations increased ($P < 0.05$) from 8 wk before puberty to 8 wk after puberty, and then decreased ($P < 0.05$) at 20 wk after puberty. Testosterone concentrations increased ($P < 0.005$) starting 4 wk after puberty, but gonadotropin concentrations remained unchanged.

There was a position effect ($P < 0.05$) on scrotal surface temperature (Figure 3.5); overall, top scrotal temperature was greater ($P < 0.0001$) than bottom temperature.

Bulls in the medium nutrition group were older ($P < 0.05$) at puberty than bulls in the low and high nutrition groups. Bulls in the medium nutrition group also had smaller ($P < 0.05$) seminiferous tubule diameter and seminiferous tubule area than bulls in the high nutrition group; bulls in the low nutrition group were intermediate (Table 3.1). Nutrition did not affect age at maturity, paired testes weight, seminiferous epithelium area, daily sperm production, epididymal sperm reserves, and proportion of normal sperm at 70 wk of age.

There were moderate to high correlations ($P < 0.05$) between body weight, backfat, SC, and paired testes volume. There were moderate correlations ($P < 0.05$) between leptin, insulin, GH, and IGF-I concentrations, but the correlations of these hormones with gonadotropins and testosterone concentrations were low. Serum concentrations of leptin, insulin, GH, and IGF-I had moderate correlations ($P < 0.05$) with body weight, backfat, SC, and paired testes volume; correlations with FSH and testosterone concentrations were low (Table 3.2). Stepwise regression models using leptin, insulin,

GH, and IGF-I concentrations as independent variables indicated that serum concentrations of these hormones accounted for 56% of the variation in body weight, 45% of the variation in backfat, 63% of the variation in SC, and 59% of the variation in paired testes volume; IGF-I was not included in the model for backfat and GH was not included in the models for SC and paired testes volume (Table 3.3).

Age at puberty was correlated ($P < 0.05$) with SC across all ages (r = -0.36 to -0.52), with paired testes volume from 26 to 58 wk of age (r = -0.38 to -0.51), and with serum insulin concentrations at 34 and 38 wk of age (r = -0.46). When SC, paired testes volume, or insulin concentrations were used as independent variables in separate stepwise regression models, a better association of age at puberty with SC, paired testes volume, and insulin concentrations at 38 wk of age was observed. In a stepwise regression model using SC, paired testes volume, and insulin concentration at 38 wk of age, SC had the best association with age at puberty (r^2 = 0.28, y-intercept: 675.0, slope: -12.8, $P < 0.01$); the other variables were not included in the model.

Age at maturity was correlated ($P < 0.05$) with body weight (r = -0.37 to -0.54) and SC (r = -0.37 to -0.64) across all ages, with paired testes volume from 26 to 66 wk of age (r = -0.31 to -0.60) and with serum leptin concentrations from 46 to 70 wk of age (r = -0.52 to -0.59). When body weight, SC, paired testes volume, or leptin concentrations were used as independent variables in separate stepwise regression models, a better association of age at maturity with body weight at 26 wk, SC and paired testes volume at 34 wk, and leptin concentration at 46 wk of age was observed. A stepwise regression model using body weight at 26 wk, SC and paired testes volume at 38 wk, and leptin

concentrations at 46 wk of age indicated that leptin concentration had the best association with age at maturity (r^2 = 0.20, *y*-intercept: 402.6, slope: -12.0, $P < 0.05$); the other variables were not included in the model.

Paired testes weight was correlated ($P < 0.05$) with GH concentrations at 42 wk of age (r = -0.50), IGF-I concentrations at 34 wk of age (r = 0.55), leptin concentrations from 38 to 46 wk and from 62 to 70 wk of age (r = 0.50 to 0.78), and insulin concentrations from 46 to 58 wk of age (r = 0.46 to 0.70). When leptin and insulin concentrations were used as independent variables in separate stepwise regression models, a better association of paired testes weight with leptin concentrations at 42 wk of age and insulin concentrations at 58 wk was observed. A stepwise regression model using GH concentrations at 42 wk of age, IGF-I concentrations at 34 wk of age, leptin concentrations at 42 wk of age, and insulin concentrations at 58 wk of age indicated that GH and leptin concentrations had the best association with paired testes weight (model r^2 = 0.64, *y*-intercept: 507.1; leptin slope: 33.9, r^2 = 0.56, $P < 0.001$; GH slope: -9.3, r^2 = 0.09, $P < 0.05$).

There were correlations ($P < 0.05$) between the proportion of normal sperm at 70 wk and SC (r = -0.46 to -0.48), LH concentration at 66 wk (r = -0.47), and testosterone concentration at 70 wk (r = -0.46). A stepwise regression model using LH concentrations at 66 wk and SC and testosterone concentrations at 70 wk indicated that testosterone concentrations had the best association with the proportion of normal sperm (r^2 = 0.18, *y*-intercept: 1.1, slope: -0.05, $P < 0.05$); the other variables were not included in the model.

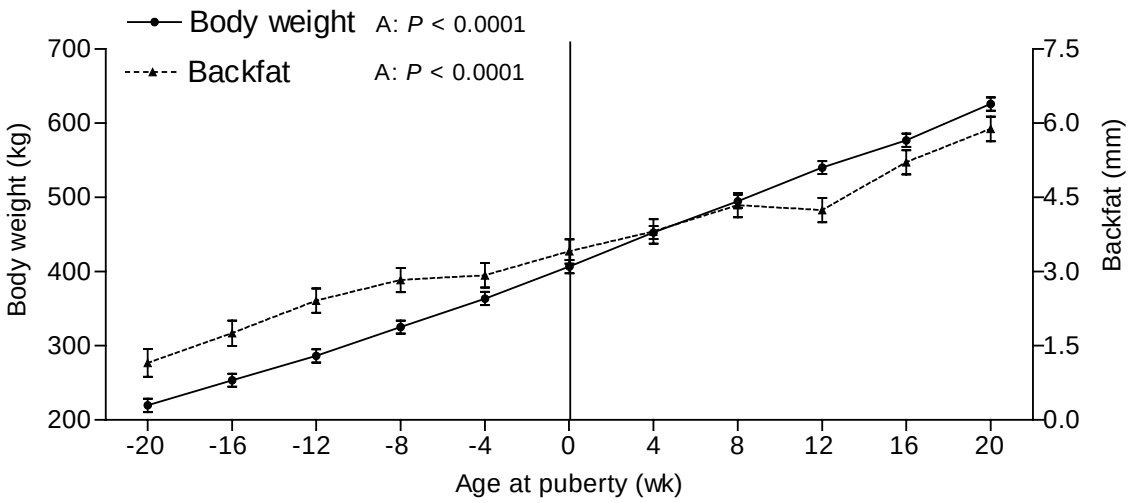
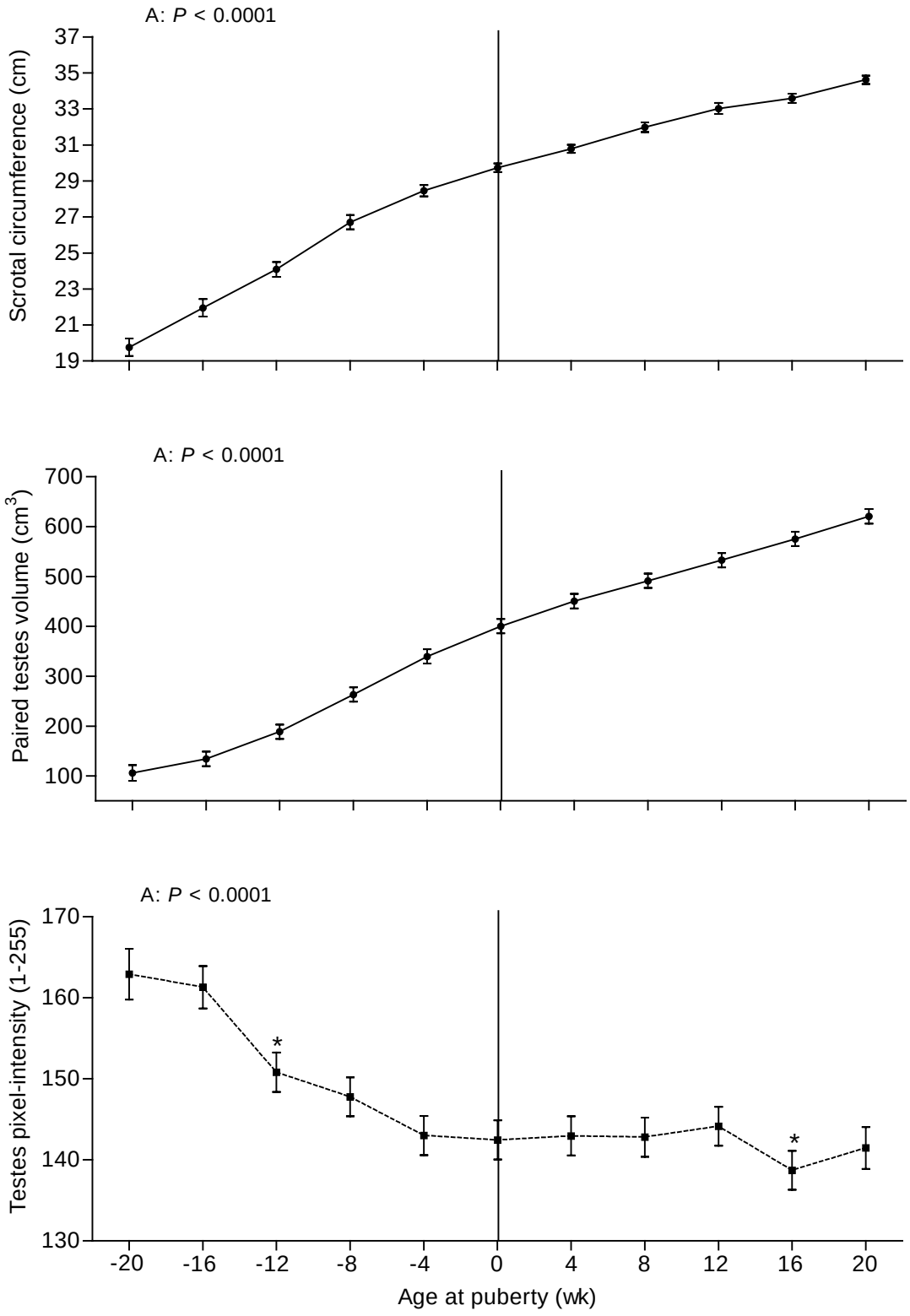


Figure 3.1. Mean (± SEM) body weight and backfat according to age at puberty in bulls (n = 40). A: age effect. BW and BF increased ($P < 0.05$) continuously during the experimental period.

Figure 3.2. Mean (± SEM) scrotal circumference, paired testes volume, and testes pixel-intensity according to age at puberty in bulls (n = 40). A: age effect. *Superscripts indicate changes ($P < 0.05$) with age. Scrotal circumference and paired testes volume increased ($P < 0.05$) continuously during the experimental period.


A: P < 0.0001
Scrotal circumference (cm)
37
35
33
31
29
27
25
23
21
19
A: P < 0.0001
Paired testes volume (cm3)
700
600
500
400
300
200
100
A: P < 0.0001
Testes pixel-intensity (1-255)
170
160
150
140
130
*
*
-20
-16
-12
-8
-4
0
4
8
12
16
20
Age at puberty (wk)

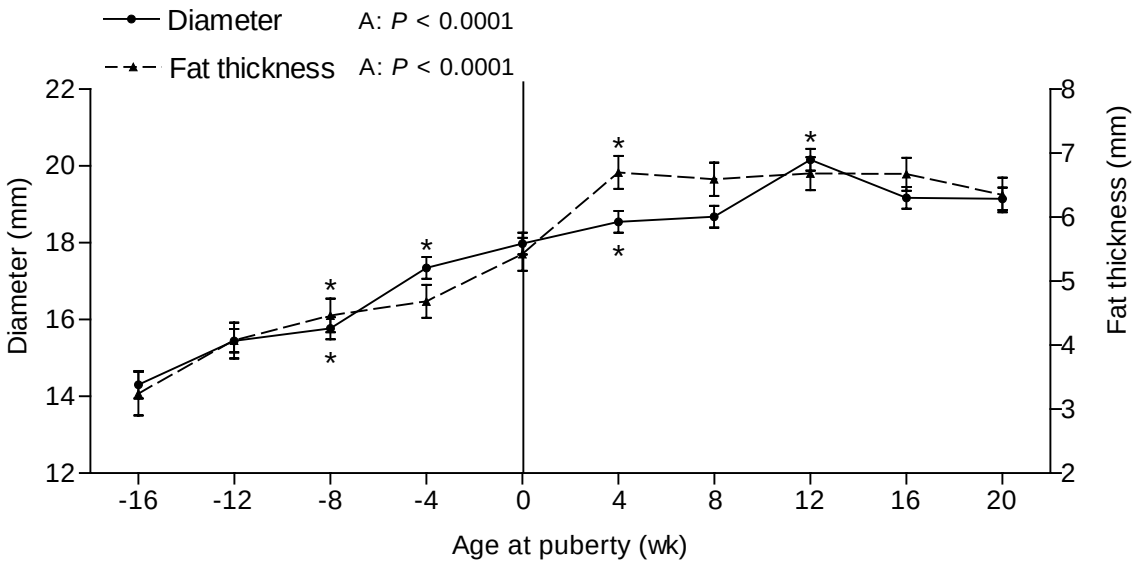
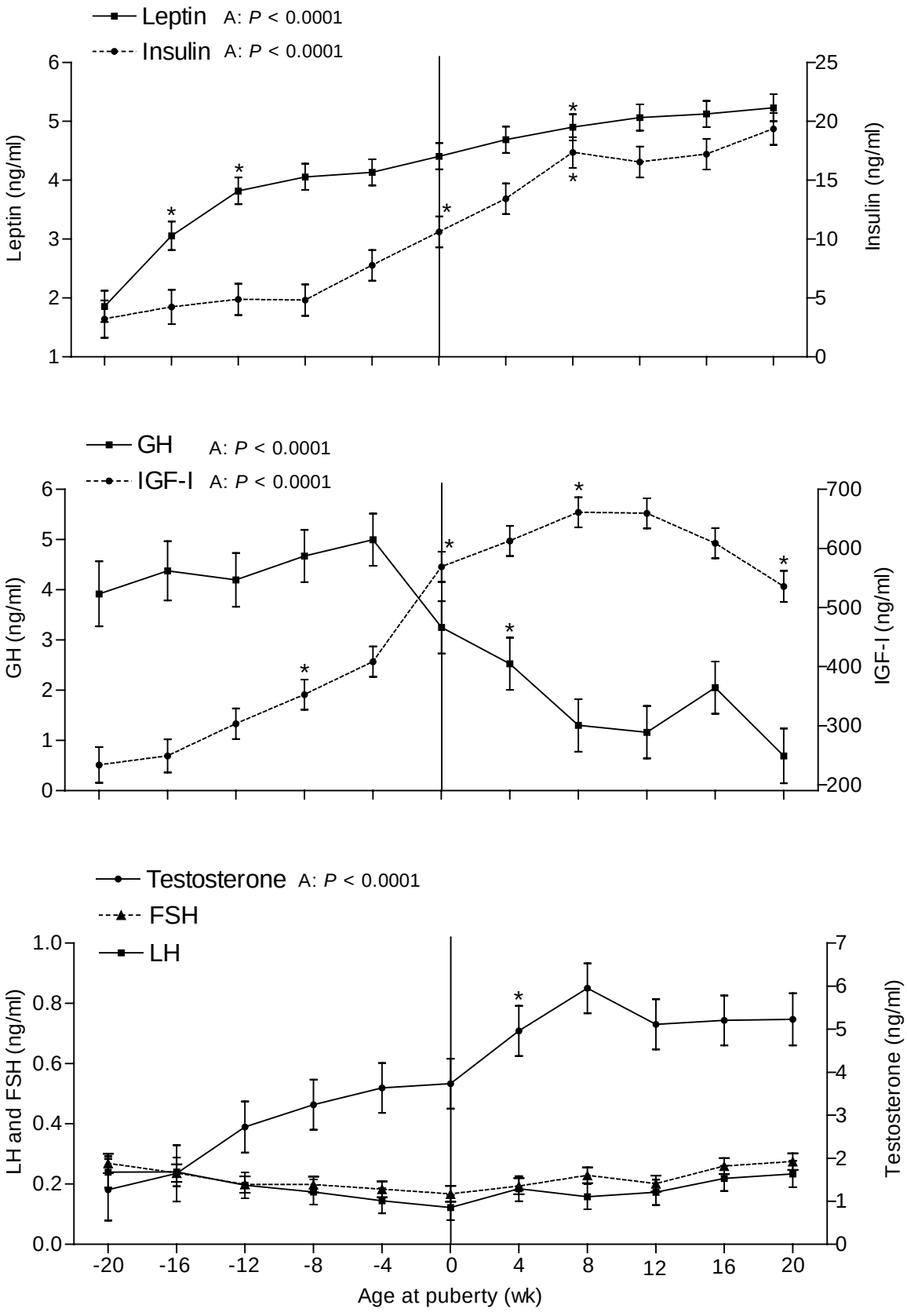


Figure 3.3. Mean (± SEM) testicular vascular cone diameter and fat thickness according to age at puberty in bulls (n = 40). A: age effect. *Superscripts indicate changes (P < 0.05) with age.

Figure 3.4. Mean (± SEM) serum leptin, insulin, growth hormone (GH), insulin-like growth factor-I (IGF-I), gonadotropins, and testosterone concentrations according to age at puberty in bulls (n = 23). A: age effect. *Superscripts indicate changes ($P < 0.05$) with age.


Leptin A: P < 0.0001
Insulin A: P < 0.0001
Leptin (ng/ml)
Insulin (ng/ml)
GH A: P < 0.0001
IGF-I A: P < 0.0001
GH (ng/ml)
IGF-I (ng/ml)
Testosterone A: P < 0.0001
FSH
LH
LH and FSH (ng/ml)
Testosterone (ng/ml)
Age at puberty (wk)

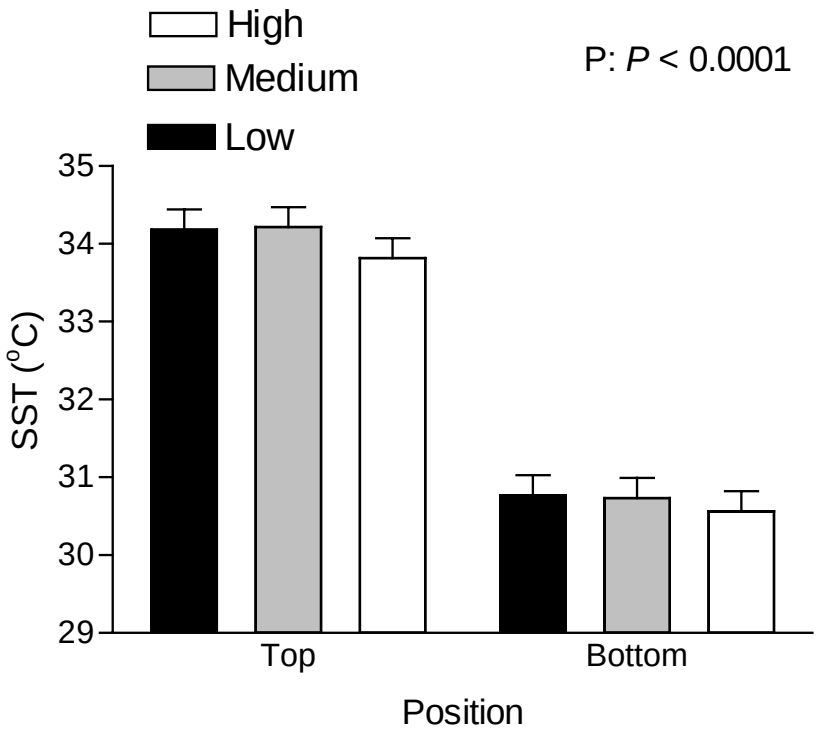


Figure 3.5. Mean (± SEM) top and bottom scrotal surface temperature (SST) at 70 wk of age in bulls receiving low (n = 14), medium (n = 13), and high (n = 13) nutrition from 26 to 70 wk of age. P: position effect.

Table 3.1.
Mean (± SEM) age at puberty and maturity, and testicular characteristics, daily sperm production (DSP), epididymal sperm reserves (ESR), and proportion of normal sperm at 70 wk of age in bulls receiving low, medium, and high nutrition from 26 to 70 wk of age.

	Low (n = 14)	Medium (n = 13)	High (n = 13)
Age at puberty (d)	301.6 ± 7.7[a]	328.4 ± 6.2[b]	299.3 ± 8.0[a]
Age at maturity (d)	364.5 ± 11.0	373.7 ± 11.4	360.8 ± 14.0
Paired testes weight (g)	618.7 ± 27.4	573.6 ± 12.7	610.8 ± 17.7
Seminiferous tubules (%)	76.5 ± 0.9	73.8 ± 0.7	74.5 ± 1.1
Seminiferous tubules diameter (μm)	241.5 ± 1.9[ab]	237.2 ± 2.5[a]	245.8 ± 2.2[b]
Seminiferous tubules area (x 10^3 μm^2)	463.9 ± 0.7[ab]	451.3 ± 1.0[a]	483.9 ± 0.9[b]
Seminiferous epithelium area (x 10^3 μm^2)	355.7 ± 0.6	345.3 ± 0.7	365.5 ± 0.7
DSP/g (x 10^6 sperm/g)	14.0 ± 1.3	14.3 ± 1.1	14.3 ± 1.4
Total DSP (x 10^6 sperm)	8.6 ± 0.9	8.3 ± 0.7	8.8 ± 0.9
ESR (x 10^9 sperm)	10.8 ± 1.1	9.7 ± 0.8	10.1 ± 0.6
Normal sperm (%)	80.2 ± 6.7	80.2 ± 5.9	80.7 ± 9.4

Columns with different superscripts differ ($P < 0.05$).

Table 3.2.
Pearson's correlation coefficients between body weight (BW), backfat (BF), scrotal circumference (SC), paired testes volume (PTV), and serum concentrations of growth hormone (GH), insulin-like growth factor-I (IGF-I), leptin, insulin, gonadotropins and testosterone in bulls (n = 23) from 26 to 70 wk of age.

	BW	BF	SC	PTV	GH	IGF-I	Leptin	Insulin	LH	FSH
BF	0.65									
SC	0.90	0.66								
PTV	0.91	0.67	0.97							
GH	-0.43	-0.46	-0.41	-0.41						
IGF-I	0.60	0.40	0.66	0.61	-0.39					
Leptin	0.66	0.60	0.69	0.67	-0.34	0.50				
Insulin	0.61	0.59	0.62	0.64	-0.44	0.45	0.62			
LH	-	-	-	-	-	-	-0.13	-		
FSH	0.14	0.29	0.15	0.16	-0.18	-	-	-	0.46	
Testosterone	0.31	0.16	0.37	0.35	-0.14	0.33	0.24	0.17	0.18	-

All described correlations are significant ($P < 0.05$).

Table 3.3.
Stepwise regression models for body weight, backfat, scrotal circumference, and paired testes volume in bulls (n = 23) from 26 to 70 wk of age using serum concentrations of leptin, insulin, GH, and IGF-I as independent variables.

	Slope	R^2	Probability
Body weight (ΣR^2 = 0.56; y-intercept = 157.7)			
Leptin	32.99	0.40	< 0.001
IGF-I	0.21	0.11	< 0.001
Insulin	3.08	0.04	< 0.005
GH	-5.08	0.01	< 0.05
Backfat (ΣR^2 = 0.45; y-intercept = 1.32)			
Insulin	0.07	0.34	< 0.001
Leptin	0.57	0.07	< 0.001
GH	-0.20	0.04	< 0.001
Scrotal circumference (ΣR^2 = 0.63; y-intercept = 19.46)			
Leptin	1.11	0.46	< 0.001
IGF-I	0.01	0.14	< 0.001
Insulin	0.10	0.03	< 0.001
Paired testes volume (ΣR^2 = 0.59; y-intercept = 15.52)			
Leptin	44.79	0.43	< 0.001
IGF-I	0.29	0.11	< 0.001
Insulin	5.10	0.05	< 0.001

ΣR^2: total model R^2.

3.2. Discussion

The effect of nutrition on body development was not consistent in the present study. Bulls in the low nutrition group seemed to be lighter than bulls in the medium nutrition group, but did not differ significantly from bulls in the high nutrition group. Moreover, nutrition did not affect backfat either. The reasons for these inconsistencies are unknown. Bulls in the medium nutrition group were older at puberty, despite being generally heavier. This was opposite from previous studies that demonstrated that early-maturing bulls (younger at puberty) were heavier than late-maturing bulls (Aravindakshan et al., 2000; Brito et al., 2004; Evans et al., 1995). Bulls in the medium nutrition group also had smaller seminiferous tubule diameters and areas, which were probably associated with the slightly (not significant) reduced paired testes weight when compared to the other nutrition groups. In the present study, SC at 38 wk of age had the best association with age at puberty, indicating that measurement of SC at this age may be useful in selecting early-maturing bulls. Metabolic hormones concentrations had strong associations with body weight and backfat, reinforcing their crucial role in body development.

Increased testicular ultrasonogram echodensity with proximity to puberty was consistent with previous reports and has been attributed to changes in testicular cellular composition and density, and fluid secretion (Aravindakshan et al., 2000; Brito et al., 2004; Chandolia et al., 1997c; Evans et al., 1996). In one study, testes pixel-intensity at puberty did not differ between early- and late-maturing bulls, indicating that a certain developmental stage of the testicular parenchyma must be reached before puberty (Brito

et al., 2004). In agreement with a previous study with mature bulls at different ages (Brito et al., 2002), testes pixel-intensity remained largely unchanged after puberty, suggesting that the testicular parenchyma remained relatively consistent after puberty.

Testicular vascular cone diameter did not increase further after 12 wk after puberty. This was consistent with a study that detected no differences in vascular cone diameter in bulls from < 36 mo to > 109 mo of age (Brito et al., 2002). Contrary to previous studies that reported increased testicular vascular cone fat thickness with age in older bulls (Brito et al., 2002; Cook et al., 1994), vascular cone fat thickness did not increase further after 4 wk after puberty; perhaps fat thickness would increase if bulls were followed for a more prolonged interval. Consistent with previous reports, top scrotal surface temperature was warmer than at the bottom (Cook et al., 1994; Kastelic et al., 1995).

Substantial testicular development occurred within the first few months after birth in bulls, coincident with the early-gonadotropin rise (Aravindakshan et al., 2000; Evans et al., 1995). However, testicular growth in beef bulls was very rapid from 6 to 16 mo of age and SC increased approximately 1.8 cm per month during this period (Barth and Ominski, 2000; Coulter, 1986). The accelerated testicular growth observed after 6 mo of age in beef bulls coincided with decreasing concentrations of circulating gonadotropins. In the present study, the peripubertal period (20 wk before to 20 wk post puberty) in bulls was characterized by increasing circulating leptin, insulin, and IGF-I concentrations, and decreasing GH concentrations. During this period, testicular growth was rapid and testosterone secretion increased substantially, without any significant

change in gonadotropin concentrations. In rams, improved nutrition (maintenance vs. 2.5 x maintenance) acutely increased concentrations of metabolic hormones and gonadotropins, as well as testicular growth. However, increased testicular growth rate was maintained well beyond the period of increased gonadotropin secretion, which usually only lasted for 3 to 4 weeks (Blache et al., 2002; Blache et al., 2000). These observations indicated the existence of an important GnRH-independent mechanism that regulates testicular development in male ruminants.

The strong association between testes size and circulating concentrations of metabolic hormones in the present study indicated that these hormones may be involved in regulating GnRH-independent testicular development in peripubertal bulls. The absence of any association between circulating metabolic hormones and gonadotropins concentrations reveals that regulation of secretion of these hormones is independent. Therefore, the possible effects of metabolic hormones on testicular growth were apparently direct and independent of the hypothalamus and pituitary. The peripubertal changes in circulating metabolic hormones and the strong association of these hormones with body and testicular development indicated the possibility of using concentrations of metabolic hormones as indicators of sexual development and reproductive function in bulls.

Testicular growth during the period of GnRH-independency in beef bulls involves increases in seminiferous tubule diameter and length, volume of testicular parenchyma occupied by seminiferous tubules, and total number of germinal cells (Abdel-Raouf, 1960; Aponte et al., 2005; Curtis and Amann, 1981). Circulating leptin and IGF-I

concentrations (and insulin concentrations to a lesser degree) had strong associations with SC and paired testes volume and weight, but there were no associations of hormonal concentrations with seminiferous tubule diameter and area, seminiferous epithelium area, or composition of the testicular parenchyma. These observations suggested that increased circulating leptin, IGF-I, and insulin concentrations were associated with increased length of the seminiferous tubules and likely with overall increases in the total number of testicular cells.

In bulls, Leydig cells multiplied between 8 and 30 wk in bulls, with a considerable increase in cell volume from 30 to 75-100 wk of age (Wrobel, 1990). Undifferentiated Sertoli cells multiplied from 12 to 20 wk and differentiates into Sertoli cells between 20 and 40 wk of age. Maximal germ cell proliferation, representing expansion of the spermatogonial stem cell line, was observed from 18 to 28 wk of age and the number of spermatogonia continued to increase until after 40 wk of age, whereas spermatocytes and spermatids number reached adult levels after 60 wk of age (Abdel-Raouf, 1960; Curtis and Amann, 1981; Sinowatz and Amselgruber, 1986; Wrobel, 2000). Considering the cellular events in the testis during the peripubertal period, the temporal patterns of hormones concentrations in the present study indicated that circulating leptin, IGF-I, and insulin could be involved in regulating Leydig cell multiplication and maturation, Sertoli cell maturation, and germ cell multiplication during the period of accelerated GnRH-independent testicular growth. Leptin has been shown to directly induce hepatic cell proliferation (Saxena et al., 2004) and IGF-I and insulin have acute metabolic actions, such as lipogenesis and glycogen synthesis, and long-term growth–promoting effects, such as DNA and RNA synthesis and cell multiplication (Soder et al., 1992).

Circulating leptin concentrations increased with proximity to puberty in humans (Garcia-Mayor et al., 1997), but leptin concentrations have not been reported in developing bulls. In the present study, leptin concentrations increased rapidly between 20 and 12 wk before puberty. Thereafter, a more gradual increase was sustained until 8 wk post puberty. The continuous increase in leptin was not accompanied by any significant change in circulating gonadotropin concentrations. This observation supported the hypothesis that leptin has only a permissive role on gonadotropin secretion (Blache et al., 2002; Blache et al., 2000). The presence of leptin receptors (Ob-R) on germ cells and Leydig cells, and the age-related changes in receptor expression in rodents indicated that leptin may have direct effects on the testis (Caprio et al., 2003; El-Hefnawy et al., 2000; Tena-Sempere et al., 2001b). There was a gradual increase in the proportion of normal sperm after puberty in bulls (Brito et al., 2004) and the negative association of leptin concentrations with age at maturity in the present study indicated that circulating leptin may affect spermatogenesis in bulls. Leptin decreased in vitro testicular steroidogenesis in rats (Tena-Sempere et al., 2001a; Tena-Sempere et al., 1999), but some authors indicated that expression of Ob-R was characteristic of mature, androgen-secreting Leydig cells (Caprio et al., 2003). In developing bulls, circulating leptin and testosterone concentrations increased concomitantly and had a low positive correlation. Leptin concentrations also had moderate positive correlations with insulin and IGF-I concentrations, indicating a possible association among the mechanisms regulating the production and secretion of these hormones.

Circulating insulin concentrations increased during sexual development in humans (Laron et al., 1988; Smith et al., 1988), but information regarding insulin concentrations

in developing bulls is scarce. One study demonstrated that insulin concentrations were stable from 1.5 to 6.5 mo of age and increased at 9 mo of age in beef bulls. However, since increased insulin concentrations were observed after bulls were offered improved nutrition after weaning, the effects of age and nutrition could not be dissociated (Arthur et al., 1990). In the present study, insulin concentrations increased from 8 wk before to 8 wk post puberty in bulls receiving the same diet after 6 mo of age, thus demonstrating developmental changes in insulin concentrations in peripubertal bulls. Insulin concentrations had a moderate positive correlation with IGF-I concentrations, indicating a possible interaction between these hormones.

A decline in GH concentrations has been reported to occur during the peripubertal period in bulls (McAndrews et al., 1993; Plouzek and Trenkle, 1991), but the relationship between GH concentrations and sexual development has not been well characterized. In contrast to rodents and primates, in which circulating GH concentrations increased during sexual development (Mauras et al., 1996; Wilson, 2001), GH concentrations decreased from 4 wk before puberty to 4 wk after puberty in the present study. Differences in the stage of body development at which each species attains puberty is likely responsible for the different GH profiles among species. For example, GH secretion decreases before puberty in bulls, indicating that a relatively advanced stage of body development must be attained before the gonads are efficiently producing sperm. This is in contrast with rodents and primates, in which circulating GH concentrations still increase for considerable periods of time after puberty (Mauras et al., 1996; Wilson, 2001). The differences in GH secretion among species may be due also to the regulatory role of steroids on GH secretion. In primates, there is considerable

evidence that steroids stimulate GH secretion in both males and females (Mauras et al., 1996; Wilson, 2001). However, mean GH concentrations did not differ between intact bulls and castrated steers (Lee et al., 1991). In the present study, decreasing GH concentrations were observed along with increasing testosterone concentrations and a small negative correlation between the concentrations of these hormones was also observed. Therefore, steroids do not have a positive feedback on GH in bulls as in other species. Moderate negative correlations between GH concentration and leptin, insulin, and IGF-I concentrations were observed, indicating that the factors that positively stimulate the secretion of leptin, insulin, and IGF-I, or these hormones themselves, may negatively affect GH secretion.

In several species, including rodents and primates, circulating IGF-I concentrations increase during the peripubertal period (Mauras et al., 1996; Wilson, 2001). In the present study, IGF-I concentrations increased from 16 wk before puberty to 8 wk post puberty, when concentrations began to decline. These results were in agreement with previous reports of increased circulating IGF-I concentrations from 2.5 to 14 mo of age in beef bulls. In addition, increasing circulating IGF-I concentrations were associated with increasing IGFBP3 and decreasing IGFBP2 circulating concentrations in these studies (Renaville et al., 1993; Renaville et al., 1996; Renaville et al., 2000). Puberty, defined by circulating testosterone > 2 ng/ml, was attained when mean IGF-I was > 150 ng/ml (Renaville et al., 1993). In the present study, when testosterone concentrations were approximately 2 ng/ml, IGF-I concentrations were comparable with this previous report; however, with puberty defined by ejaculate characteristics, IGF-I concentrations at puberty were greater than 550 ng/ml. A high positive correlation between IGF-I and

testosterone concentrations was described (Renaville et al., 1993), but only a small positive correlation was observed in the present study.

Peripubertal rises in GH and IGF-I were concomitant in rodents and primates (Mauras et al., 1996; Wilson, 2001), but circulating GH concentrations decreased whereas IGF-I concentrations increased during development in bulls. This observation questions the origin and control of circulating IGF-I in cattle. The main source of circulating IGF-I before puberty (when GH concentrations were high) was probably the liver rather than the testes, since LH and testosterone do not affect circulating IGF-I concentrations during this period. Treatment of bulls 2.5 to 6 mo old with a GnRH agonist or testosterone resulted in great variations in LH and testosterone concentrations without any effect on circulating IGF-I concentration (Renaville et al., 1996). Therefore, the testes may be a substantial contributor to circulating IGF-I after GH concentrations decreased in the peripubertal period. In support of this possibility, intact bulls tended to have greater IGF-I concentrations than castrated steers at 12 mo of age (Lee et al., 1991). Gonadotropins stimulate IGF-I secretion by Leydig and Sertoli cells in vitro, demonstrating the existence of a GH-independent mechanism of testicular IGF-I production (Chandrashekar et al., 1999; Spiteri-Grech and Nieschlag, 1992). However, circulating IGF-I concentrations increased continuously during the peripubertal period in the present study, despite decreasing GH concentrations and unchanged secretions of gonadotropins.

Insulin-like growth factor-I and insulin receptors have been identified in Leydig cells in several species (Abele et al., 1986; Bellve and Zheng, 1989; Lin, 1995; Rouiller-Fabre

et al., 1998; Spiteri-Grech and Nieschlag, 1992). Insulin-like growth factor-I increases the proliferation of Leydig cell precursors from immature animals and increases the differentiation of mesenchymal precursors into Leydig cells when combined with LH. Studies with the IGF-I-null mice indicated that IGF-I is an essential complement to LH for promoting Leydig cell development. Expression of steroidogenic enzymes was decreased in IGF-I-null mice and circulating testosterone concentration was only 30% of that observed in adult controls (Wang et al., 2003). Leydig cell numbers increased by 200% with IGF-I replacement in IGF-I-null mice and additive effects on testicular cell numbers were observed in response to IGF-I plus LH treatments, indicating that the two hormones used separate signaling pathways (Wang and Hardy, 2004). Insulin-like growth factor-I increased hCG (LH) binding to Leydig cells and increased in vitro basal and stimulated testosterone secretion in a dose-dependent manner, indicating that IGF-I may regulate steroidogenesis by regulating the number of LH receptors (Lin, 1995; Spiteri-Grech and Nieschlag, 1992). Rat Sertoli cells also have IGF-I receptors and IGF-I stimulated DNA synthesis and up-regulated metabolism (Borland et al., 1984; Oonk et al., 1989). The results of the present study support a possible role for IGF-I and insulin in regulating Leydig cell and Sertoli cell multiplication and function in peripubertal bulls.

Leydig and Sertoli cells produced IGF-I, indicating the existence of a paracrine/autocrine mechanism of testicular regulation involving IGF-I (Bellve and Zheng, 1989; Rouiller-Fabre et al., 1998; Spiteri-Grech and Nieschlag, 1992). It is assumed that most of the IGF-I in the testes is produced locally and that circulating IGF-I may play a secondary role in regulating testicular development and function. The

temporal patterns and strong associations between circulating IGF-I concentrations and testes size observed in the present study argued for a primary role for this hormone. The primary role of increased circulating IGF-I during the peripubertal period may be to promote the peripubertal increase in testosterone concentrations by regulating Leydig cell multiplication, differentiation, and maturation. In support of this view, it has been demonstrated that IGF-I treatment increases gonadal sensitivity to gonadotropins and hastens puberty in female monkeys (Wilson, 1998). Since testosterone up-regulated IGF-I production and IGF-I receptor expression by Leydig and Sertoli cells (Cailleau et al., 1990), the establishment of a positive feedback loop between IGF-I secretion and testosterone production may be important for testicular development.

In conclusion, the peripubertal period was characterized by increasing concentrations of circulating leptin, insulin, and IGF-I and decreasing GH concentrations. Secretion of metabolic hormones was not associated with changes in gonadotropin concentrations and circulating leptin, IGF-I, and insulin were probably involved in a GnRH-independent mechanism regulating testicular development in peripubertal bulls.

4. EXPERIMENT II - EFFECT OF NUTRITION DURING CALFHOOD AND PERIPUBERTAL PERIOD ON SERUM METABOLIC HORMONES, GONADOTROPINS AND TESTOSTERONE, AND ON SEXUAL DEVELOPMENT, SPERM PRODUCTION AND SEMEN QUALITY IN BULLS

4.1. Results

One bull in the medium nutrition group became severely lame and no data were recorded from that bull after 54 wk of age. There were nutrition, age, and nutrition-by-age interaction effects ($P < 0.001$) on body weight and backfat (Figure 4.1). Body weight increased ($P < 0.05$) continuously during the experimental period in all groups, whereas backfat thickness increased ($P < 0.05$) from 26 to 38 wk in the low nutrition group, from 22 to 30 wk in the medium nutrition group, and from 18 to 34 wk of age in the high nutrition group. Bulls in the low nutrition group were lighter ($P < 0.05$) than bulls in the high nutrition group between 26 and 30 wk of age, and than bulls in both medium and high nutrition groups thereafter. Backfat was thinner ($P < 0.05$) in the low nutrition group when compared to the high nutrition group after 26 wk, and when compared to the medium nutrition group between 30 and 42 wk of age. Bulls in the high nutrition group had thicker ($P < 0.05$) backfat than bulls in the medium nutrition group after 62 wk of age.

There were nutrition, age, and nutrition-by-age interaction effects ($P < 0.001$) on SC and paired testes volume. There were also age and nutrition-by-age interaction effects ($P < 0.001$) on testes pixel-intensity (Figure 4.2). Scrotal circumference increased ($P < 0.05$) until 62 wk in the low and high nutrition groups, and until 58 wk of age in the medium nutrition group. Bulls in the low nutrition group had smaller ($P < 0.05$) SC than bulls in medium and high nutrition groups between 22 and 50 wk of age, and smaller than bulls in the high nutrition group thereafter. Bulls in the high nutrition group had larger ($P < 0.05$) SC than bulls in the medium nutrition group after 62 wk of age. Paired testes volume increased ($P < 0.05$) from 38 to 58 wk in the low nutrition group, from 30 to 62 wk in the medium nutrition group, and from 26 to 66 wk of age in the high nutrition group. Bulls in the low nutrition group had smaller ($P < 0.05$) paired testes volume than bulls in the high nutrition group after 30 wk and than bulls in the medium nutrition group from 38 to 42 wk of age. Overall, testis pixel-intensity decreased ($P < 0.05$), i.e. ultrasonograms became more echodense, after 30 wk and again after 46 wk of age; there were no differences among groups within age.

There were nutrition and age effects on testicular vascular cone diameter and fat thickness ($P < 0.05$) and a nutrition-by-age interaction effect ($P < 0.01$) on fat thickness (Figure 4.3). Overall, testicular vascular cone diameter increased ($P < 0.05$) until 62 wk of age in all groups; bulls in the low nutrition group had smaller ($P = 0.01$) diameter than bulls in the high nutrition group. Testicular vascular cone fat thickness increased ($P < 0.05$) from 30 to 70 wk in the low nutrition group, from 22 to 62 wk in the medium nutrition group, and from 18 to 46 wk of age in the high nutrition group. Overall, testicular vascular cone fat thickness was smaller ($P < 0.005$) in bulls in the low

nutrition group than in bulls in the medium and high nutrition groups, but there were no differences among groups within age.

There were age and nutrition-by-age interaction effects ($P < 0.01$) on serum leptin and insulin concentrations. There was also a nutrition effect ($P < 0.0001$) on insulin concentrations (Figure 4.4). Overall, leptin concentrations increased ($P < 0.05$) after 30 wk of age, but there were no differences among groups within age. Insulin concentrations did not change with age in the low nutrition group, whereas it increased ($P < 0.05$) at 30 wk in the medium nutrition group and from 30 to 50 wk of age in the high nutrition group. Bulls in the low nutrition group had lower ($P < 0.05$) serum insulin concentrations than bulls in the medium nutrition group at 30 wk and than bulls in the high nutrition group from 30 to 50 wk of age. Insulin concentrations were also lower ($P < 0.05$) in the medium nutrition group than in the high nutrition group at 50 wk of age.

There were nutrition and age effects ($P < 0.001$) on serum IGF-I and GH concentrations. There was also a nutrition-by-age interaction effect ($P < 0.0001$) on IGF-I concentrations (Figure 4.5). Serum IGF-I concentrations increased ($P < 0.05$) after 43 wk of age in bulls in the low nutrition group and did not vary thereafter. In bulls in the medium and high nutrition groups, IGF-I concentrations increased ($P < 0.05$) from 30 to 43 wk and from 22 to 39 wk of age, respectively. In both of these groups, IGF-I concentrations decreased ($P < 0.05$) after 61 wk of age. Bulls in the low nutrition group had lower ($P < 0.05$) IGF-I concentrations than bulls in the medium nutrition group from 30 to 48 wk and than bulls in the high nutrition group from 22 to 48 wk of age. Serum IGF-I concentrations were lower in the medium nutrition group than in the high

nutrition group at 26 wk of age. There were great variations in GH concentrations with age within nutrition group; this variation seemed more pronounced in the low nutrition group and less pronounced in the high nutrition group. Overall, bulls in the low nutrition group had greater ($P < 0.01$) GH concentrations (6.65 ± 0.5 ng/ml) than bulls in the medium and high nutrition groups.

There were age and nutrition-by-age interaction effects ($P < 0.0001$) on serum FSH, LH, and testosterone concentrations. There was also a nutrition effect ($P < 0.05$) on FSH concentrations (Figure 4.6). Serum FSH concentrations decreased ($P < 0.05$) after 26 wk of age in all groups. In the medium nutrition group, FSH concentrations increased ($P < 0.05$) at 59 wk, whereas in the high nutrition group concentrations increased from 57 to 61 wk of age. Bulls in the low nutrition group had lower ($P < 0.05$) FSH concentrations than bulls in the medium and high nutrition groups from 61 to 67 wk of age. Serum LH concentrations decreased ($P < 0.05$) after 26 wk of age in all groups, but there was great variation in concentrations thereafter (an apparent increase after 60 wk was observed). Bulls in the low nutrition group had greater ($P < 0.05$) LH concentration than bulls in the medium and high nutrition groups at 39 wk of age. Testosterone concentrations increased ($P < 0.05$) after 37 wk in the low nutrition group and after 30 wk of age in the medium and high nutrition groups. Bulls in the low nutrition group had lower testosterone concentrations than bulls in the high nutrition group at 30 wk of age ($P < 0.05$).

There were nutrition and age effects ($P < 0.05$) on LH basal concentrations and pulse frequency. There was also a nutrition-by-age interaction effect ($P < 0.05$) on LH pulse frequency and age effects ($P < 0.0001$) on total LH secretion and pulse amplitude (Figure 4.7). Basal LH concentration and total LH secretion were augmented ($P < 0.05$) from 10 to 18 wk of age. Overall, LH basal concentrations were lower in bulls in the low nutrition group than in bulls in the medium and high nutrition groups. LH pulse frequency did not change significantly with age in the low nutrition group, but increased ($P < 0.05$) after 14 wk of age in the medium and high nutrition groups. Bulls in the high nutrition group had greater ($P < 0.05$) LH pulse frequency than bulls in low nutrition group from 18 to 26 wk of age. Increased LH pulse frequency subsided after 18 wk in the medium nutrition group, but was sustained until at least 26 wk of age in the high nutrition group. The increased LH pulse frequency coincided with a decrease ($P < 0.05$) in pulse amplitude after 10 wk of age.

There were age and nutrition-by-age interaction effects ($P < 0.05$) on mean LH concentration and total secretion, and age effects ($P < 0.0001$) on mean FSH concentration and total secretion after GnRH challenge (Figure 4.8). Mean LH concentration and total secretion were greater ($P < 0.05$) at 44 wk than at 10 and 14 wk of age in bulls in the low nutrition. Overall, mean FSH concentration decreased ($P < 0.05$) after 22 wk of age, whereas total secretion was greater at 10 and 18 wk of age. A significant effect of nutrition on GnRH-stimulated gonadotropin secretion was not observed at any age.

There were age and nutrition-by-age interaction effects ($P < 0.005$) on mean testosterone concentration and total secretion after GnRH challenge (Figure 4.9). Testosterone secretion after GnRH increased ($P < 0.05$) only after 44 wk in the low nutrition group. In the medium nutrition group, testosterone secretion increased ($P < 0.05$) from 22 to 44 wk of age, whereas testosterone secretion increased ($P < 0.05$) after 18 wk and reached maximum levels at 26 wk of age in the high nutrition group.

There were nutrition and position effects ($P < 0.005$) on scrotal surface temperature (Figure 4.10). Overall, bulls in the low nutrition group had lower ($P = 0.01$) scrotal temperature than bulls in the high nutrition group and top scrotal temperature was greater ($P < 0.0001$) than bottom temperature.

Bulls in the low nutrition group were older ($P < 0.05$) at puberty than bulls in the medium and high nutrition groups. Bulls in the high nutrition group had greater ($P < 0.05$) paired testes weight and epididymal sperm reserves at 70 wk of age than bulls in the low and medium nutrition groups (Table 4.1). Two bulls in the medium nutrition group and one bull in the high nutrition group did not produce > 70% morphologically normal sperm and could not be classified as mature; age at maturity did not differ significantly among groups. There were no effects of nutrition on the proportion of the testicular parenchyma occupied by seminiferous tubules, seminiferous tubules diameter and area, seminiferous epithelium area, daily sperm production/g, total daily sperm production, and proportion of normal sperm at 70 wk of age.

There were high correlations ($P < 0.05$) between body weight, backfat, SC, and paired testes volume. IGF-I concentrations were moderately correlated with insulin and testosterone concentrations and highly correlated with body weight, backfat, SC, and paired testes volume. Testosterone concentrations also had moderate correlations with body weight, backfat, SC, and paired testes volume. Correlations between concentrations of gonadotropins and metabolic hormones were low (Table 4.2). Stepwise regression models, using hormone concentrations as independent variables, indicated that serum concentrations of these hormones accounted for 78% of the variation in body weight, 66% of the variation in backfat, 79% of the variation in SC, and 69% of the variation in paired testes weight. IGF-I concentration was the single best predictor of body weight, backfat, SC, and paired testes volume. Other metabolic hormones and gonadotropins were included in the final model, but their contributions were very small (Table 4.3).

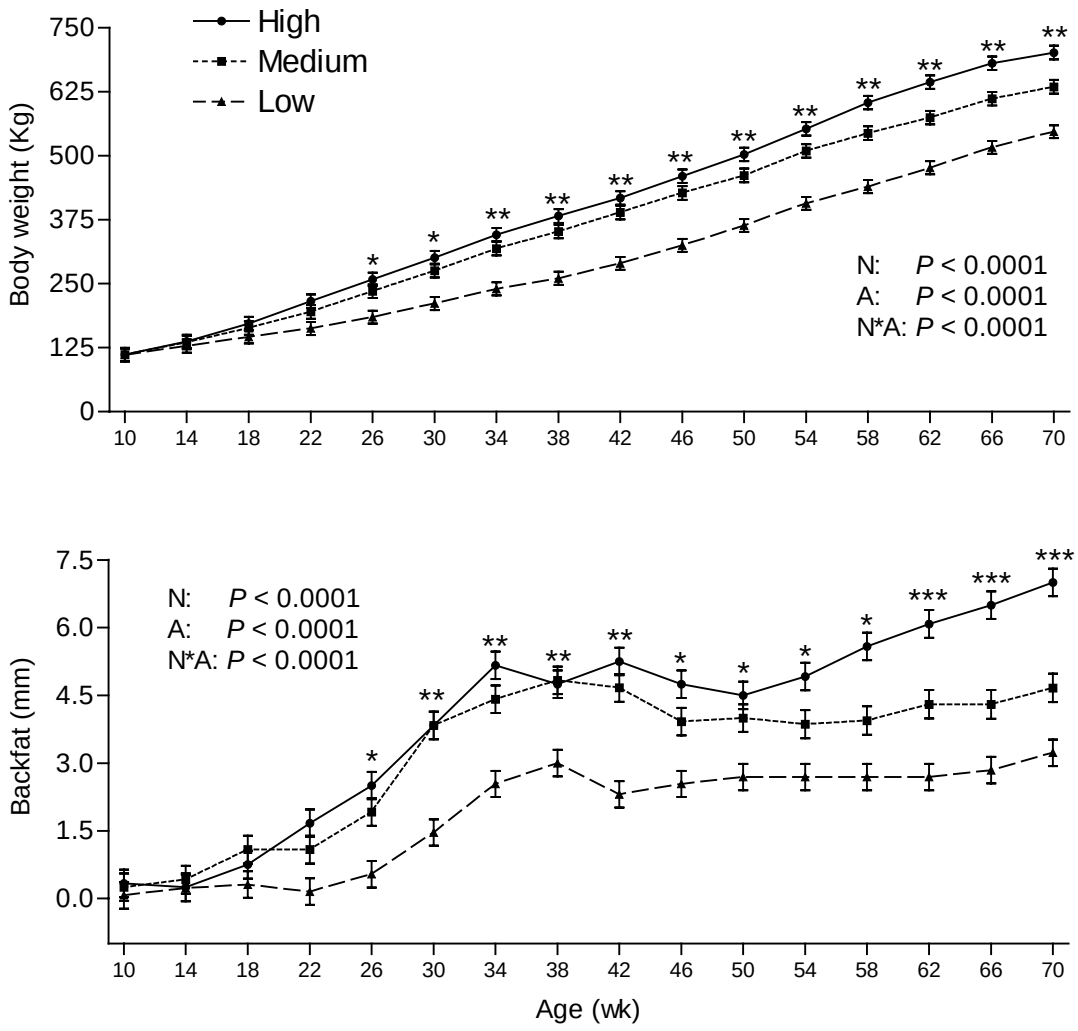
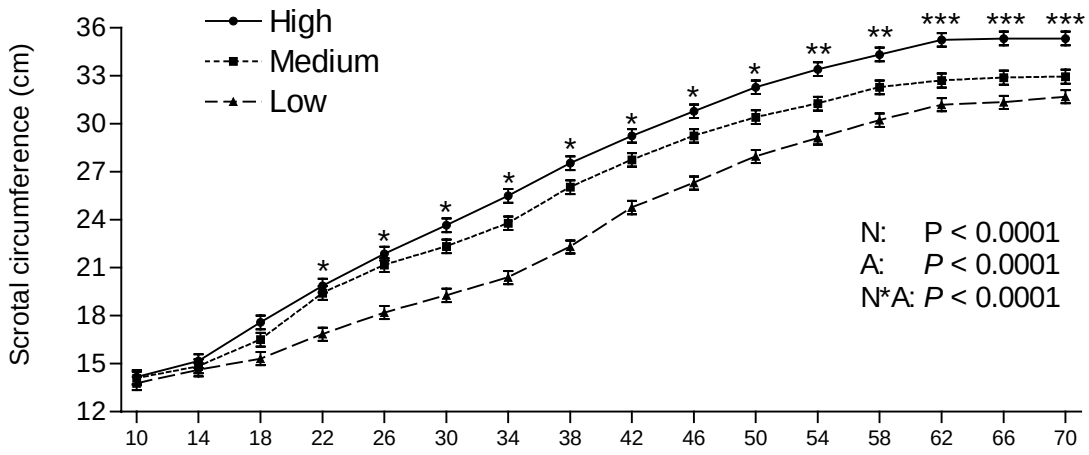
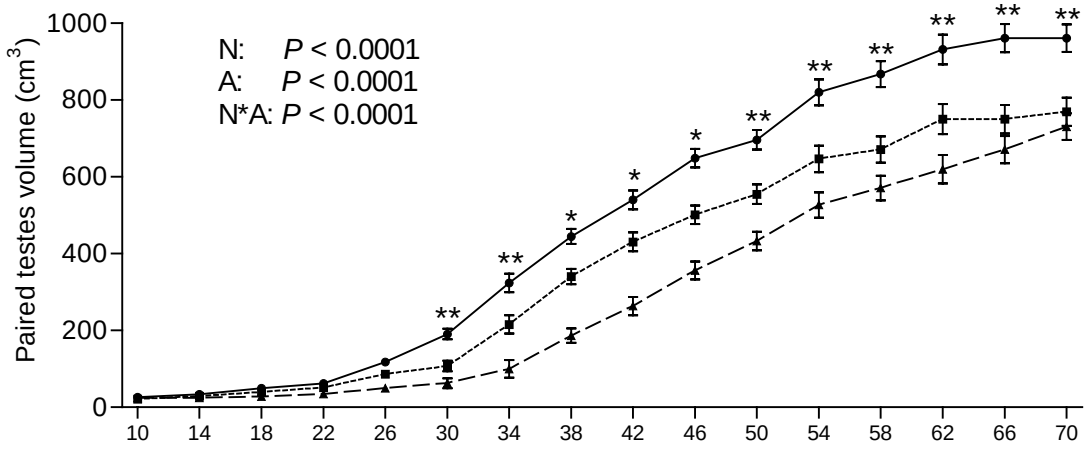
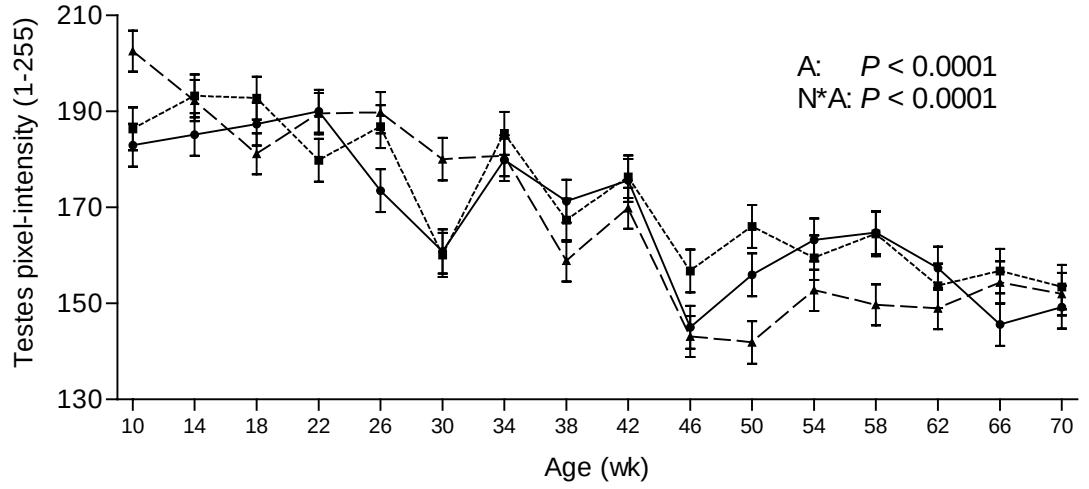


Figure 4.1. Mean (± SEM) body weight and backfat in bulls receiving low (n = 13), medium (n = 12), and high (n = 12) nutrition from 10 to 70 wk of age. N: nutrition effect; A: age effect; N*A: nutrition-by-age interaction effect. Superscripts indicate differences ($P < 0.05$) among groups within age (*low differs from high; **low differs from medium and high; ***all groups differ).

Figure 4.2. Mean (± SEM) scrotal circumference, paired testes volume, and testes pixel-intensity in bulls receiving low (n = 13), medium (n = 12), and high (n = 12) nutrition from 10 to 70 wk of age. N: nutrition effect; A: age effect; N*A: nutrition-by-age interaction effect. Superscripts indicate differences ($P < 0.05$) among groups within age (*low differs from medium and high; **low differs from high; ***all groups differ).


High
Medium
Low
Scrotal circumference (cm)
N: P < 0.0001
A: P < 0.0001
N*A: P < 0.0001


Paired testes volume (cm3)
N: P < 0.0001
A: P < 0.0001
N*A: P < 0.0001


Testes pixel-intensity (1-255)
A: P < 0.0001
N*A: P < 0.0001
Age (wk)

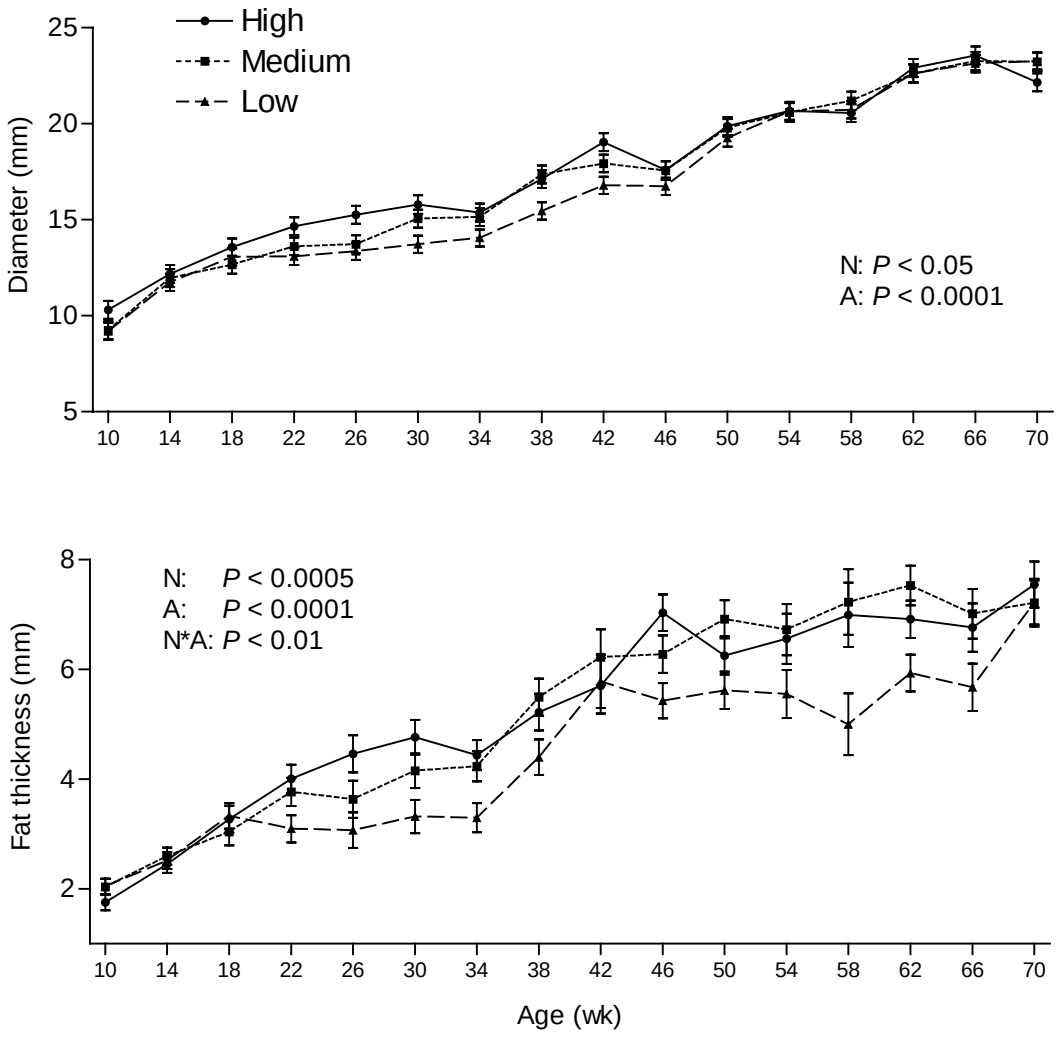


Figure 4.3. Mean (± SEM) testicular vascular cone diameter and fat thickness in bulls receiving low (n = 13), medium (n = 12), and high (n = 12) nutrition from 10 to 70 wk of age. N: nutrition effect; A: age effect; N*A: nutrition-by-age interaction effect.

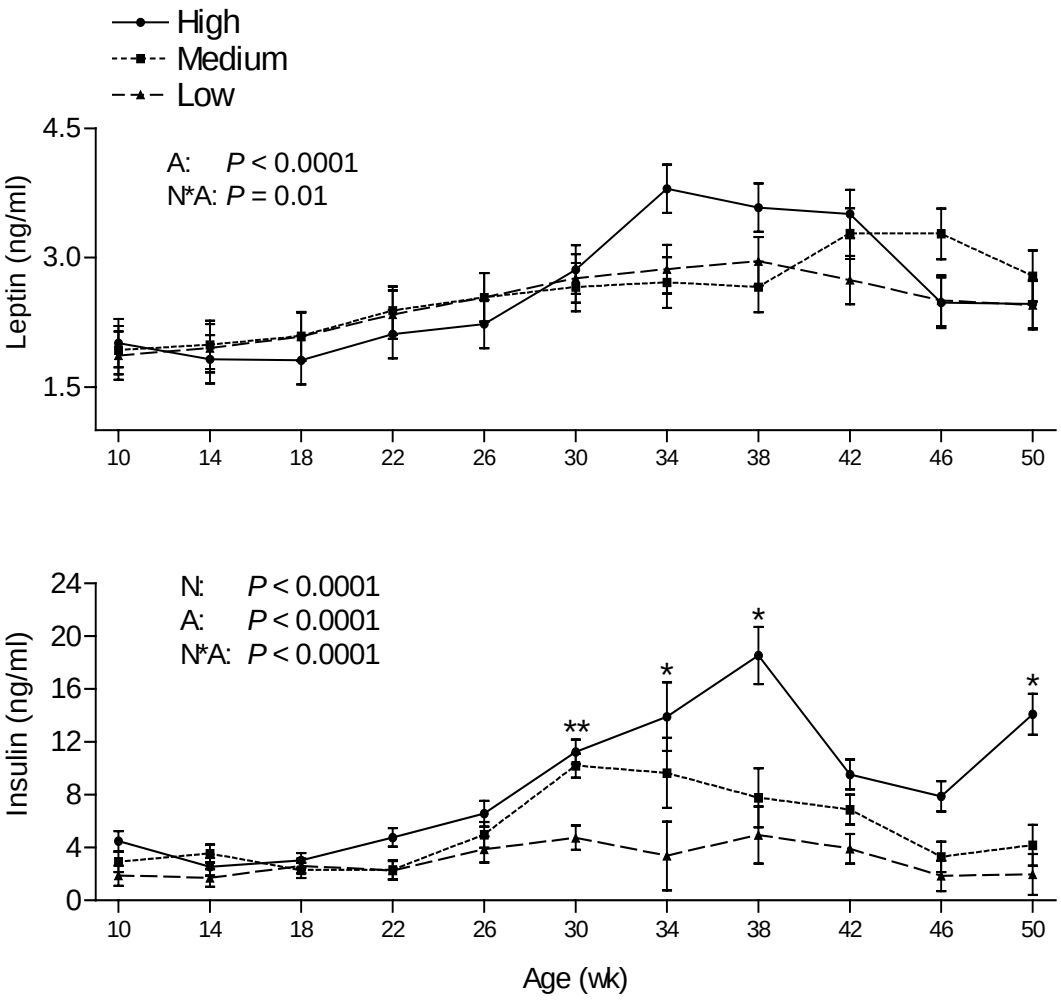


Figure 4.4. Mean (± SEM) serum leptin and insulin concentrations in bulls receiving low (n = 8), medium (n = 7), and high (n = 8) nutrition from 10 to 70 wk of age. N: nutrition effect; A: age effect; N*A: nutrition-by-age interaction effect. Superscripts indicate differences ($P < 0.05$) among groups within age (*low differs from high; **low differs from medium and high).

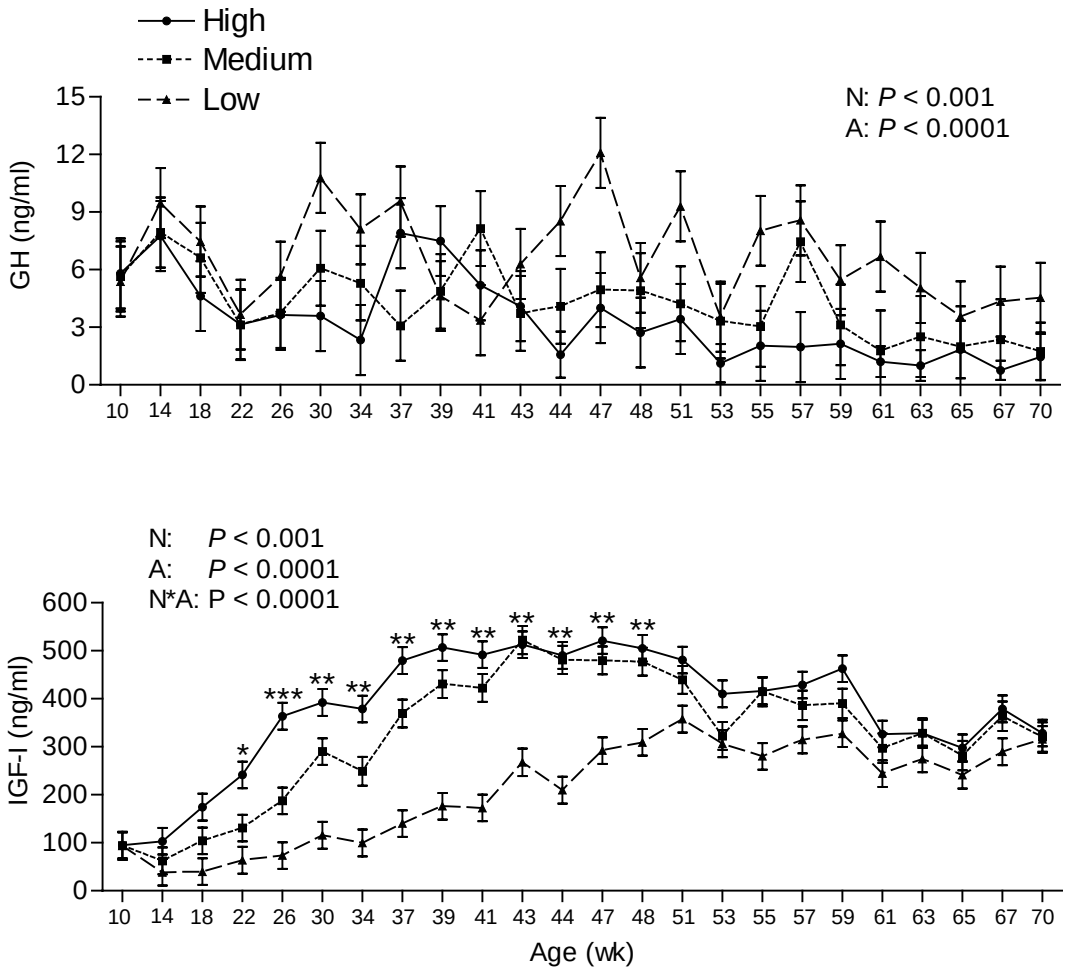
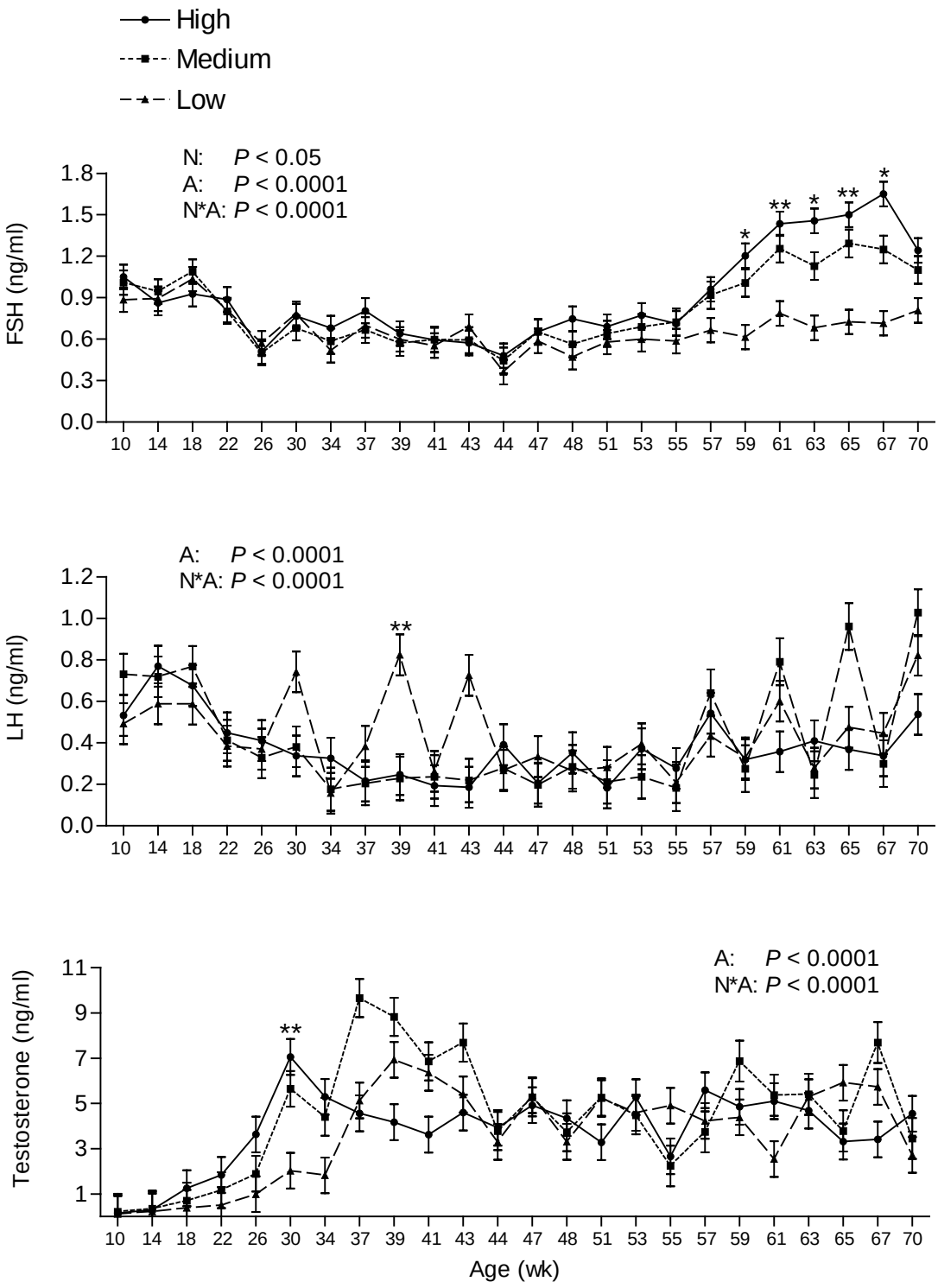


Figure 4.5. Mean (± SEM) serum growth hormone (GH) and insulin-like growth factor-I (IGF-I) concentrations in bulls receiving low (n = 8), medium (n = 7), and high (n = 8) nutrition from 10 to 70 wk of age. N: nutrition effect; A: age effect; N*A: nutrition-by-age interaction effect. Superscripts indicate differences ($P < 0.05$) among groups within age (*low differs from high; **low differs from medium and high; ***all groups differ).

Figure 4.6. Mean (± SEM) serum FSH, LH, and testosterone concentrations in bulls receiving low (n = 8), medium (n = 7), and high (n = 8) nutrition from 10 to 70 wk of age. N: nutrition effect; A: age effect; N*A: nutrition-by-age interaction effect. Superscripts indicate differences ($P < 0.05$) among groups within age (*low differs from high; **low differs from medium and high).


High
Medium
Low
N: P < 0.05
A: P < 0.0001
N*A: P < 0.0001
FSH (ng/ml)
LH (ng/ml)
Testosterone (ng/ml)
Age (wk)

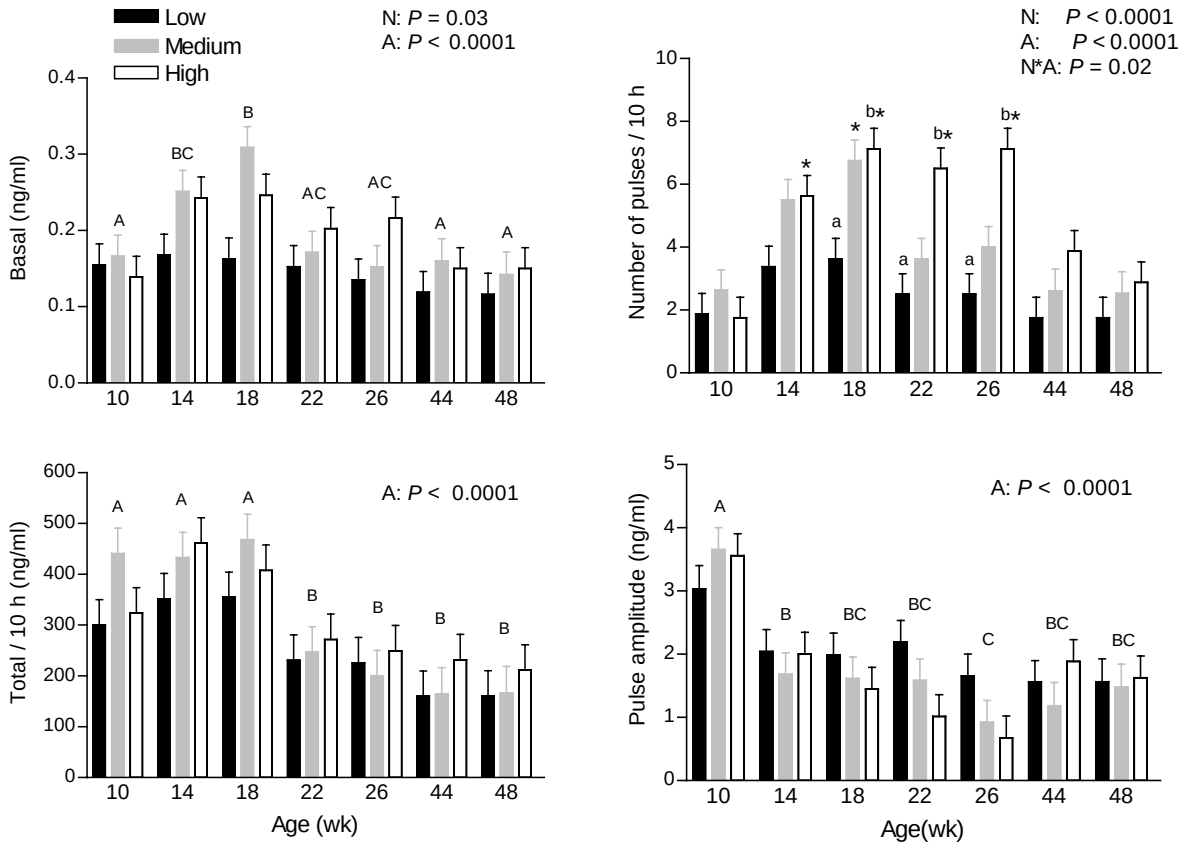


Figure 4.7. Mean (± SEM) serum LH basal concentration, total secretion, number of pulses, and pulse amplitude during 10 h intensive samplings (every 15 min) in bulls receiving low (n = 8), medium (n = 7), and high (n = 8) nutrition from 10 to 70 wk of age. N: nutrition effect; A: age effect; N*A: nutrition-by-age interaction effect. ^{A,B,C}Superscripts indicate overall time differences ($P < 0.05$). ^{a,b}Superscripts indicate group differences ($P < 0.05$) within age. *Superscripts indicate age differences ($P < 0.05$) within group.

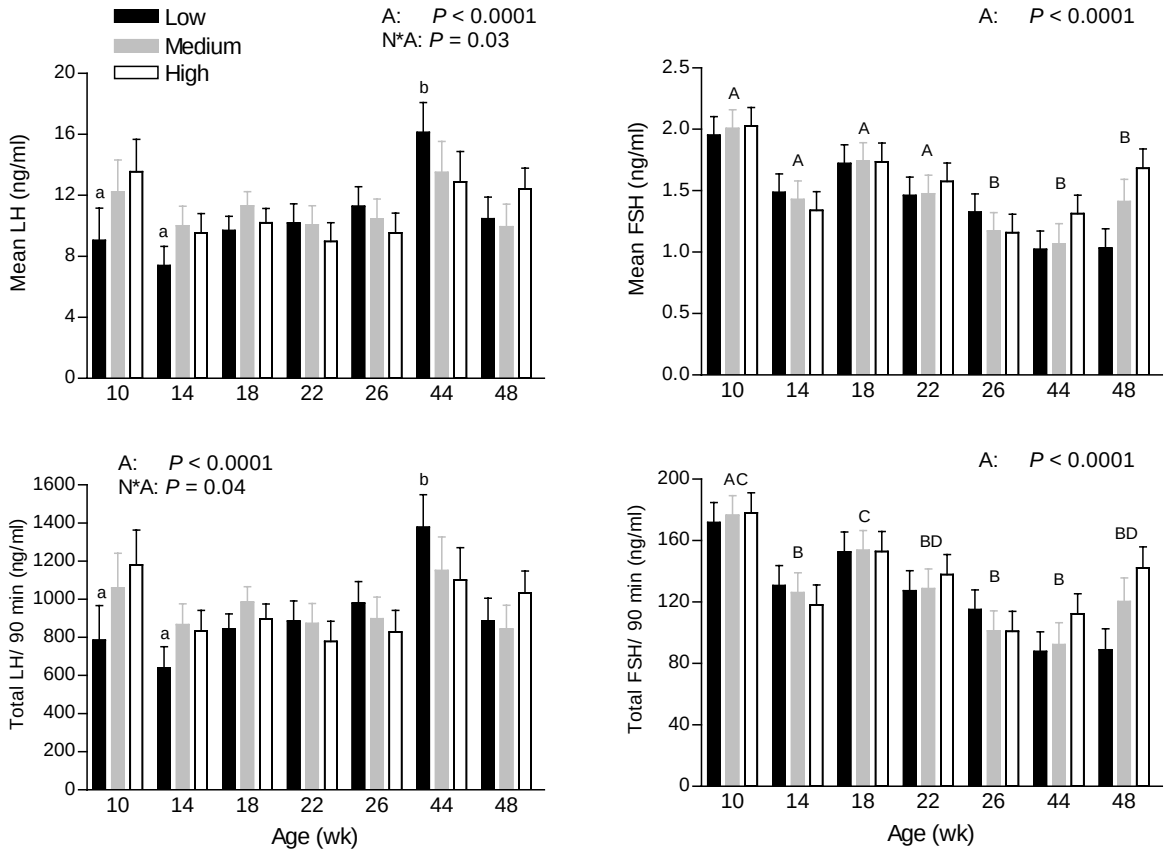


Figure 4.8. Mean (± SEM) serum LH and FSH mean concentrations and total secretion after a GnRH challenge (0.04 μg/kg, IV) in bulls receiving low (n = 8), medium (n = 7), and high (n = 8) nutrition from 10 to 70 wk of age. Serum samples were collected every 15 min for 90 min after GnRH treatment. A: age effect; N*A: nutrition-by-age interaction effect. Superscripts indicate age differences ($P < 0.05$) within group (^{a,b}for low nutrition group; ^{x,y}for high nutrition group). ^{A,B,C,D}Superscripts indicate overall differences ($P < 0.05$) among ages.

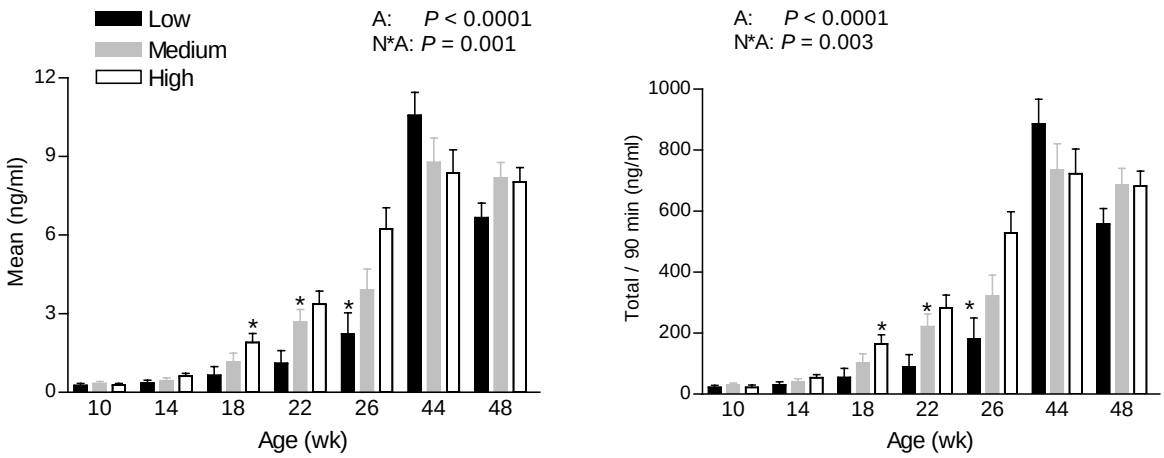


Figure 4.9. Mean (± SEM) serum testosterone mean concentration and total secretion after a GnRH challenge (0.04 μg/kg, IV) in bulls receiving low (n = 8), medium (n = 7), and high (n = 8) nutrition from 10 to 70 wk of age. Serum samples were collected every 15 min for 90 min after GnRH treatment. N: nutrition effect; A: age effect; N*A: nutrition-by-age interaction effect. *Superscripts indicate first difference ($P < 0.05$) with age within group.

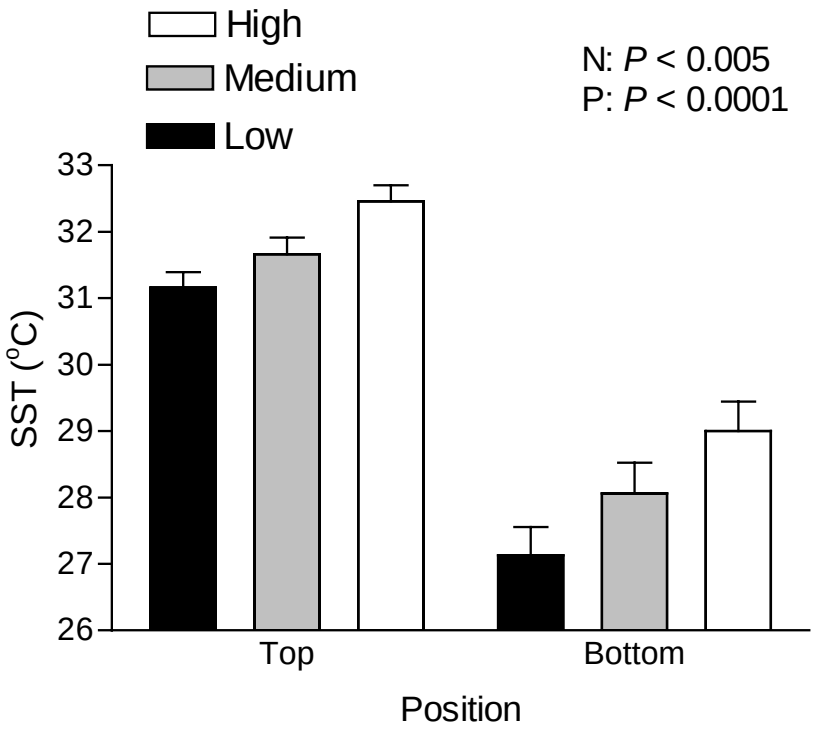


Figure 4.10. Mean (± SEM) top and bottom scrotal surface temperature (SST) at 70 wk of age in bulls receiving low (n = 13), medium (n = 12), and high (n = 12) nutrition from 10 to 70 wk of age. N: nutrition effect; P: position effect.

Table 4.1.
Mean (± SEM) age at puberty and maturity, and testicular characteristics, daily sperm production (DSP), epididymal sperm reserves (ESR), and proportion of normal sperm at 70 wk of age in bulls receiving low, medium, and high nutrition from 10 to 70 wk of age.

	Low (n = 13)	Medium (n = 12)	High (n = 12)
Age at puberty (d)	326.9 ± 5.5^{a}	304.7 ± 7.4^{b}	292.3 ± 4.6^{b}
Age at maturity (d)	390.5 ± 7.9	384.3 ± 6.8	397.3 ± 11.4
Paired testes weight (g)	523.9 ± 25.8^{a}	552.4 ± 21.1^{a}	655.2 ± 21.2^{b}
Seminiferous tubules (%)	81.5 ± 0.7	79.7 ± 1.0	78.5 ± 1.2
Seminiferous tubules diameter (μm)	251.1 ± 2.9	253.6 ± 6.7	254.2 ± 3.6
Seminiferous tubules area (x 10^3 μm^2)	506.9 ± 1.1	517.3 ± 2.8	515.2 ± 1.6
Seminiferous epithelium area (x 10^3 μm^2)	366.0 ± 0.8	378.3 ± 1.8	373.0 ± 1.5
DSP/g (x 10^6 sperm/g)	11.4 ± 0.8	12.0 ± 1.0	11.3 ± 1.2
Total DSP (x 10^9 sperm)	5.9 ± 0.4	6.6 ± 0.6	7.6 ± 0.9
ESR (x 10^9 sperm)	9.8 ± 0.8^{a}	10.7 ± 1.0^{a}	13.9 ± 1.3^{b}
Normal sperm (%)	76.3 ± 6.0	77.5 ± 5.5	72.4 ± 6.0

Columns with different superscripts differ ($P < 0.05$).

Table 4.2.
Pearson's correlation coefficients between body weight (BW), backfat (BF), scrotal circumference (SC), paired testes volume (PTV), and serum concentrations of growth hormone (GH), insulin-like growth factor-I (IGF-I), leptin, insulin, gonadotropins and testosterone in Angus and Angus x Charolais bulls (n = 23) from 10 to 70 wk of age.

	BW	BF	SC	PTV	GH	IGF-I	Leptin	Insulin	LH	FSH
BF	0.78									
SC	0.95	0.81								
PTV	0.92	0.75	0.93							
GH	-0.24	-0.29	-0.25	-0.23						
IGF-I	0.83	0.77	0.85	0.82	-0.28					
Leptin	0.39	0.36	0.30	0.27	-	0.26				
Insulin	0.46	0.55	0.45	0.43	-0.20	0.58	0.24			
LH	-0.49	-0.44	-0.49	-0.43	0.17	-0.37	-0.20	-0.23		
FSH	-0.47	-0.40	-0.50	-0.38	-	-0.32	-0.21	-0.13	0.56	
Testosterone	0.61	0.54	0.62	0.49	-0.16	0.61	0.26	0.36	-0.32	-0.21

All described correlations are significant ($P < 0.05$).

Table 4.3.
Stepwise regression models for body weight, backfat, scrotal circumference, and paired testes volume in Angus and Angus x Charolais bulls (n = 23) from 10 to 70 wk of age using serum concentrations of insulin-like growth factor-I (IGF-I), leptin, insulin, gonadotropins, and testosterone as independent variables.

	Slope	R^2	Probability
Body weight (ΣR^2 = 0.78; y-intercept = 157.1)			
IGF-I	0.43	0.69	< 0.0001
FSH	-54.7	0.05	< 0.005
Leptin	19.5	0.02	< 0.0001
LH	-52.4	0.01	< 0.001
Testosterone	4.4	0.01	< 0.005
Backfat (ΣR^2 = 0.66; y-intercept = 0.55)			
IGF-I	0.007	0.59	< 0.0001
Leptin	0.3	0.03	< 0.005
LH	-0.7	0.02	< 0.05
Insulin	0.06	0.01	< 0.001
FSH	-0.9	0.01	< 0.05
Scrotal circumference (ΣR^2 = 0.79; y-intercept = 19.4)			
IGF-I	0.02	0.72	< 0.0001
FSH	-3.9	0.05	< 0.0001
Testosterone	0.23	0.01	< 0.001
LH	-2.2	0.01	< 0.01
Paired testes volume (ΣR^2 = 0.69; y-intercept = 27.1)			
IGF-I	0.94	0.67	< 0.0001
Insulin	-108.9	0.02	< 0.001

ΣR^2: total model R^2.

4.2. Discussion

Different nutrition during calfhood and the peripubertal period affected body and sexual development in bulls. Bulls receiving low nutrition were lighter, and had smaller testes and delayed puberty when compared with bulls receiving medium or high nutrition. Moreover, bulls receiving high nutrition were slightly heavier and had significantly larger testes at 16 mo of age than bulls receiving medium nutrition. In previous reports in bulls, testicular ultrasonogram echodensity increased from 20-30 to 40-46 wk of age; these changes in testes pixel-intensity were probably related to testicular cellular proliferation, changes in cellular density, fluid secretion, and release of sperm into the seminiferous tubules (Aravindakshan et al., 2000; Chandolia et al., 1997c; Evans et al., 1996). These observations were consistent with the results of the present experiment, in which testicular echodensity increased from 30 to 46 wk of age. Some studies also reported that testicular echodensity in early-maturing bulls was greater than in late-maturing bulls before puberty (Aravindakshan et al., 2000; Brito et al., 2004). In the present study however, the initial increase in testicular echodensity in bulls in the low nutrition group, which were older at puberty, seemed delayed when compared to bulls in the medium and high nutrition groups, but there were no significant differences among groups were observed within age.

Testicular vascular cone diameter did not increase further after 62 wk of age, consistent with a study that revealed no differences in vascular cone diameter in mature bulls (Brito et al., 2002). The overall greater vascular cone diameter in bulls in the low nutrition group when compared to bulls in the high nutrition group seemed to be a result

of delayed growth of the vascular cone in the former group, since by 46 wk of age all groups showed similar diameter and there was no difference among groups within age. Testicular vascular cone fat thickness increased until 46, 62, and 70 wk of age in bulls in the low, medium, and high nutrition groups, indicating that higher nutrition favors faster deposition of fat around the vascular cone. Again, the overall greater vascular cone fat thickness in bulls in the low nutrition group when compared to bulls in the medium and high nutrition groups seemed to be a result of delayed fat deposition in the former group, since by 70 wk of age all groups had a similar diameter and there was no difference among groups within age. Previous studies have reported increased testicular vascular cone fat thickness with age in older bulls (Brito et al., 2002; Cook et al., 1994) and perhaps fat thickness would have increased if bulls in the present study had been monitored for a longer interval.

Bulls in the high nutrition group had greater scrotal surface temperature than bulls in the low nutrition group, but sperm production and semen quality were not adversely affected. This was in disagreement with previous reports that failed to show differences in scrotal temperature between bulls receiving high and medium nutrition diets and showed impaired sperm production and semen quality in bulls receiving high nutrition (Coulter et al., 1987; Coulter et al., 1997; Coulter and Kozub, 1984); differences in diets and period of feeding probably accounted for the discrepancies with the present study. Perhaps deleterious effects of increased scrotal temperature in bulls receiving high nutrition would be manifested at older ages. Despite that testicular vascular cone fat thickness had been associated with scrotal temperature (Cook et al., 1994), differences in scrotal temperature in the present study were not related to differences in vascular cone

diameter and fat thickness. Consistent with previous reports, top scrotal surface temperature was warmer than at the bottom (Cook et al., 1994; Kastelic et al., 1995).

The present study is the first to document the temporal relationships among metabolic hormones, gonadotropins, and testosterone during the entire period of sexual development in bulls. In agreement with previous reports (Aravindakshan et al., 2000; Evans et al., 1995; Moura and Erickson, 1997), the early gonadotropin rise in bulls was characterized by an increase in LH pulse frequency, mean and basal concentrations, and total secretion from 10 to 22-26 wk of age. Mean FSH concentrations were also increased during this period. Insulin-like growth factor-I concentrations increased during the early gonadotropin rise, suggesting a role for this hormone in regulating the early gonadotropin rise in bulls. Leptin and insulin concentrations only increased after 30 wk of age and therefore were not involved in regulating GnRH secretion during the early gonadotropin rise. Similarly, in intact and castrated male monkeys, the gonadotropin rise occurred without any significant change in circulating leptin concentrations (Plant and Durrant, 1997).

Metabolic hormones changes after the period of the early gonadotropin rise were similar to those previously reported in bulls (McAndrews et al., 1993; Renaville et al., 1993), but differed somewhat from humans and rodents, especially the somatotropic axis. Both GH and IGF-I concentrations increased during the peripubertal period in humans and rodents (Mauras et al., 1996; Wilson, 2001), but IGF-I concentrations increased with decreasing GH concentrations in bulls. Therefore, either drastic changes in liver sensitivity to GH, or other sources (perhaps the testes) contributed substantially

to circulating IGF-I concentrations during the peripubertal period in bulls. Leptin decreased in vitro testicular steroidogenesis in rats, but this suppression diminished with age (Tena-Sempere et al., 2001a; Tena-Sempere et al., 1999). Circulating leptin and testosterone concentrations increased concomitantly in developing bulls, indicating that leptin either does not adversely affects steroidogenesis or the effect is also age-dependent in bulls.

Nutrition affected LH secretion, with consequent effects on sexual development. Gonadotropin secretion after GnRH challenge was not affected by nutrition, indicating that the effects of nutrition were not mediated through the pituitary. Therefore, during the period of the early gonadotropin rise in bulls, nutrition regulated the hypothalamus-pituitary-testes axis by modulating the GnRH pulse generator in the hypothalamus. The primary effect of nutrition on the hypothalamus has also been demonstrated in rams receiving low nutrition; frequent GnRH treatments prevented the decrease in circulating concentrations of gonadotropins (Hotzel et al., 1995). Signaling of nutritional status to the brain involves metabolic hormones, as well as other gut hormones, like ghrelin, neuromedin U, and amylin. Orexins, neuropeptide Y (NPY), and other neurotransmitters are possibly involved with signaling nutritional status within the brain (Blache et al., 2002; Blache et al., 2003). The neural apparatus that gauges metabolic rate has been called the “metabolic sensor”. The metabolic sensor translates signals provided by circulating (peripheral) concentrations of certain hormones into neuronal signs that ultimately regulate the GnRH pulse generator. The hormonal links between metabolism and gonadotropin secretion are not clear, but probably involve a number of interactions

among several hormones and neuropeptides that are also involved in controlling appetite and metabolism (Blache et al., 2002; Blache et al., 2003).

Analysis of temporal hormone patterns and their association with parameters of sexual development suggested that circulating IGF-I may have direct effects on the hypothalamus-pituitary-testes axis. Insulin-like growth factor-I may act on the hypothalamus, since the differences in IGF-I concentrations among groups during the early gonadotropin rise coincided with differences in LH pulse secretion. Receptors for IGF-I have been identified in immortalized GnRH-secreting cells (GT-17 cells), and in the median eminence, preoptic area-anterior hypothalamus, and mediobasal hypothalamus in rodents (Bohannon et al., 1988; Daftary and Gore, 2004; Miller and Gore, 2001; Olson et al., 1995). GnRH-secreting neurons express IGF-I receptors and expression changes with age, indicating a possible direct role for IGF-I on GnRH release by the hypothalamus (Daftary and Gore, 2004). GnRH neurons also express mRNA for IGF-I, IGF-II, and IGFBPs in an age-dependent manner, indicating the existence of an autocrine regulatory mechanism of neuronal GnRH secretion involving the IGF system (Anderson et al., 1999; Miller and Gore, 2001).

However, the regulatory role of circulating IGF-I on GnRH secretion has been questioned in rams, since IGF-I concentrations did not increase in the cerebrospinal fluid after improved nutrition, despite increased plasma concentrations. Furthermore, IGF-I infusion into the third ventricle did not affect GnRH secretion (Blache et al., 2000; Martin and Walkden-Brown, 1995; Miller et al., 1998). Temporal changes in cerebrospinal fluid and plasma IGF-I concentrations have not been reported in bulls and

the possibility of differences among species should be considered. Another possibility is that circulating IGF-I concentrations may affect circumventricular parts of the brain, rather than other parts of the brain that are likely on the brain side of the blood-brain barrier and that are therefore less influenced by circulating IGF-I concentrations (Bohannon et al., 1988).

Developmental and nutritional differences in LH pulse frequency were not related to differences in leptin concentrations. Other studies have demonstrated that leptin does not stimulate in vitro GnRH secretion from hypothalamic explants or gonadotropin secretion from adenohypophyseal cells collected from bulls and steers maintained at an adequate level of nutrition (Amstalden et al., 2005). Moreover, treatment with exogenous leptin did not affect LH pulse frequency in adequately fed peripubertal rams (Morrison et al., 2002) and heifers (Maciel et al., 2004a). Differences in LH pulse frequency were not related to differences in insulin concentrations either. In contrast, plasma and cerebrospinal fluid insulin concentrations in rams increased concomitantly with increased LH pulse frequency after increases in nutrition plane and intra-cerebral infusion of insulin increased LH pulse frequency (Blache et al., 2002; Blache et al., 2000; Martin and Walkden-Brown, 1995). The large differences in LH pulse frequency without differences in leptin or insulin concentrations among groups in the present study supported the assertion that the role of these metabolic hormones on GnRH secretion is permissive. That LH pulse frequency did not increase with age in the low nutrition group, despite no differences in circulating leptin and insulin concentrations among groups, indicated that GnRH pulse secretion was regulated by other systems, at least when minimal concentrations of leptin and insulin were present.

After the early gonadotropin rise, testicular growth may continue to be regulated by gonadotropins, but GnRH-independent mechanisms may also be present (Blache et al., 2002; Blache et al., 2000). GnRH-independent testicular growth during the peripubertal period may be regulated by leptin, IGF-I, and insulin in bulls. In the present study, bulls in the medium and high nutrition groups had similar circulating concentrations of metabolic hormones, gonadotropins, and testosterone after 30 wk of age, but paired testes weight at 70 wk of age was greater in the high nutrition group. This difference in testes size in mature bulls was apparently caused by a more prolonged period of increased testicular growth in the high nutrition group. Perhaps the putative effects of metabolic hormones and gonadotropins on testicular growth after the early gonadotropin rise may be largely dependent on the previous LH secretion pattern during the gonadotropin rise. The only endocrine difference between bulls in the medium and high nutrition groups was the more sustained increase in LH pulse frequency during the early gonadotropin rise. Therefore, the LH secretion pattern may "prime" testicular development and may dictate maximum adult testicular size. Testicular development in the low nutrition group was delayed in relation to the medium nutrition group, but testicular size was similar at 70 wk of age. Therefore, differences between the low and medium nutrition groups in LH pulse frequency and metabolic hormones concentrations may have affected testicular growth rate, but did not affect maximum testicular size, as in the high nutrition group.

Circulating IGF-I may have direct effects on Leydig cell multiplication, differentiation, and function, since reduced circulating IGF-I in the low nutrition group was associated with a delay in the rise of circulating testosterone concentrations during

the peripubertal period. IGF-I receptors have been identified in Leydig cells in several species and IGF-I increases the proliferation of mesenchymal precursors and the differentiation of these cells into Leydig cells (Lin, 1995; Spiteri-Grech and Nieschlag, 1992). Leydig cell numbers and expression of Leydig cell-specific steroidogenic enzymes is decreased in IGF-I-null mice (Wang et al., 2003) and additive effects of LH and IGF-I treatments on testicular cell numbers were observed, indicating that the two hormones use separate signaling pathways (Wang and Hardy, 2004). Adding IGF-I to Leydig cells in vitro increased hCG binding without changes in receptor affinity, indicating that IGF-I regulates Leydig cell steroidogenesis by regulating the number of LH receptors (Lin, 1995; Spiteri-Grech and Nieschlag, 1992). Leydig cells have also been demonstrated to produce IGF-I, indicating the existence of a paracrine/autocrine mechanism of testicular regulation involving the IGF-I system (Rouiller-Fabre et al., 1998; Spiteri-Grech and Nieschlag, 1992). Interestingly, IGF-I up-regulates LH receptors and testosterone secretion, whereas testosterone in turn up-regulates IGF-I receptors and IGF-I production by Leydig cells (Cailleau et al., 1990). Therefore, circulating IGF-I may initially up-regulate testosterone production, which in turn stimulates IGF-I secretion and the establishment of a positive feedback loop between IGF-I and testosterone secretion in Leydig cells in peripubertal bulls. In support of this view, it has been demonstrated that IGF-I treatment increased gonadal sensitivity to gonadotropins and hastened puberty in female monkeys (Hiney et al., 1996; Wilson, 1998).

The strong positive associations of circulating IGF-I with testes size suggested that this growth factor might have direct mitogenic effects on the testes. Substantial testicular development occurs within the first few months after birth in bulls, coincident with increased secretion of LH and FSH during the early-gonadotropin rise (Aravindakshan et al., 2000; Evans et al., 1995; Moura and Erickson, 1997). However, testicular growth in beef bulls is accelerated from 6 to 16 months of age and scrotal circumference increases approximately 1.8 cm per month during this period (Barth and Ominski, 2000; Coulter, 1986). Differences in circulating IGF-I concentrations accounted for a significant proportion of the variation observed in testicular size in the present study. Therefore, this hormone may regulate not only testicular steroidogenesis, but also cell proliferation in peripubertal bulls. The positive effects of IGF-I on testicular cell proliferation may be direct or involve up-regulation of its own receptors, own local secretion, or production of other local growth factors (Soder et al., 1992).

There were moderate correlations between leptin, insulin, GH, and IGF-I concentrations and age at puberty. Insulin and IGF-I had negative correlations, indicating that greater circulating concentrations were associated with earlier puberty. Surprisingly, leptin had positive correlations with age at puberty, indicating that greater leptin concentrations were associated with delayed puberty in bulls; GH also had positive correlations with age at puberty. However, in the final regression model, backfat accounted for most of the variation in age at puberty. Scrotal circumference and paired testes volume had moderate positive correlations similar to that of metabolic hormones, but were not included in the model for age at puberty. Insulin and IGF-I also had positive correlations with epididymal sperm reserve and paired testes weight,

respectively. Circulating IGF-I concentrations also had strong associations with body weight and backfat, reinforcing the important role of IGF-I in body development. Surprisingly, IGF-I concentrations accounted for a greater proportion of the variation in backfat than leptin concentrations. Body weight, testes size, testosterone, insulin, and IGF-I were also positively correlated in rams (Adam and Findlay, 1997). Circulating leptin concentrations have been associated with body fat in sheep (Adam et al., 2003), but correlations of backfat and leptin concentrations were low in the present study.

In conclusion, nutrition regulated the hypothalamus-pituitary-testes axis through effects on the GnRH pulse generator in the hypothalamus and effects on the testes; pituitary function was not affected. Nutrition modulated LH pulse frequency and basal concentration during the period of the early gonadotropin rise. Close temporal associations suggested that circulating IGF-I may be involved in regulating the timing and the duration of the early gonadotropin rise in bulls. A delayed peripubertal increase in testosterone concentration was associated with low circulating IGF-I concentrations, suggesting a role for IGF-I in regulating Leydig cell function. Moreover, IGF-I concentrations accounted for a high proportion of the variation in testes size, indicating that IGF-I may be a potent testicular mitogen. Based on differences in testicular growth without differences in blood concentrations of metabolic hormones, GnRH-independent testicular growth may be dependent on previous exposure to gonadotropins.

5. EXPERIMENT III - EFFECT OF FEED RESTRICTION DURING CALFHOOD ON SERUM METABOLIC HORMONES, GONADOTROPINS AND TESTOSTERONE CONCENTRATIONS, AND ON SEXUAL DEVELOPMENT IN BULLS

5.1. Results

There were age effects ($P < 0.0001$) during Period 1, and nutrition, age, and nutrition-by-age interaction effects ($P \leq 0.07$) during Period 2 on body weight and backfat (Figure 5.1). Body weight increased ($P < 0.05$) continuously during Periods 1 and 2 in all groups. Overall, bulls in the low/medium nutrition group were lighter ($P < 0.05$) than bulls in the medium/medium nutrition group during Period 2, but there were no differences among groups within age. Backfat increased at 22 and 26 wk of age during Period 1 in all groups. During period 2, backfat increased ($P < 0.05$) from 58 to 70 wk in the low/medium nutrition group, from 54 to 62 wk in the low/high nutrition group, and at 58 wk of age in the medium/medium nutrition group. Overall, bulls in the low/medium nutrition group had thinner ($P < 0.05$) backfat than bulls in the low/high nutrition group during Period 2, but there were no differences among groups within age.

There were age effects ($P < 0.0001$) during Period 1, and nutrition and age effects ($P < 0.01$) during Period 2 on SC and paired testes volume (5.2). Scrotal circumference and paired testes volume increased ($P < 0.05$) continuously during Periods 1 and 2 in all groups. Overall, bulls in the low/medium nutrition group had smaller ($P < 0.01$) SC and paired testes volume than bulls in the medium/medium nutrition group during Period 2. There were age effects ($P < 0.0001$) during Periods 1 and 2, and nutrition-by-age interaction effects ($P < 0.05$) during Period 2 on testes pixel-intensity. Overall, testes pixel-intensity decreased ($P < 0.05$), i.e. ultrasonograms became more echodense, after 22 wk of age during Period 1. During Period 2, testes pixel intensity decreased ($P < 0.05$) at 34 wk in the low/high nutrition group and at 42 wk of age in the low/medium and medium/medium nutrition groups; there were no differences among groups within age.

There were age effects ($P < 0.0001$) on serum leptin and insulin concentrations, and nutrition, age, and nutrition-by-age interaction effects ($P < 0.01$) on IGF-I concentrations during Period 1 (Figure 5.3). Serum leptin and insulin concentrations increased ($P < 0.05$) after 18 and 26 wk of age, respectively. Serum IGF-I concentrations remained unchanged in bulls in the low nutrition group and IGF-I concentrations were lower ($P < 0.05$) in bulls in this group than in bulls in the medium nutrition group after 18 wk of age. There was also an age effect ($P < 0.01$) on serum GH concentrations during Period 1; concentrations decreased from approximately 10 ng/ml between 14 and 22 wk to approximately 7 ng/ml at 26 wk of age (data not shown).

There were age and nutrition by age effects ($P < 0.01$) on serum leptin, insulin, and IGF-I concentrations, and nutrition effects on insulin and IGF-I concentrations during Period 2 (Figure 5.3). Leptin concentrations increased ($P < 0.05$) from 54 to 58 wk of age in the low/high and medium/medium nutrition groups, but did not change significantly in the low/medium nutrition group. Insulin concentrations increased from 36 to 58 wk in the low/high nutrition group, and at 42 and 54 wk of age in the medium/medium and low/medium nutrition groups, respectively. Serum IGF-I concentrations increased ($P < 0.05$) at 34 wk in the medium/medium nutrition group. In the low/medium and low/high nutrition groups, IGF-I concentrations increased until 54 wk of age and decreased thereafter. Overall, bulls in the low/high nutrition group had greater ($P \leq 0.07$) insulin concentrations than bulls in the low/medium and medium/medium nutrition groups, and greater ($P < 0.05$) IGF-I concentrations than bulls in the low/medium nutrition group. Serum GH concentrations were not affected by nutrition or age after Period 1 (concentrations from 30 and 34 wk of age were approximately 8 ng/ml).

There were nutrition effects ($P \leq 0.07$) on LH pulse frequency, peak concentration, and total secretion, and age effects ($P < 0.005$) on LH pulse frequency and total secretion during Period 1 (Figure 5.4). Overall, LH pulse frequency, peak concentration, and total secretion were lower ($P \leq 0.07$) in bulls in the low nutrition group than in bulls in the medium nutrition group and LH pulse frequency and total secretion decreased ($P < 0.05$) after 22 wk of age. There was an age effect ($P < 0.01$) on LH total secretion during Period 2; total secretion increased from 30 to 34 wk of age (Figure 5.4). Neither

nutrition nor age affected LH basal concentrations or LH pulse amplitude during Periods 1 and 2 (data not shown).

There were nutrition effects ($P < 0.05$) on mean serum LH and testosterone concentrations and age effects ($P < 0.005$) on mean serum LH, FSH, and testosterone concentrations during Period 1 (Figure 5.5). Overall, LH and testosterone concentrations were lower ($P < 0.05$) in bulls in the low nutrition group than in bulls in the medium nutrition group. Gonadotropin concentrations decreased ($P < 0.05$) after 18 wk of age, whereas testosterone concentrations increased ($P < 0.05$) after this age. There were age effects ($P < 0.0005$) on serum LH, FSH, and testosterone concentrations, and a nutrition-by-age interaction effect ($P < 0.0001$) on LH concentrations during Period 2 (Figure 5.5). Serum LH concentrations increased ($P < 0.05$) at 50 and 66 wk of age only in bulls in the low/high nutrition group. Overall, FSH concentrations increased ($P < 0.05$) after 54 wk and testosterone concentrations increased ($P < 0.05$) after 38 wk of age.

There were nutrition and age effects ($P < 0.01$) on mean LH concentrations and total secretion, and age effects ($P < 0.0001$) on mean FSH concentrations and total secretion after GnRH treatment during Period 1 (Figures 5.6 and 5.7). GnRH-induced mean LH concentrations and total secretion were lower ($P < 0.01$) in bulls in the low nutrition group than in bulls in the medium nutrition group. Mean LH concentrations and total secretion increased at 18 wk of age, whereas mean FSH concentrations and total secretion decreased ($P < 0.05$) after 18 wk of age. Neither group nor age affected GnRH-induced LH and FSH secretion during Period 2.

There were nutrition and age effects ($P < 0.05$) on mean testosterone concentrations and total secretion after GnRH treatment during Period 1 (Figure 5.8). Overall, GnRH-induced mean testosterone concentrations and total secretion increased ($P < 0.05$) with age and was lower ($P < 0.05$) in bulls in the low nutrition group than in bulls in the medium nutrition group. There were age and nutrition-by-age interaction effects ($P < 0.005$) on mean testosterone concentrations and total secretion after GnRH treatment during Period 2 (Figure 5.8). GnRH-induced mean testosterone concentrations and total secretion increased ($P < 0.05$) from 30 to 34 wk of age only in bulls in the low/medium group.

There were nutrition and position effects ($P < 0.0005$) on scrotal surface temperature (Figure 5.9). Overall, bulls in the low/high nutrition group had lower ($P < 0.0005$) scrotal temperature than bulls in the high nutrition group and top scrotal temperature was greater ($P < 0.0001$) than bottom temperature.

Bulls in the low/medium and low/high nutrition group were older ($P < 0.05$) at puberty than bulls in the medium/medium nutrition group. Bulls in the medium/medium nutrition group had greater ($P < 0.05$) paired testes weight than bulls in the low/medium nutrition group; bulls in the low/high nutrition group were intermediate (Table 5.1). One bull in the low/high nutrition group constantly produced high proportions of sperm with knobbed acrosomes and was excluded from the analysis of age at maturity. One bull in the medium/medium nutrition group and one bull in the low/high nutrition group did not produce > 70% morphologically normal sperm and could not be classified as mature; age at maturity did not differ among groups. There were no effects of nutrition on

testicular vascular cone diameter and fat thickness, daily sperm production/g, total daily sperm production, epididymal sperm reserves, and proportion of normal sperm at 70 wk of age.

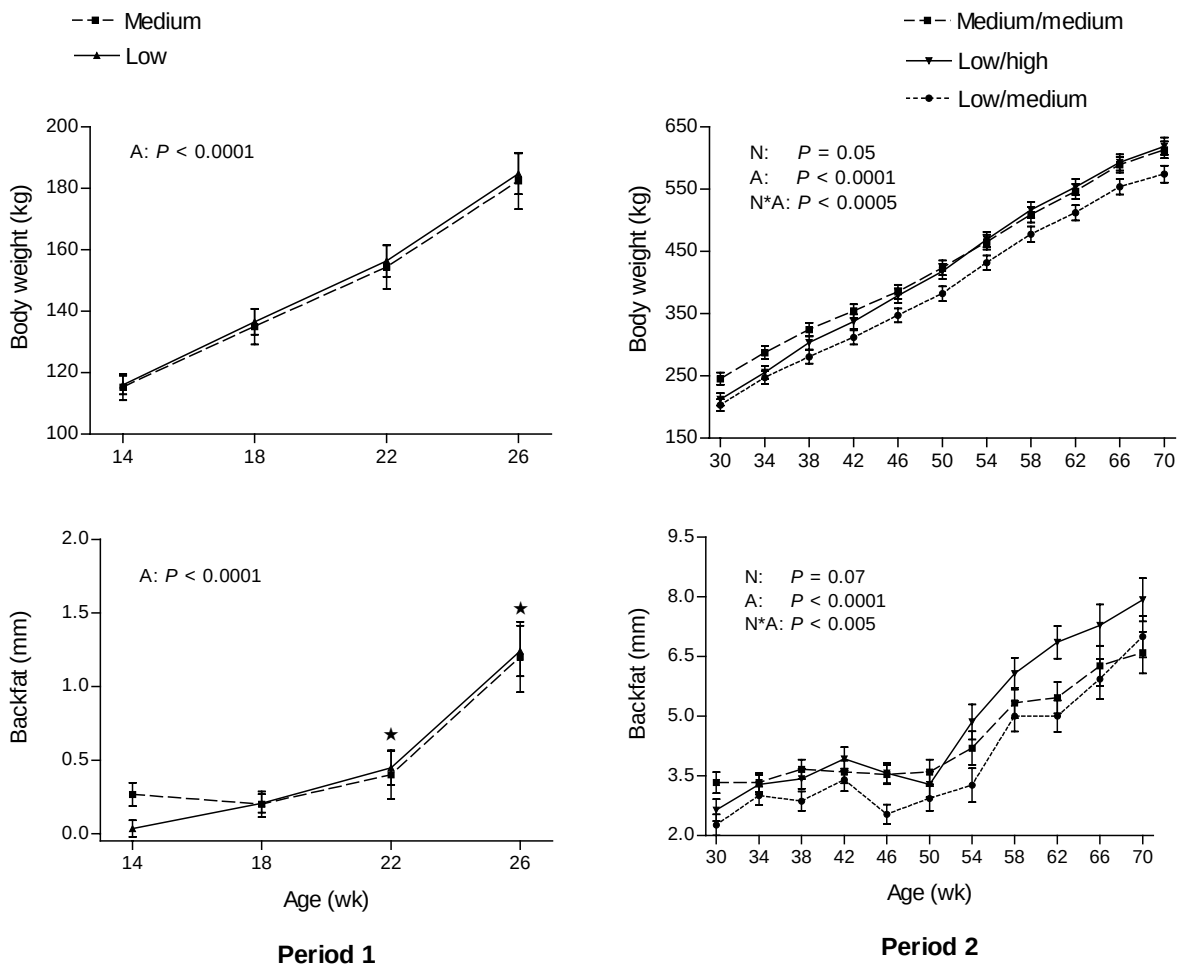


Figure 5.1. Mean (± SEM) body weight and backfat in bulls receiving low (n = 29) or medium nutrition (n = 15) from 10 to 26 wk of age (Period 1) and medium or high nutrition (medium/medium, n = 15; low/high, n = 14; and low/medium; n = 15) after 27 wk (Period 2). N: nutrition effect; A: age effect; N*A: nutrition-by-age interaction effect. ★Superscripts indicate overall changes ($P < 0.05$) with age. Overall, body weight increased ($P < 0.05$) continuously during Period 1.

Figure 5.2. Mean (± SEM) scrotal circumference, paired testes volume, and testes pixel-intensity in bulls receiving low (n = 29) or medium nutrition (n = 15) from 10 to 26 wk of age (Period 1) and medium or high nutrition (medium/medium, n = 15; low/high, n = 14; and low/medium; n = 15) after 27 wk (Period 2). N: nutrition effect; A: age effect; N*A: nutrition-by-age interaction effect. ★Superscripts indicate overall changes ($P <$ 0.05) with age. Overall, scrotal circumference and paired testes volume increased ($P <$ 0.05) continuously during Periods 1 and 2.

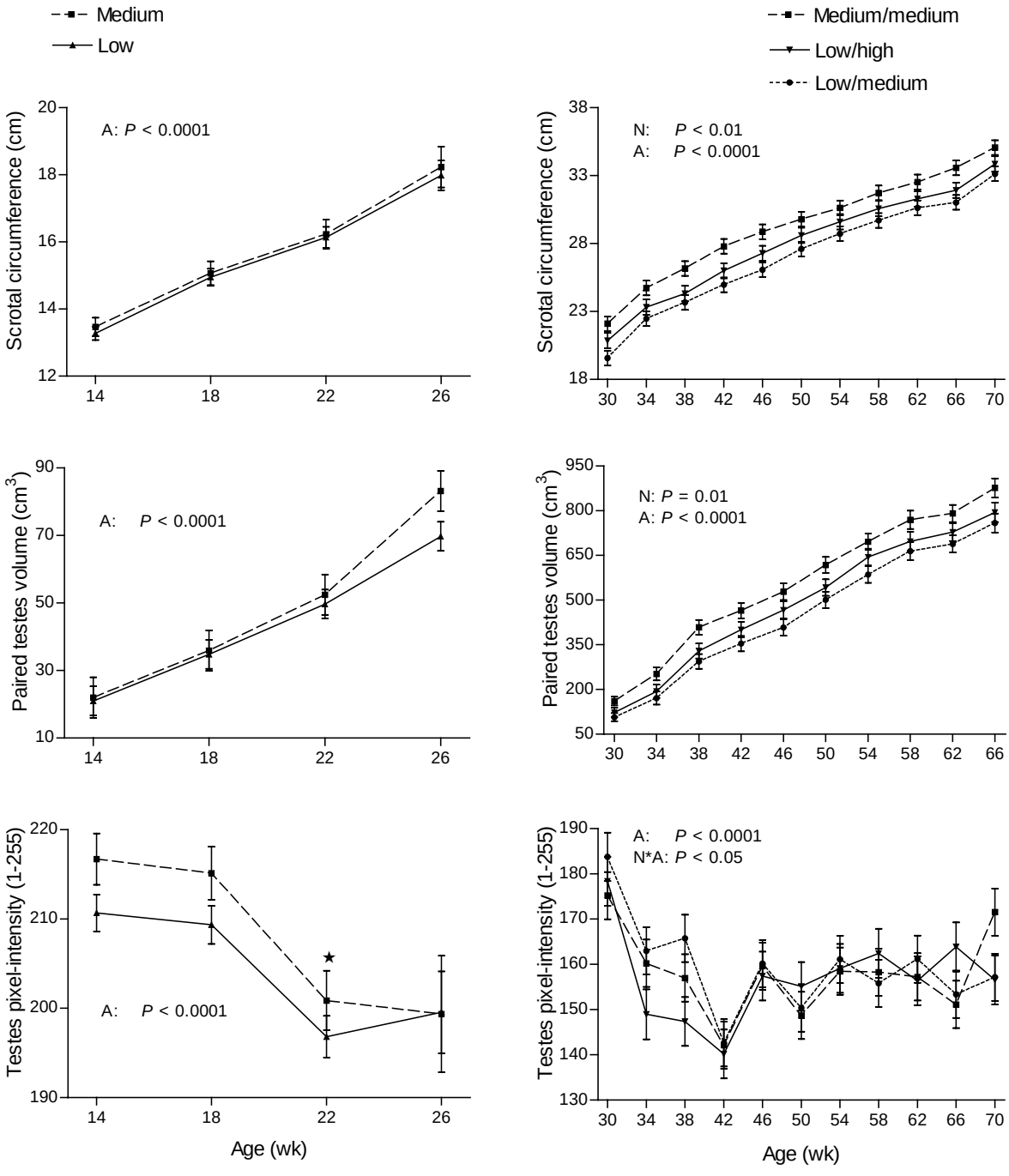

Medium
Low
Scrotal circumference (cm)
A: P < 0.0001
Paired testes volume (cm3)
A: P < 0.0001
Testes pixel-intensity (1-255)
A: P < 0.0001
Age (wk)
Period 1
Medium/medium
Low/high
Low/medium
N: P < 0.01
A: P < 0.0001
N: P = 0.01
A: P < 0.0001
A: P < 0.0001
N*A: P < 0.05
Age (wk)
Period 2

Figure 5.3. Mean (± SEM) serum leptin, insulin, and IGF-I concentrations in bulls receiving low (n = 16) or medium nutrition (n = 8) from 10 to 26 wk of age (Period 1) and medium or high nutrition (medium/medium, low/high, and low/medium; n = 8/group) after 27 wk (Period 2). N: nutrition effect; A: age effect; N*A: nutrition-by-age interaction effect. ★Superscripts indicate overall changes ($P < 0.05$) with age. [a,b]Superscripts indicate differences ($P < 0.05$) between groups within age.

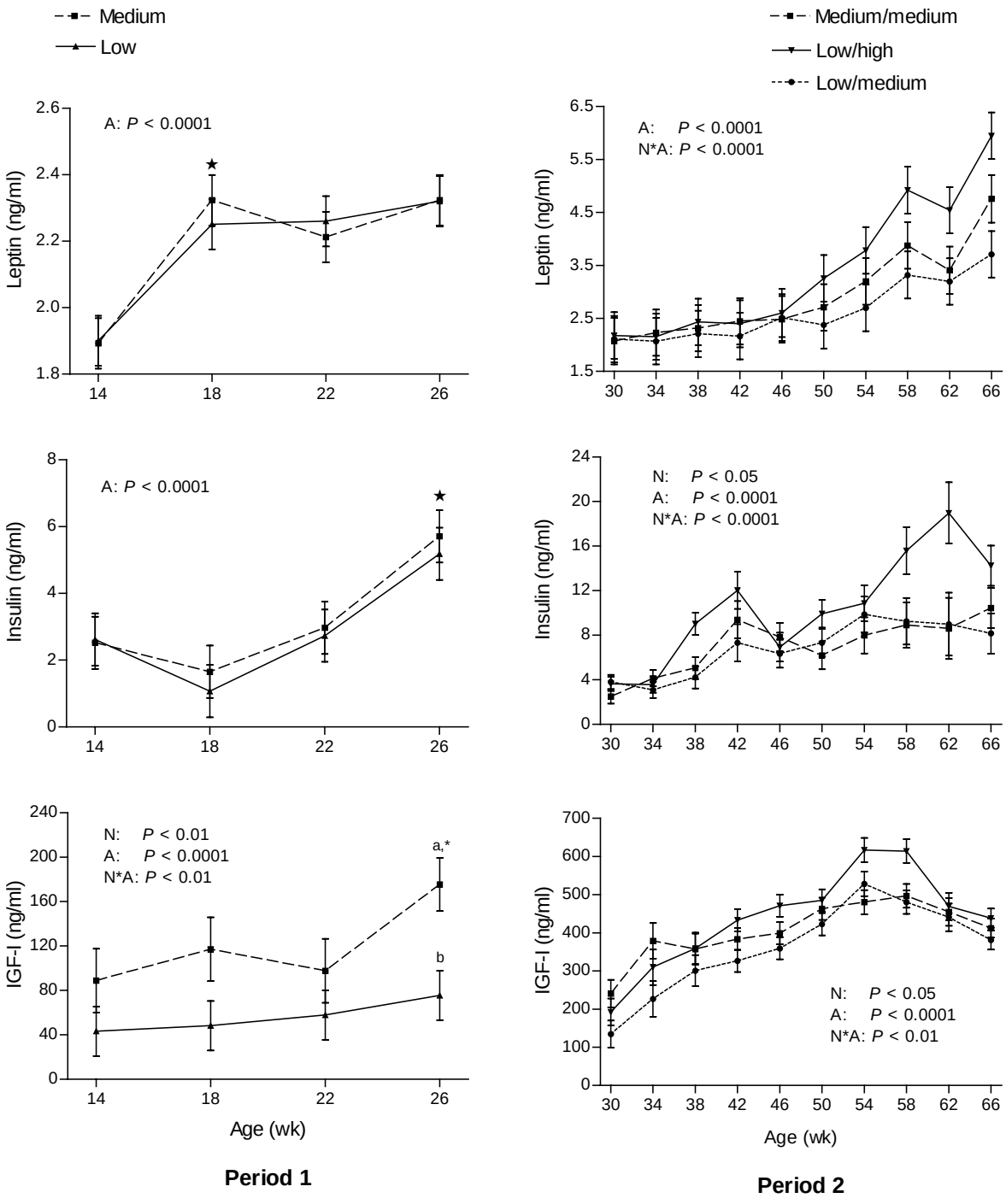

Medium
Low
Leptin (ng/ml)
A: P < 0.0001
Insulin (ng/ml)
A: P < 0.0001
IGF-I (ng/ml)
N: P < 0.01
A: P < 0.0001
N*A: P < 0.01
a,*
b
Age (wk)
Period 1
Medium/medium
Low/high
Low/medium
Leptin (ng/ml)
A: P < 0.0001
N*A: P < 0.0001
Insulin (ng/ml)
N: P < 0.05
A: P < 0.0001
N*A: P < 0.0001
IGF-I (ng/ml)
N: P < 0.05
A: P < 0.0001
N*A: P < 0.01
Age (wk)
Period 2

Figure 5.4. Mean (± SEM) number of LH pulses, peak concentration, and total secretion in 10 h in bulls receiving low (n = 16) or medium nutrition (n = 8) from 10 to 26 wk of age (Period 1) and receiving medium or high nutrition (medium/medium, low/high, and low/medium; n = 8/group) after 27 wk (Period 2). N: nutrition effect; A: age effect. ★Superscripts indicate overall changes ($P < 0.05$) with age.

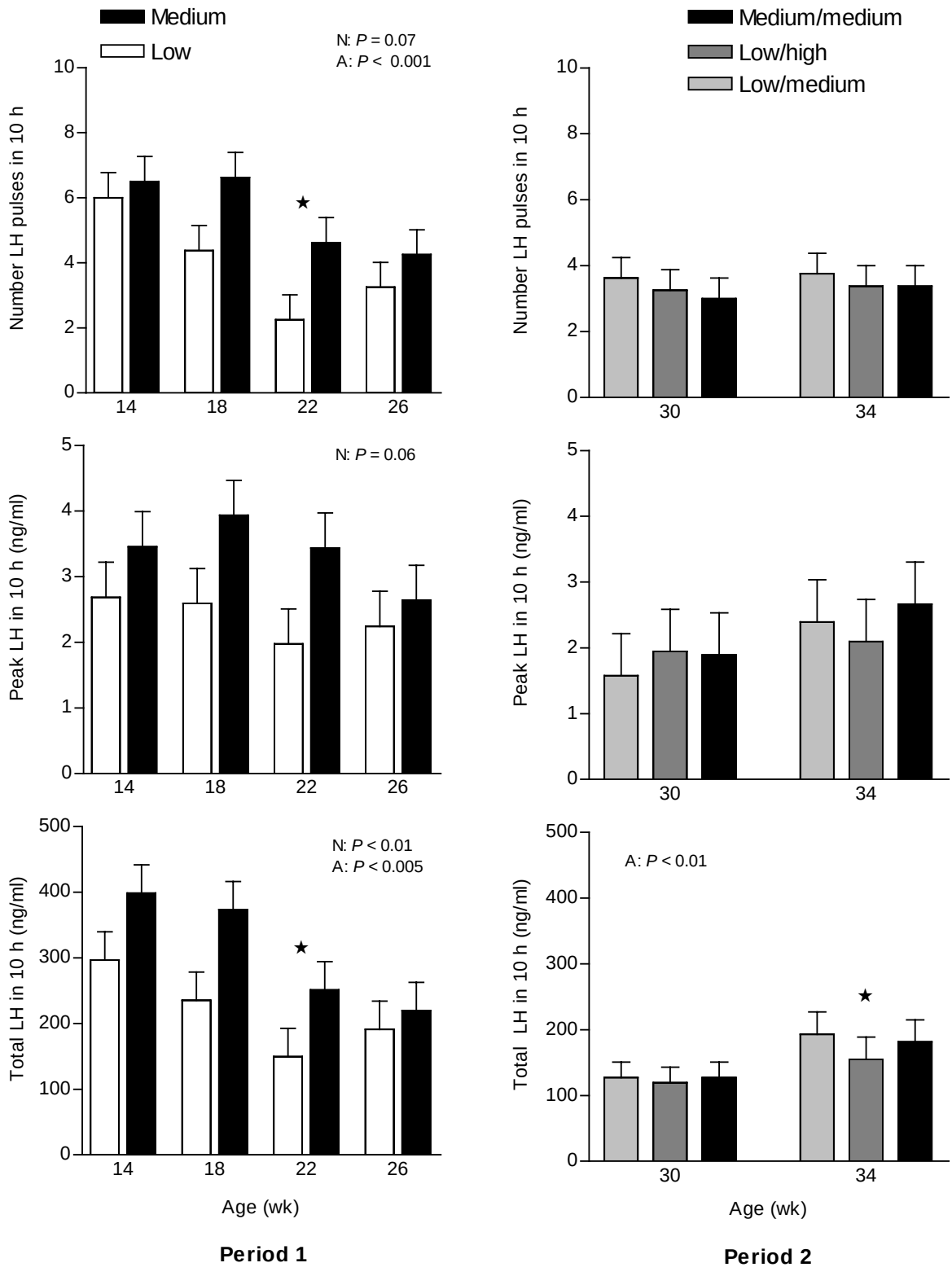
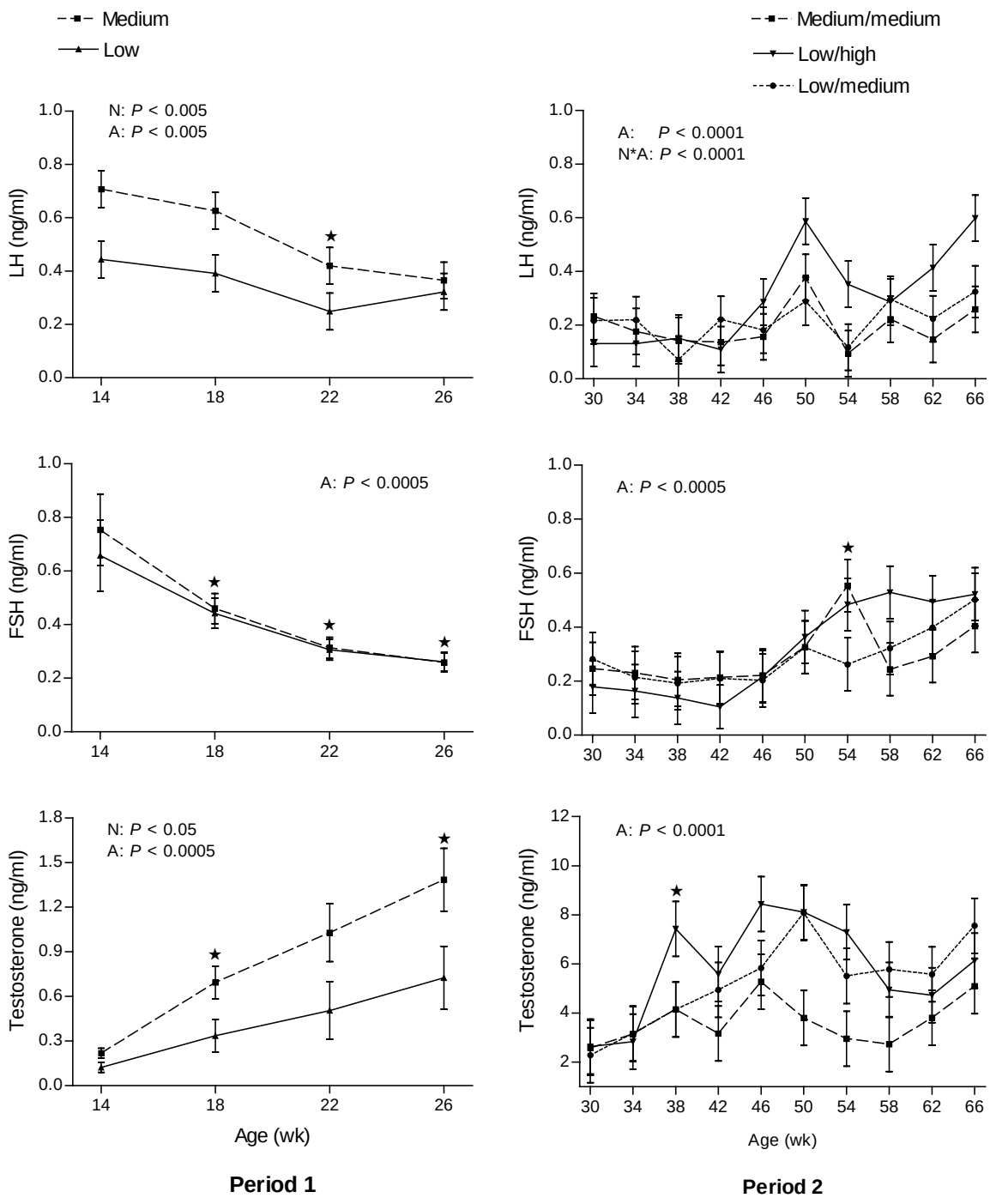

Medium
Low
N: P = 0.07
A: P < 0.001
Number LH pulses in 10 h
Peak LH in 10 h (ng/ml)
N: P = 0.06
Total LH in 10 h (ng/ml)
N: P < 0.01
A: P < 0.005
Age (wk)
Period 1
Medium/medium
Low/high
Low/medium
A: P < 0.01
Age (wk)
Period 2

Figure 5.5. Mean (± SEM) serum LH, FSH, and testosterone concentrations in bulls receiving low (n = 16) or medium nutrition (n = 8) from 10 to 26 wk of age (Period 1) and receiving medium or high nutrition (medium/medium, low/high, and low/medium; n = 8/group) after 27 wk (Period 2). N: nutrition effect; A: age effect; N*A: nutrition-by-age interaction effect. ★Superscripts indicate overall changes ($P < 0.05$) with age. *Superscripts indicate changes ($P < 0.05$) with age within group.


Medium
Low
Medium/medium
Low/high
Low/medium
N: P < 0.005
A: P < 0.005
A: P < 0.0001
N*A: P < 0.0001
A: P < 0.0005
A: P < 0.0005
N: P < 0.05
A: P < 0.0005
A: P < 0.0001
LH (ng/ml)
FSH (ng/ml)
Testosterone (ng/ml)
Age (wk)
Age (wk)
Period 1
Period 2

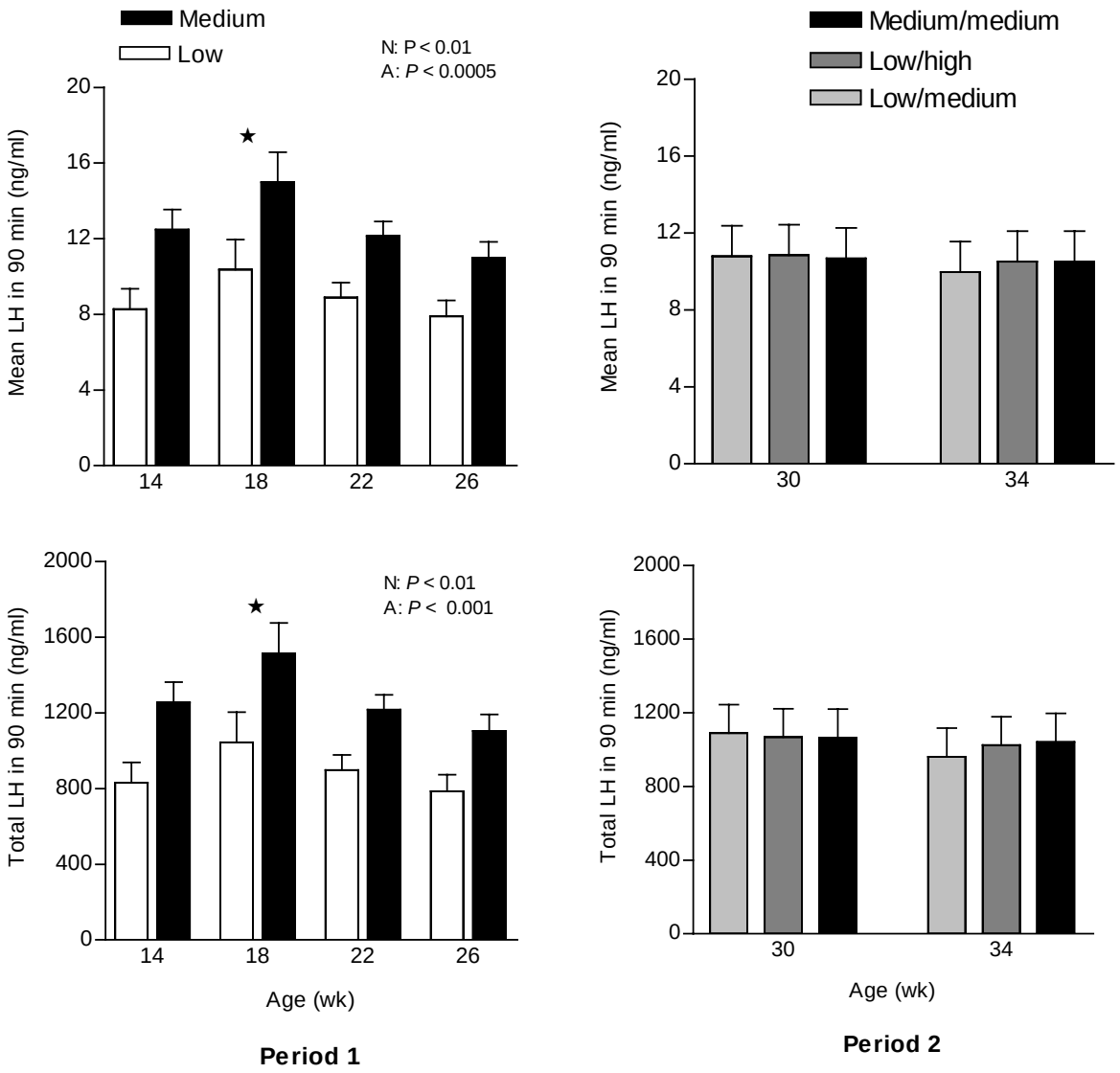


Figure 5.6. Mean (± SEM) serum LH concentration and total secretion after GnRH challenge (0.4 µg/kg, iv) in bulls receiving low (n = 16) or medium nutrition (n = 8) from 10 to 26 wk of age (Period 1) and receiving medium or high nutrition (medium/medium, low/high, and low/medium; n = 8/group) after 27 wk (Period 2). N: nutrition effect; A: age effect. ★Superscripts indicate overall changes (P < 0.05) with age.

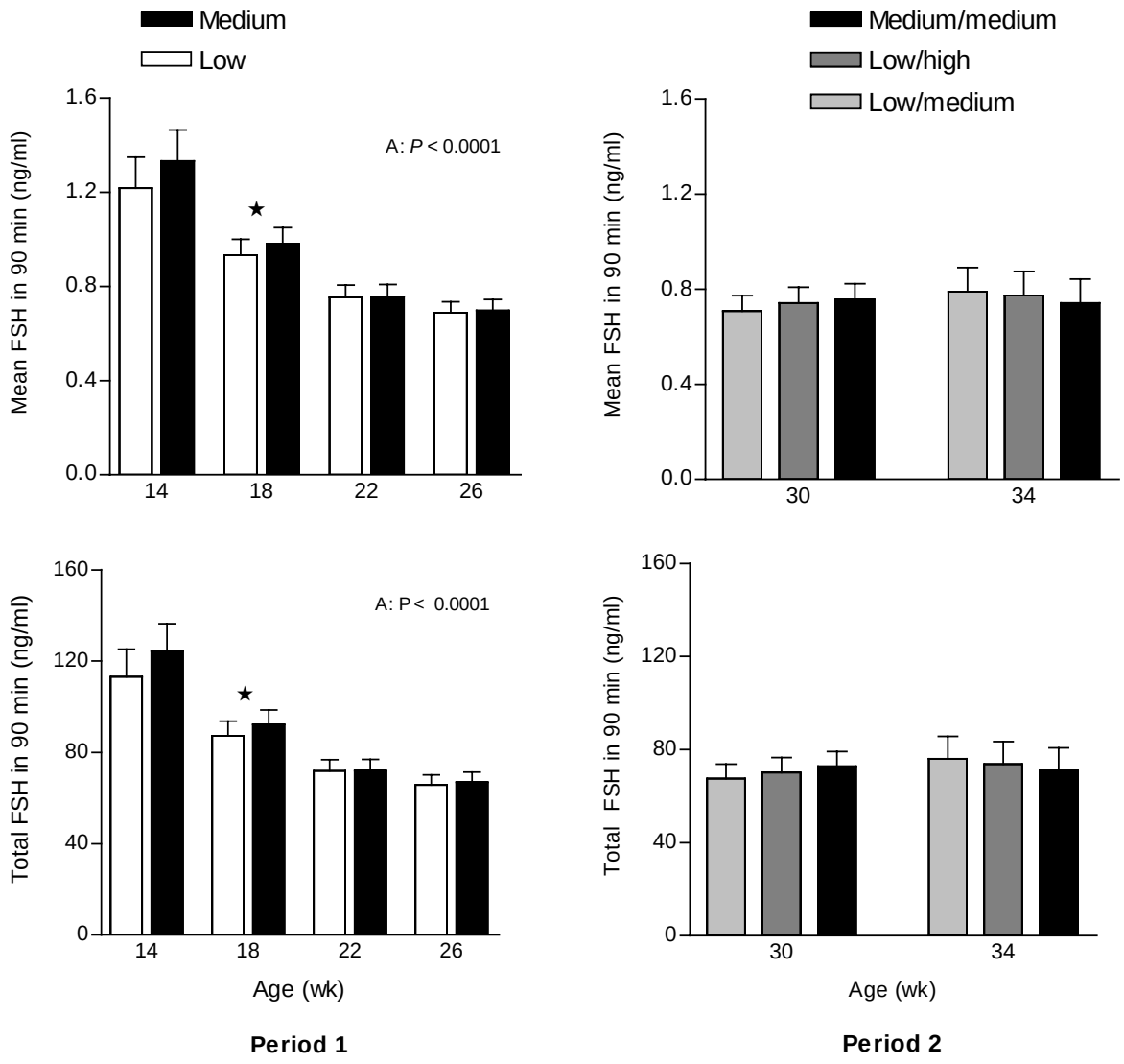


Figure 5.7. Mean (± SEM) serum FSH concentration and total secretion after GnRH challenge (0.4 µg/kg, iv) in bulls receiving low (n = 16) or medium nutrition (n = 8) from 10 to 26 wk of age (Period 1) and receiving medium or high nutrition (medium/medium, low/high, and low/medium; n = 8/group) after 27 wk (Period 2). A: age effect. ★Superscripts indicate overall changes ($P < 0.05$) with age.

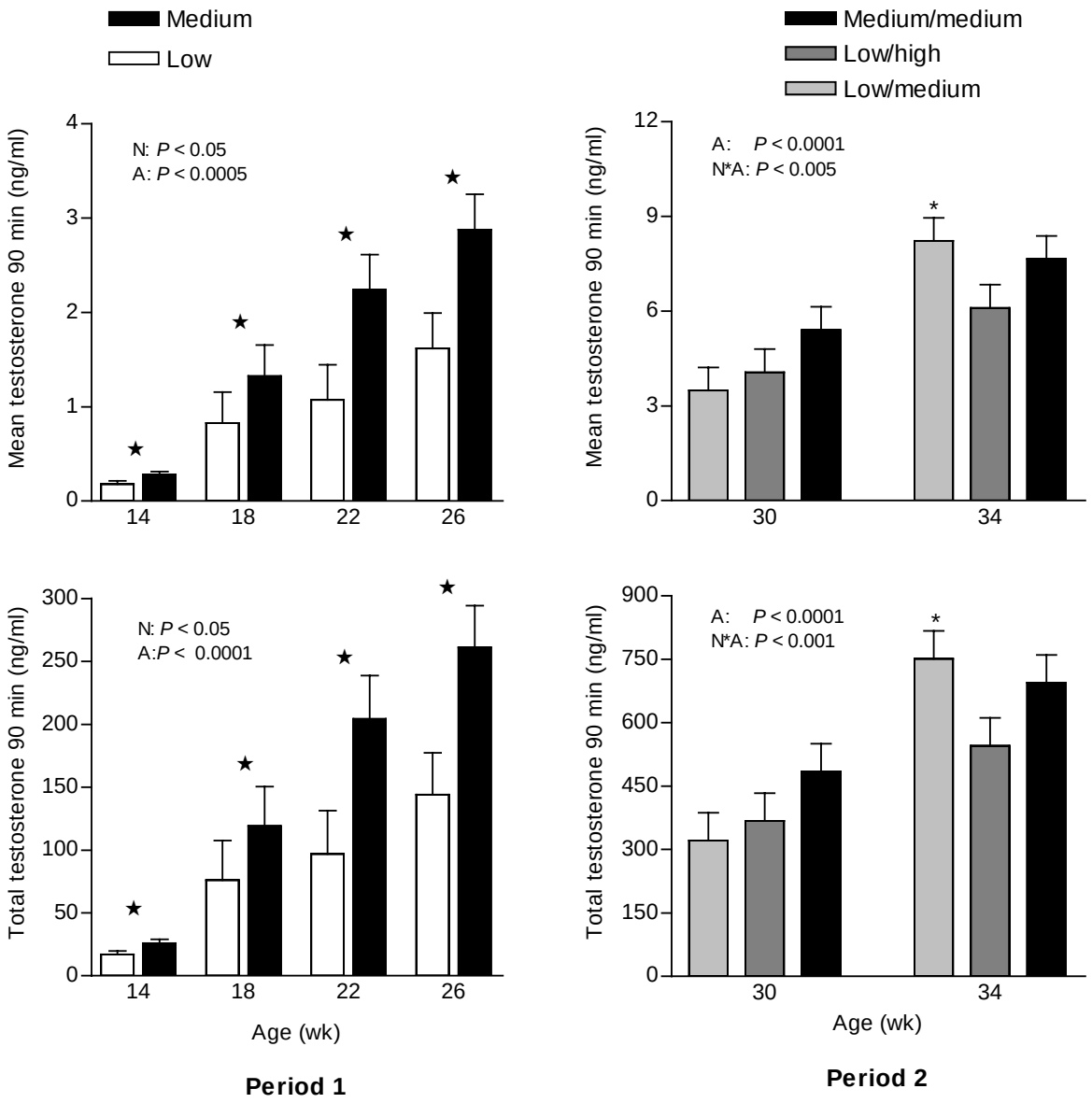


Figure 5.8. Mean (± SEM) serum testosterone concentrations and total secretion after GnRH challenge (0.4 µg/kg, iv) in bulls receiving low (n = 16) or medium nutrition (n = 8) from 10 to 26 wk of age (Period 1) and receiving medium or high nutrition (medium/medium, low/high, and low/medium; n = 8/group) after 27 wk (Period 2). N: nutrition effect; A: age effect; N*A: nutrition-by-age interaction effect. ★Superscripts indicate overall changes (P < 0.05) with age. *Superscripts indicate changes (P < 0.05) with age within group.

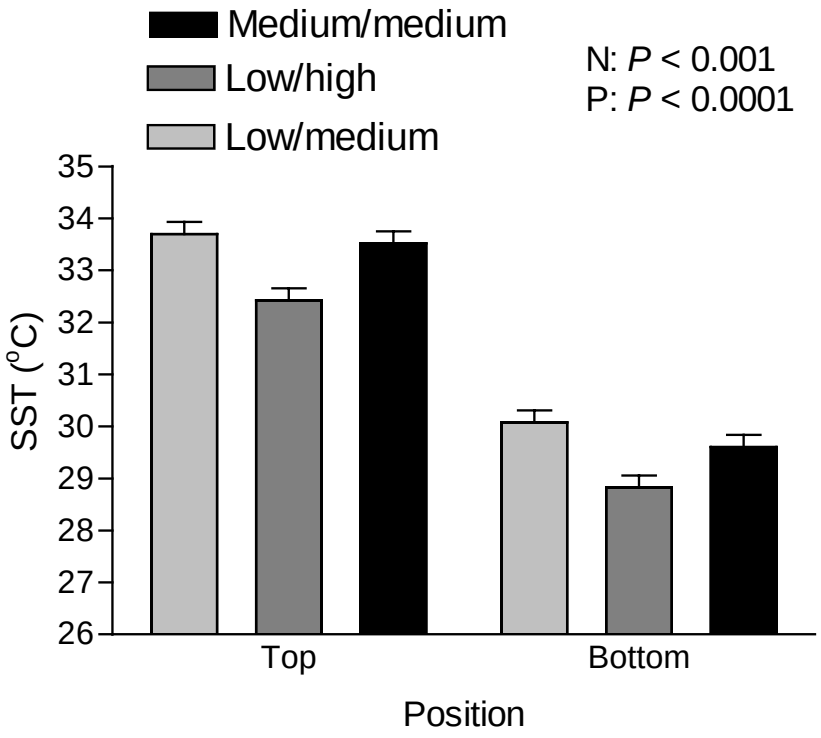


Figure 5.9. Mean (± SEM) top and bottom scrotal surface temperature (SST) at 70 wk of age in bulls receiving low (n = 29) or medium nutrition (n = 15) from 10 to 26 wk of age and medium or high nutrition (medium/medium, n = 15; low/high, n = 14; and low/medium; n = 15) after 27 wk. N: nutrition effect; P: position effect.

Table 5.1.
Mean (± SEM) age at puberty and maturity, testes weight, testicular vascular cone (TVC) characteristics, daily sperm production (DSP), epididymal sperm reserves (ESR), and proportion of normal sperm at 70 wk of age in bulls receiving medium or low nutrition from 10 to 26 wk of age and medium or high nutrition from 27 to 70 wk of age.

	Medium/medium (n = 15)	Low/high (n = 14)	Low/medium (n = 15)
Age at puberty (d)	293.0 ± 8.0[a]	333.7 ± 12.1[b]	334.0 ± 8.5[b]
Age at maturity (d)	366.4 ± 12.8	392.6 ± 10.6	388.7 ± 10.8
Paired testes weight (g)	597.4 ± 11.2[a]	547.6 ± 18.6[ab]	503.1 ± 22.0[b]
TVC diameter (mm)	22.3 ± 0.4	21.8 ± 0.5	22.3± 0.4
TVC fat thickness (mm)	5.2 ± 0.5	5.5 ± 0.6	4.8 ± 0.3
DSP/g (x 10^6 sperm/g)	12.4 ± 0.6	12.3 ± 0.6	12.6 ± 0.7
Total DSP (x 10^9 sperm)	7.4 ± 0.4	6.7 ± 0.4	6.3 ± 0.4
ESR (x 10^9 sperm)	13.1 ± 0.9	13.2 ± 0.8	12.0 ± 0.7
Normal sperm (%)	78.1± 3.5	77.3 ± 5.9	78.1 ± 3.5

Columns with different superscripts differ ($P < 0.05$).

5.2. Discussion

Restricted feed (low nutrition) during calfhood delayed body and testicular development even after feed supplementation during the peripubertal period. Bulls that received restricted feed during calfhood were older at puberty and had smaller testes at 16 mo of age. Therefore, nutrition during calfhood had long-term effects on sexual development, regardless of the nutrition offered during the peripubertal period. In bulls, restricted feed during calfhood had negative effects on sexual development that were not compensated by supplementation during the peripubertal period.

Similar to previous reports in bulls, testicular ultrasonogram echodensity increased from 22 to 42 wk of age, possibly associated with changes occurring in the testicular parenchyma such as cellular proliferation and fluid secretion (Aravindakshan et al., 2000; Chandolia et al., 1997c; Evans et al., 1996). In the present study, testicular parenchyma of bull in the control group (medium/medium nutrition) seemed more echodense than bulls receiving restricted nutrition during Period 1, although the difference was not significant. Moreover, during Period 2, testicular echodensity increased earlier in control bulls (34 wk) than in bulls that had received restricted feed (low/medium and low/high nutrition groups) and that were older at puberty. These observations were in agreement with previous studies that demonstrated that early-maturing bulls have more echodense testes than late-maturing bulls until before puberty (Aravindakshan et al., 2000; Brito et al., 2004) and indicated hastened testicular development in control bulls.

Surprisingly, bulls in the low/high nutrition group had lower scrotal surface temperature than bulls in the low/medium and medium/medium nutrition groups; differences in the testicular vascular cone diameter and fat thickness were not related to differences in scrotal temperature in the present study. Consistent with previous reports, top scrotal surface temperature was warmer than at the bottom (Cook et al., 1994; Kastelic et al., 1995).

The effects of restricted feed on sexual development were probably related to the effects on gonadotropins secretion, more specifically LH. Late-maturing bulls had lower LH secretion than early-maturing bulls during the period of early gonadotropin rise (Aravindakshan et al., 2000; Evans et al., 1995) and suppressing LH secretion during calfhood delayed testicular development in bulls (Chandolia et al., 1997a). In the present study, bulls receiving restricted feed had lower LH pulse frequency, indicating that nutrition affected the GnRH pulse generator in the hypothalamus during the period of the early gonadotropin rise in bulls. The GnRH pulse generator is the primary regulator of reproductive function and is the primary locus of inhibition by restricted food in males and females (Schneider, 2004). The neural apparatus designed to monitor body metabolism and energy (the "metabolic sensor") is a central link between food and reproduction. Metabolic signals, hormonal mediators and modulators, and neuropeptides are involved in an intricate mechanism in which circulating (peripheral) signals are translated within the brain and ultimately regulate the function of GnRH neurons (Blache et al., 2003; Jobst et al., 2004; Schneider, 2004). Within the brain, regulation of the GnRH pulse generator by food may involve the neurotransmitters orexins and

neuropeptide Y, since food restriction increases their gene expression in sheep (Blache et al., 2002; Blache et al., 2003).

Circulating IGF-I concentrations did not increase with age in bulls receiving restricted feed (low nutrition) and concentrations were lower in these bulls than in control bulls (medium nutrition). Therefore, IGF-I may have been associated with the differences in body and sexual development observed in the present experiment. Moreover, differences in IGF-I concentrations may be associated with differences in LH pulse frequency. Insulin-like growth factor-I receptors have been detected in several areas of the brain in rodents and in immortalized GnRH-secreting neurons (GTI-7 cell line). Moreover, dual-label immunohistochemistry specifically demonstrated that GnRH neurons expressed IGF-I receptors (Anderson et al., 1999; Bohannon et al., 1988; Daftary and Gore, 2003, 2004; Miller and Gore, 2001). Expression of IGF-I receptors was affected by sex, age, and nutrition; feed restriction (with concomitant decreases in circulating IGF-I concentrations) up-regulated IGF-I receptors in the median eminence in rats (Bohannon et al., 1988; Daftary and Gore, 2003). In vitro, IGF-I stimulated GnRH secretion from female rat brain and from GTI-7 cells (Anderson et al., 1999; Hiney et al., 1991). Insulin-like growth factor-I may also control GnRH secretion by regulating hypothalamus response to steroids. In female monkeys, decreased hypothalamic hypersensitivity to negative steroid feedback associated with pubertal development was accelerated by exogenous IGF-I treatment (Wilson, 1995).

Restricted feed did not reduce concentrations of leptin, insulin, and GH; therefore, delayed body and testicular development was not associated with these hormones. That

leptin and insulin concentrations did not differ during the period of restricted food supported the hypothesis that these hormones may have only a permissive role on gonadotropin secretion (Blache et al., 2002; Blache et al., 2000). Similar results have been observed in intact and castrated male monkeys, in which the developmental gonadotropin rise occurred without any significant change in circulating leptin concentrations (Plant and Durrant, 1997). Furthermore, feed restriction in ewes resulted in decreased LH secretion concomitant with decreased leptin and insulin concentrations, but leptin and insulin concentrations returned to control levels at the end of the feed restriction period without any rebound in LH secretion (Recabarren et al., 2004). The results of the present experiment indicated that feed intake restricted to 75% of the normal, ad libitum intake resulted in circulating leptin and insulin concentrations that permitte the early-gonadotropin rise to occurr. Therefore, reduced gonadotropin secretion due to reduced leptin and insulin concentrations must result from severe undernutrition. Alternatively, short-term mechanisms involving leptin and insulin may also be important, as a drastic decrease in circulating concentrations of these hormones promoted by fasting also inhibited LH secretion in cattle (Amstalden et al., 2002; Amstalden et al., 2000).

Restricted feed during calfhood resulted in pronounced LH suppression with reduced LH pulse frequency, mean and peak LH concentrations, and total LH secretion. Moreover, GnRH-stimulated LH secretion was also reduced in bulls receiving restricted feed, indicating that restricted feed also affected pituitary function. Insulin-like growth factor-I receptors have been detected in the pituitary and IGF-I stimulated gonadotropin

secretion from rat pituitary in vitro (Kanematsu et al., 1991; Rosenfeld et al., 1984; Soldani et al., 1995; Soldani et al., 1994).

Testicular growth rate was maximal from 6 to 16 mo of age in bulls (Barth and Ominski, 2000; Coulter, 1986), a period that coincided with decreasing gonadotropin concentrations (Amann, 1983; Amann and Walker, 1983; Rawlings et al., 1978). In rams, improved nutrition had positive effects on testicular growth that were maintained beyond the period of the gonadotropin rise promoted by improved nutrition (Blache et al., 2002; Blache et al., 2000). These observations indicated the existence of GnRH-independent mechanisms regulating testicular development in male ruminants. Circulating metabolic hormones may be part of this mechanism and leptin, insulin, and IGF-I may be involved in regulating testicular growth during the peripubertal period in bulls. In agreement with the present study, circulating GH concentrations in bulls decreased during the peripubertal period in bulls (McAndrews et al., 1993). In general, insulin and IGF-I concentrations were similar to those previously reported in bulls (Renaville et al., 1993). During the peripubertal period, insulin concentrations increased around 8 mo of age, whereas IGF-I concentrations increased until approximately 12 mo of age and declined slightly thereafter.

In the present study, IGF-I concentrations had positive correlations with SC and paired testes weight (data not shown). However, although circulating concentrations of leptin, insulin, IGF-I, and testosterone after the improved diet in bulls that had received restricted feed where either similar or greater than in control bulls, testes weight at 16 mo of age was still less in bulls that had received restricted food than in control bulls. In

Experiment I, bulls receiving medium and high diets had similar circulating concentrations of metabolic hormones during the peripubertal period, but increased LH pulse frequency during calfhood was sustained longer and testes weight at 16 mo of age was greater in the high diet group. These observations indicated that the GnRH-independent mechanism of testicular development that may involve metabolic hormones was affected by previous exposure to gonadotropins. The pattern of LH secretion during calfhood may "prime" testicular development and dictate maximum adult testicular size in bulls.

Restricted feed during calfhood also reduced both physiological and GnRH-stimulated testosterone secretion. These observations indicated that nutrition may also have direct effects on Leydig cells function and/or number. These effects could be mediated by IGF-I, since receptors have been identified in Leydig cells in several species and IGF-I increases Leydig cells proliferation and differentiation (Lin, 1995; Spiteri-Grech and Nieschlag, 1992). Leydig cell numbers and expression of steroidogenic enzymes were decreased in IGF-I-null mice; IGF-I treatments in combination with LH were necessary for normal Leydig cell development in these animals (Wang and Hardy, 2004). In vitro, IGF-I up-regulated Leydig cells LH receptors and increased basal and stimulated testosterone secretion (Lin, 1995; Spiteri-Grech and Nieschlag, 1992). Leydig cells also produced IGF-I, indicating the existence of a paracrine/autocrine mechanism of testicular regulation involving the IGF-I system (Rouiller-Fabre et al., 1998; Spiteri-Grech and Nieschlag, 1992). Interestingly, IGF-I stimulated testosterone secretion and testosterone in turn up-regulated IGF-I receptors and IGF-I production by Leydig cells (Cailleau et al., 1990). These observations

indicated that circulating IGF-I may have initially up-regulated testosterone production, which in turn stimulated IGF-I secretion and the establishment of a positive feedback loop between IGF-I and testosterone secretion in Leydig cells in peripubertal bulls. In support of this view, IGF-I treatment increased gonadal sensitivity to gonadotropins and hastened puberty in female monkeys (Hiney et al., 1996; Wilson, 1998).

In conclusion, restricted feed during calfhood affected sexual development in bulls as result of adverse effects on every level of the hypothalamic-pituitary-gonadal axis. Restricted feed inhibited the hypothalamic GnRH pulse generator, reduced the pituitary response to GnRH, and adversely affected testicular steroidogenesis. Temporal associations indicated that the effects of nutrition on the hypothalamic-pituitary-gonadal axis could be mediated by circulating IGF-I. Bulls receiving restricted feed had lower LH secretion during calfhood and lower testicular mass at 16 mo of age, independent of the increased circulating concentrations of metabolic hormones and testosterone after improved nutrition during the peripubertal period. This observation supported the hypothesis that GnRH-independent mechanisms involved in testicular growth are affected by previous LH exposure.

6. EXPERIMENT IV - EFFECT OF FEED SUPPLEMENTATION DURING CALFHOOD ON SERUM METABOLIC HORMONES, GONADOTROPINS AND TESTOSTERONE CONCENTRATIONS, AND ON SEXUAL DEVELOPMENT IN BULLS

6.1. Results

There were nutrition and age effects ($P < 0.01$) on body weight and backfat. There was also a nutrition-by-age effect ($P < 0.0001$) on body weight (Figure 6.1). Body weight increased continuously during the experimental period and backfat increased until 70 wk of age in the medium and high nutrition groups. Bulls in the high nutrition group were heavier ($P < 0.05$) than bulls in the medium nutrition group from 26 to 58 wk of age. Overall, bulls in the high nutrition group had thicker ($P < 0.01$) backfat than bulls in the medium nutrition group.

There were age and nutrition-by-age effects ($P < 0.05$) on SC, paired testes volume, and testes pixel-intensity. There were also nutrition effects ($P < 0.005$) on SC and paired testes volume (Figure 6.2). Scrotal circumference increased ($P < 0.05$) continuously during the experimental period in bulls in the medium nutrition group, whereas SC in the high nutrition group increased ($P < 0.05$) until 66 wk of age. Paired testes volume

increased ($P < 0.05$) until 66 wk of age in both groups. Bulls in the high nutrition group had greater ($P < 0.05$) SC than bulls in the medium nutrition group at 30 wk, and greater ($P < 0.05$) paired testes volume at 42 and 46 wk of age. Testes pixel-intensity decreased ($P < 0.05$), i.e. ultrasonograms became more echodense, at 46 wk and increased ($P < 0.05$) after 66 wk of age in bulls in the medium nutrition group, whereas pixel-intensity decreased ($P < 0.05$) from 46 to 50 wk and increased after 62 wk of age in bulls in the high nutrition group.

There were age and nutrition-by-age effects ($P < 0.05$) on testicular vascular cone diameter and an age effect ($P < 0.0001$) on vascular cone fat thickness (Figure 6.3). Testicular vascular cone diameter increased ($P < 0.05$) until 58 wk of age in both groups; there were no significant differences between groups within age. Overall, testicular vascular cone fat thickness increased from 26 to 62 wk and decreased at 74 wk of age.

There were nutrition and age effects ($P < 0.05$) on insulin and IGF-I serum concentrations, and a nutrition-by-age interaction ($P < 0.0001$) on IGF-I concentrations (Figure 6.4). Overall, insulin concentrations increased after 22 and 66 wk of age and were greater ($P < 0.05$) in bulls in the high nutrition group than in bulls in the medium nutrition group. Serum IGF-I concentrations increased until 50 wk of age in both groups and were greater ($P \leq 0.08$) in bulls in the high nutrition group than in bulls in the medium nutrition group from 26 to 30 wk and from 42 to 50 wk of age.

There were age and nutrition-by-age interaction effects ($P < 0.005$) on LH pulse frequency, pulse amplitude, and peak concentration. There was also an age effect ($P < 0.0001$) on total LH secretion (Figure 6.5). Luteinizing hormone pulse frequency decreased ($P < 0.05$) after 18 and 22 wk of age in bulls in the medium and high nutrition groups, respectively; pulse frequency tended to be greater ($P = 0.09$) in bulls in the high nutrition group at 18 wk of age. Luteinizing hormone pulse amplitude and peak concentration did not change significantly with age in bulls in the medium nutrition group, but decreased after 26 wk in bulls in the high nutrition group. Bulls in the medium nutrition group tended to have greater ($P \leq 0.09$) LH pulse amplitude and peak concentration than bulls in the high nutrition group at 26 and 30 wk of age, respectively. Overall, total LH secretion decreased after 22 wk of age. Although total LH secretion seemed greater in bulls in the high nutrition group at 18 wk of age, the difference only approached significance ($P = 0.11$).

There were age effects ($P < 0.0001$) on mean serum LH, FSH, and testosterone concentrations. There was also a tendency ($P \leq 0.08$) for nutrition and nutrition-by-age interaction effects on testosterone concentrations (Figure 6.6). Overall, LH and FSH concentrations decreased from 14 to 22 wk of age and testosterone concentrations increased after 42 wk of age. Despite the nutrition-by-age interaction effect on LH concentrations, there were no significant differences between groups within age. Overall, bulls in the high nutrition group tended to have greater testosterone concentrations than bulls in the medium nutrition group.

There were age effects ($P < 0.0001$) on mean and peak LH concentrations and total LH secretion after GnRH challenge (Figure 6.7). Overall, GnRH-induced mean and peak LH concentrations and total LH secretion decreased after 22 wk of age. There were nutrition, age, and nutrition-by-age interaction effects ($P < 0.0005$) on mean and peak testosterone concentrations and total testosterone secretion after GnRH challenge (Figure 6.7). GnRH-induced mean and peak testosterone concentrations and total testosterone secretion increased ($P < 0.05$) from 22 to 26 wk of age in bulls in the high nutrition group, but only increased at 30 wk of age in bulls in the medium nutrition group. Moreover, mean and peak testosterone concentrations and total testosterone secretion were greater in bulls in the high nutrition group than in bulls in the medium nutrition group at 26 and 30 wk of age.

There was a position effect ($P < 0.0001$) on scrotal surface temperature (Figure 6.8). Overall, top scrotal temperature was greater ($P < 0.0001$) than bottom temperature.

Bulls in the high nutrition group had greater ($P < 0.05$) paired testes weight and total daily sperm production than bulls in the medium nutrition group. There were no effects of nutrition on age at puberty, daily sperm production/g, epididymal sperm reserves, and proportion of normal sperm at 70 wk of age. One bull in the medium nutrition group constantly produced high proportions of sperm with knobbed acrosomes and was excluded from the analysis of age at maturity. Three bulls in the medium nutrition group and two bulls in the high nutrition group did not produce > 70% morphologically normal sperm and could not be classified as mature; age at maturity did not differ among groups.

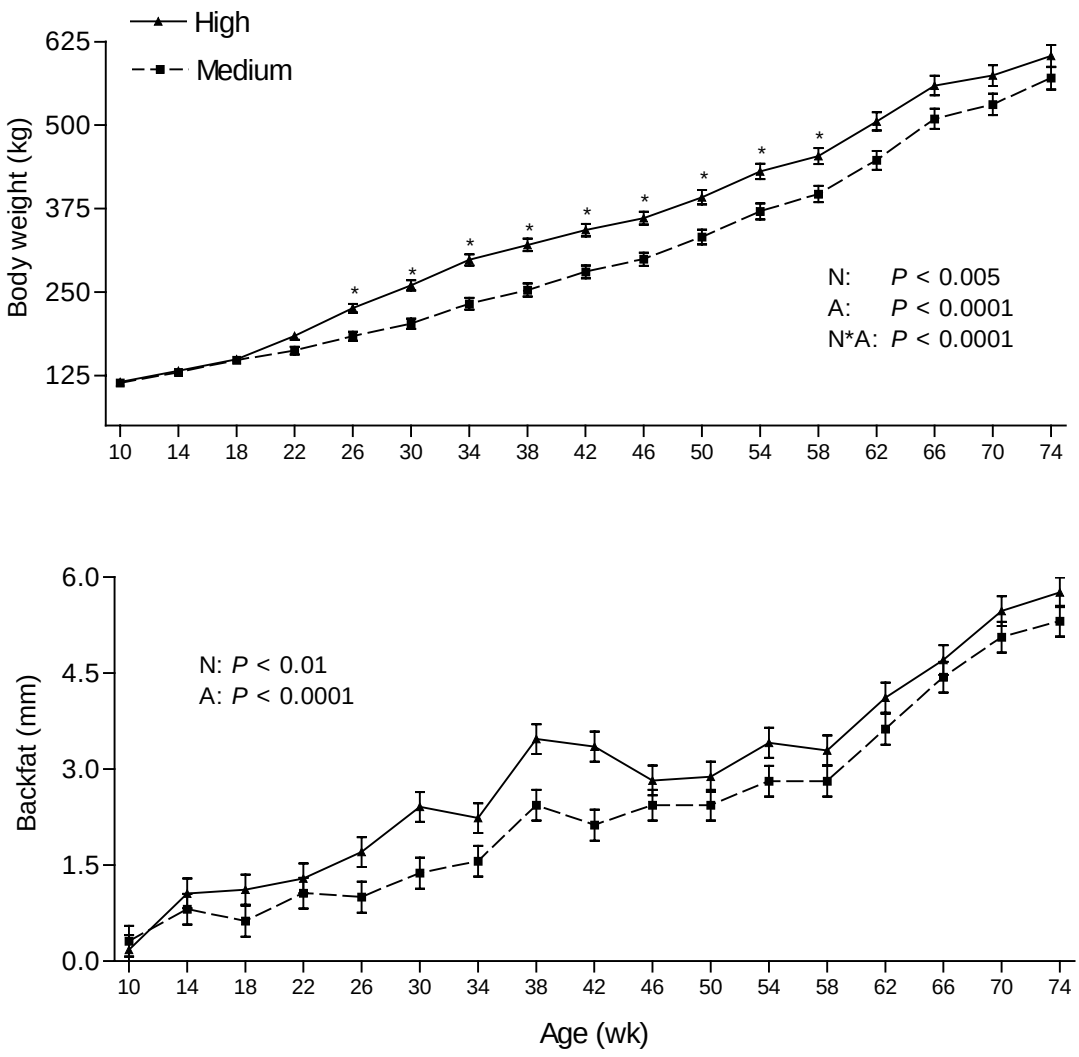
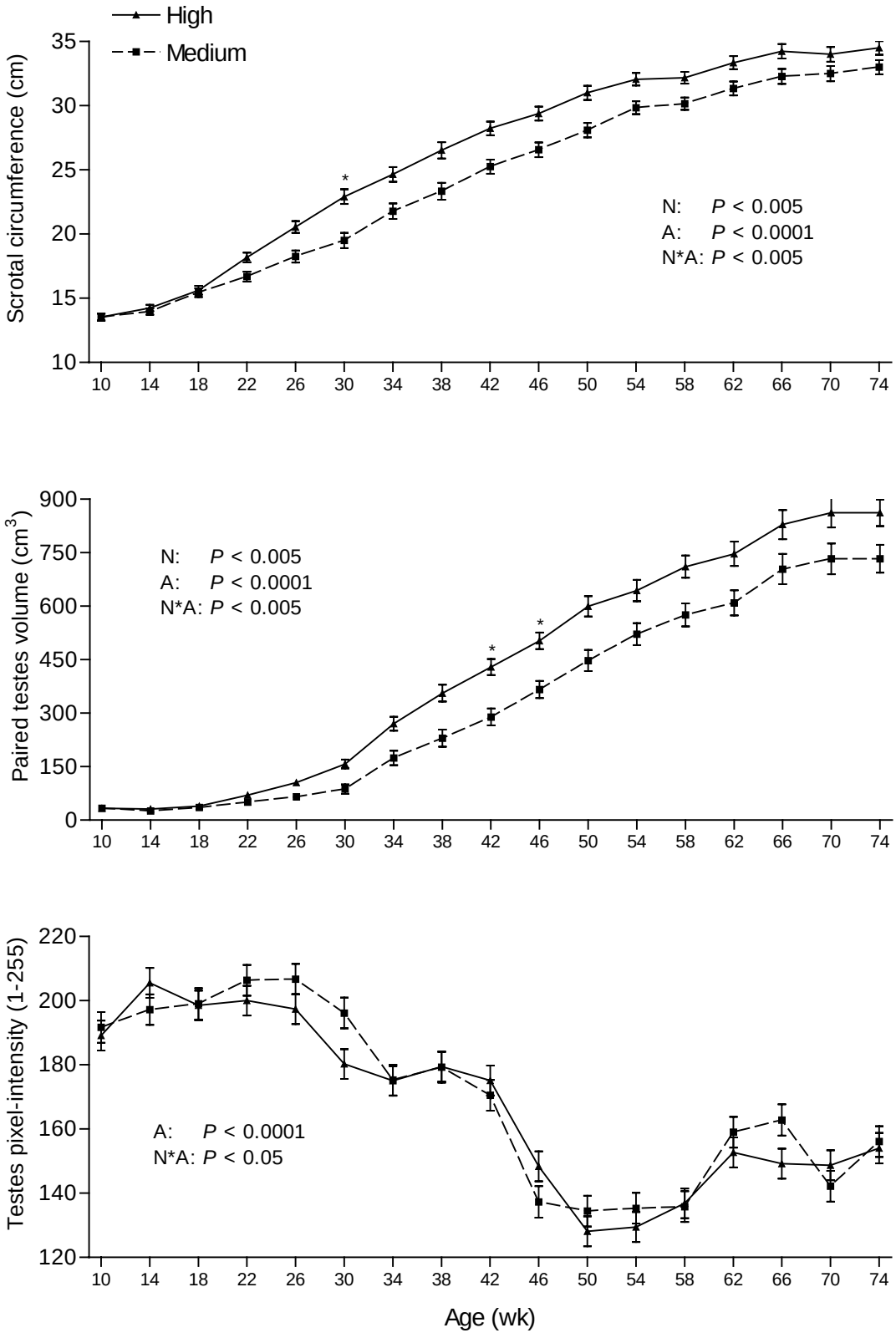


Figure 6.1. Mean (± SEM) body weight and backfat in bulls receiving high (n = 17) or medium nutrition (n = 16) from 8 to 30 wk of age and medium nutrition from 31 to 74 wk of age. N: nutrition effect; A: age effect; N*A: nutrition-by-age interaction effect. *Superscripts indicate differences ($P < 0.05$) between groups within age.

Figure 6.2. Mean (± SEM) scrotal circumference, paired testes volume, and testes pixel-intensity in bulls receiving high (n = 17) or medium nutrition (n = 16) from 8 to 30 wk of age and medium nutrition from 31 to 74 wk of age. N: nutrition effect; A: age effect; N*A: nutrition-by-age interaction effect. *Superscripts indicate differences ($P < 0.05$) between groups within age.


High
Medium
Scrotal circumference (cm)
N: P < 0.005
A: P < 0.0001
N*A: P < 0.005
Paired testes volume (cm3)
N: P < 0.005
A: P < 0.0001
N*A: P < 0.005
Testes pixel-intensity (1-255)
A: P < 0.0001
N*A: P < 0.05
Age (wk)

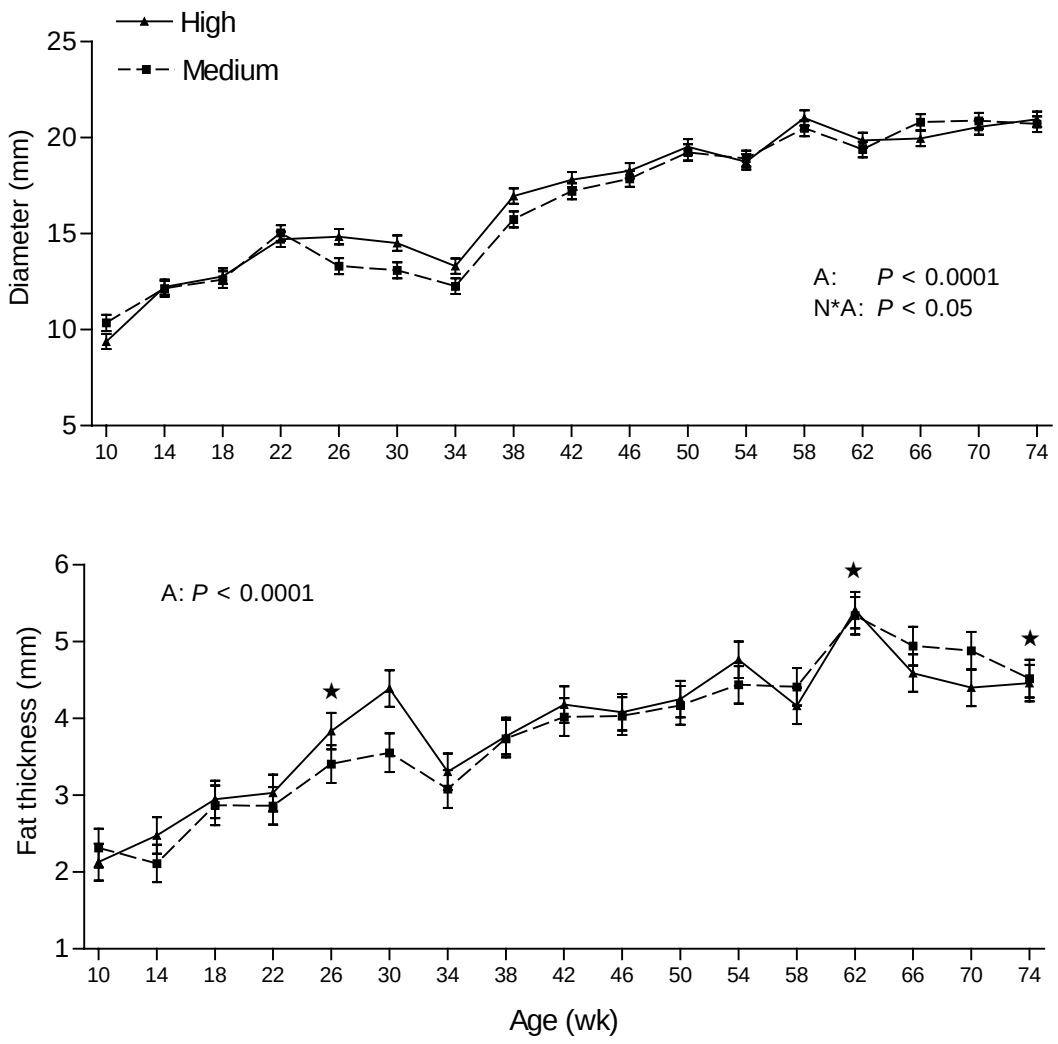


Figure 6.3. Mean (± SEM) testicular vascular cone diameter and fat thickness in bulls receiving high (n = 17) or medium nutrition (n = 16) from 8 to 30 wk of age and medium nutrition from 331 to 74 wk of age. N: nutrition effect; A: age effect; N*A: nutrition-by-age interaction effect. ★Superscripts indicate overall differences ($P < 0.05$) among ages.

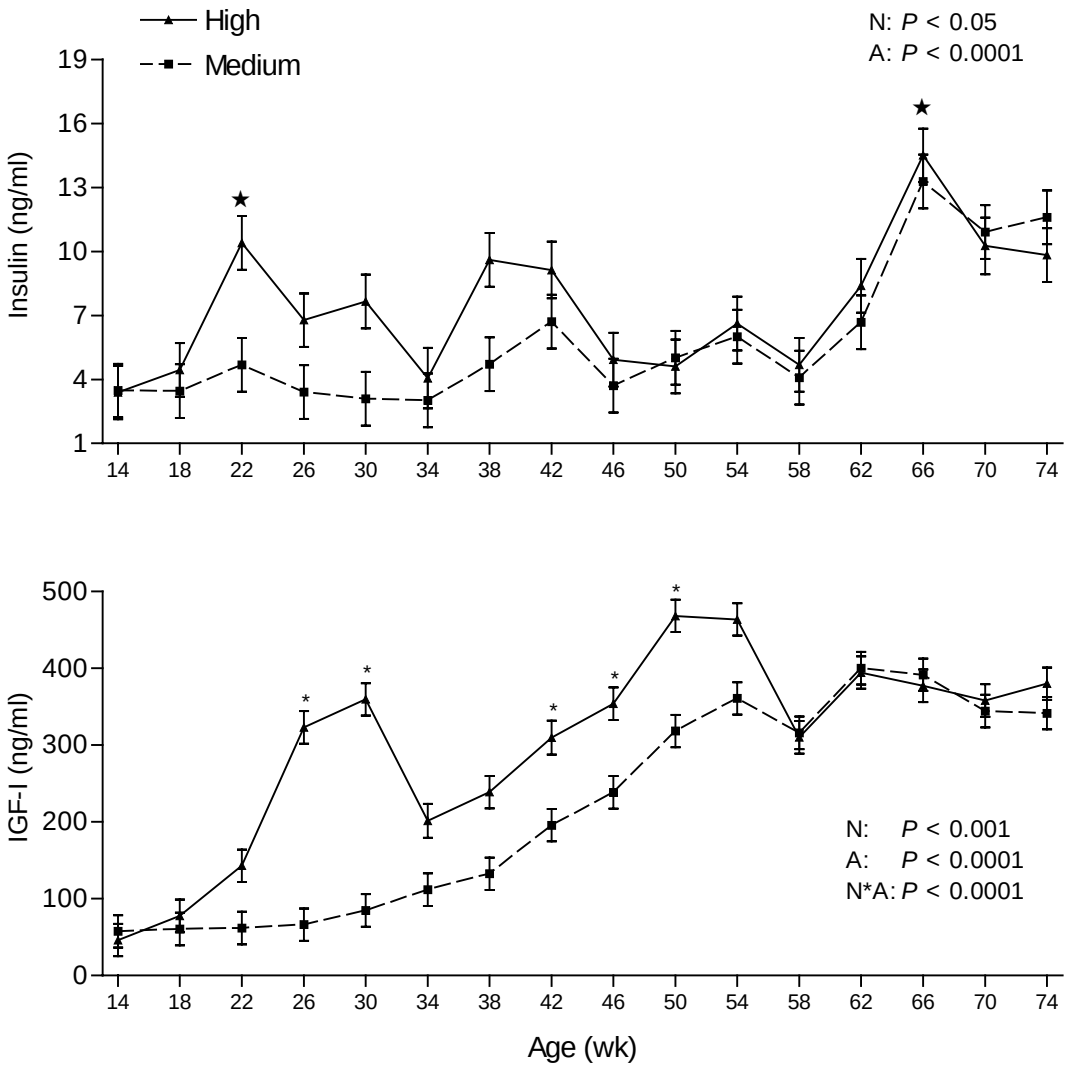


Figure 6.4. Mean (± SEM) serum insulin and IGF-I concentrations in bulls receiving high or medium nutrition (n = 8/group) from 8 to 30 wk of age and medium nutrition from 31 to 74 wk of age. N: nutrition effect; A: age effect; N*A: nutrition-by-age interaction effect. ★Superscripts indicate overall differences ($P < 0.05$) among ages. *Superscripts indicate differences ($P < 0.05$) between groups within age.

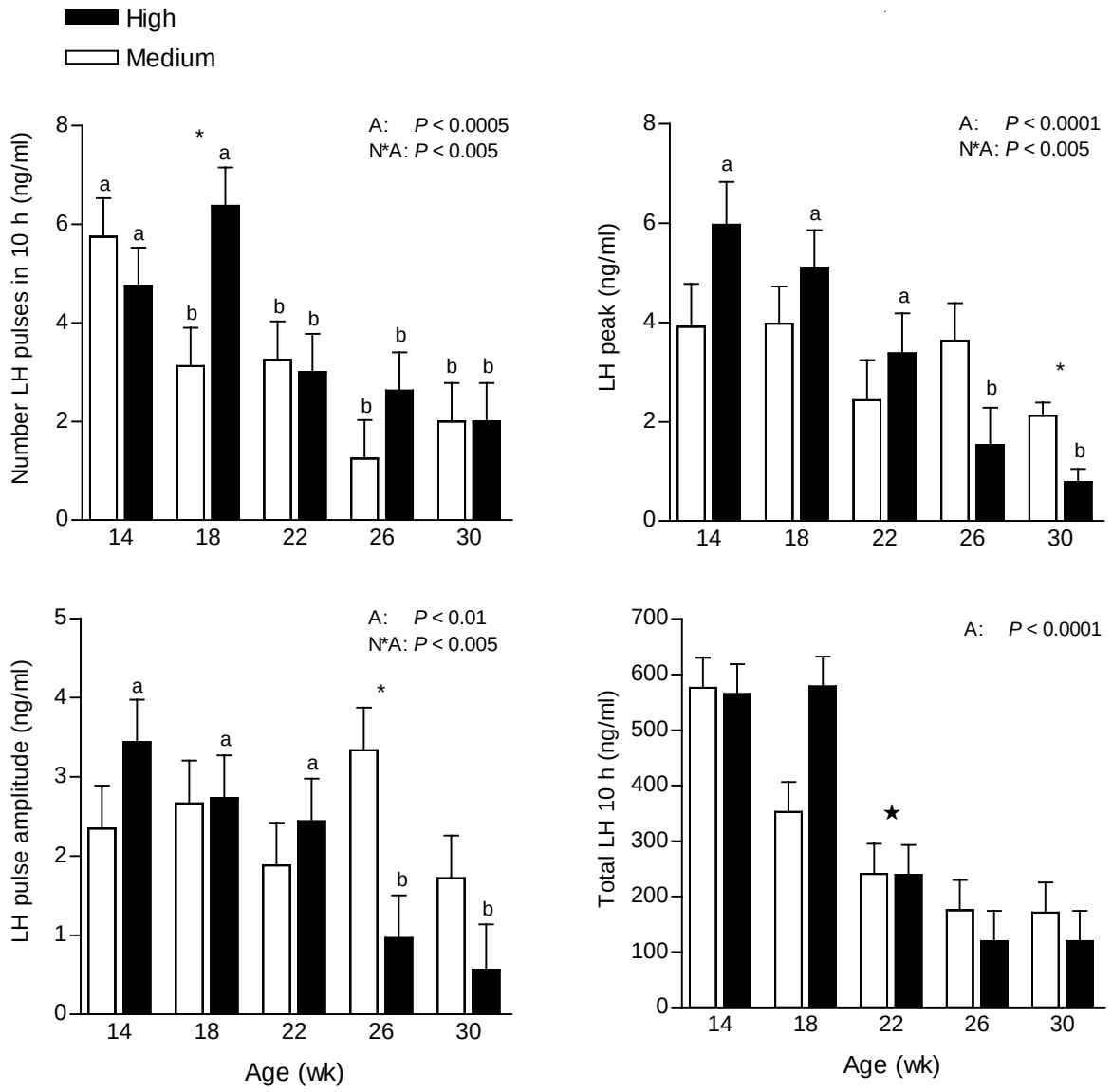


Figure 6.5. Mean (± SEM) serum LH pulse frequency, pulse amplitude, peak concentration, and total secretion during 10 h of intensive sampling (15 min intervals) in bulls receiving high or medium nutrition (n = 8/group) from 8 to 30 wk of age and medium nutrition from 31 to 74 wk of age. N: nutrition effect; A: age effect; N*A: nutrition-by-age interaction effect. [a,b]Superscripts indicate differences ($P < 0.05$) among ages within group. *Superscripts indicate differences ($P \leq 0.08$) between groups within age. ★Superscripts indicate overall differences ($P < 0.05$) among ages.

Figure 6.6. Mean (± SEM) serum LH, FSH, and testosterone concentrations in bulls receiving high or medium nutrition (n = 8/group) from 8 to 30 wk of age and medium nutrition from 31 to 74 wk of age. N: nutrition effect; A: age effect; N*A: nutrition-by-age interaction effect. ★Superscripts indicate overall differences ($P < 0.05$) among ages.

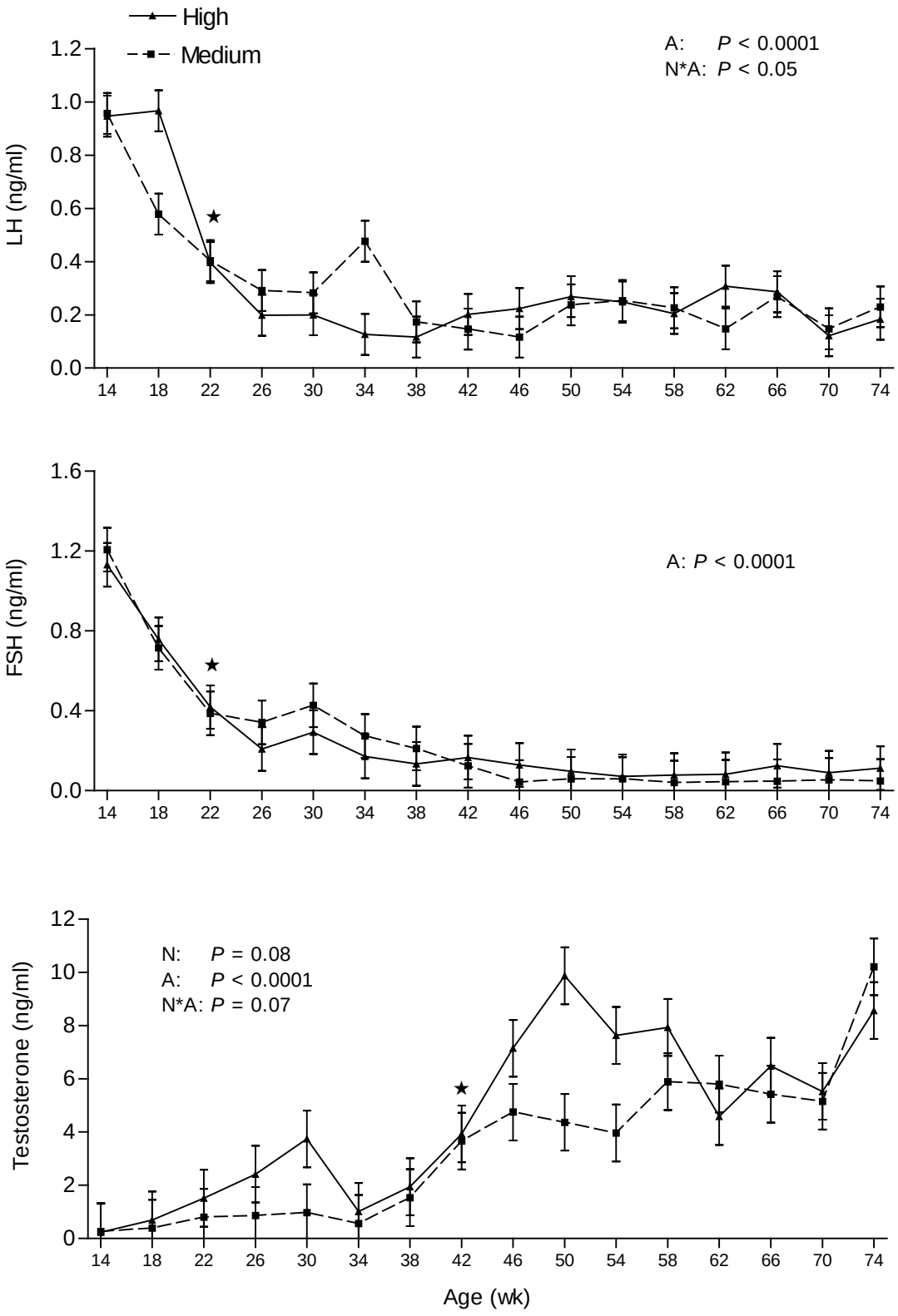
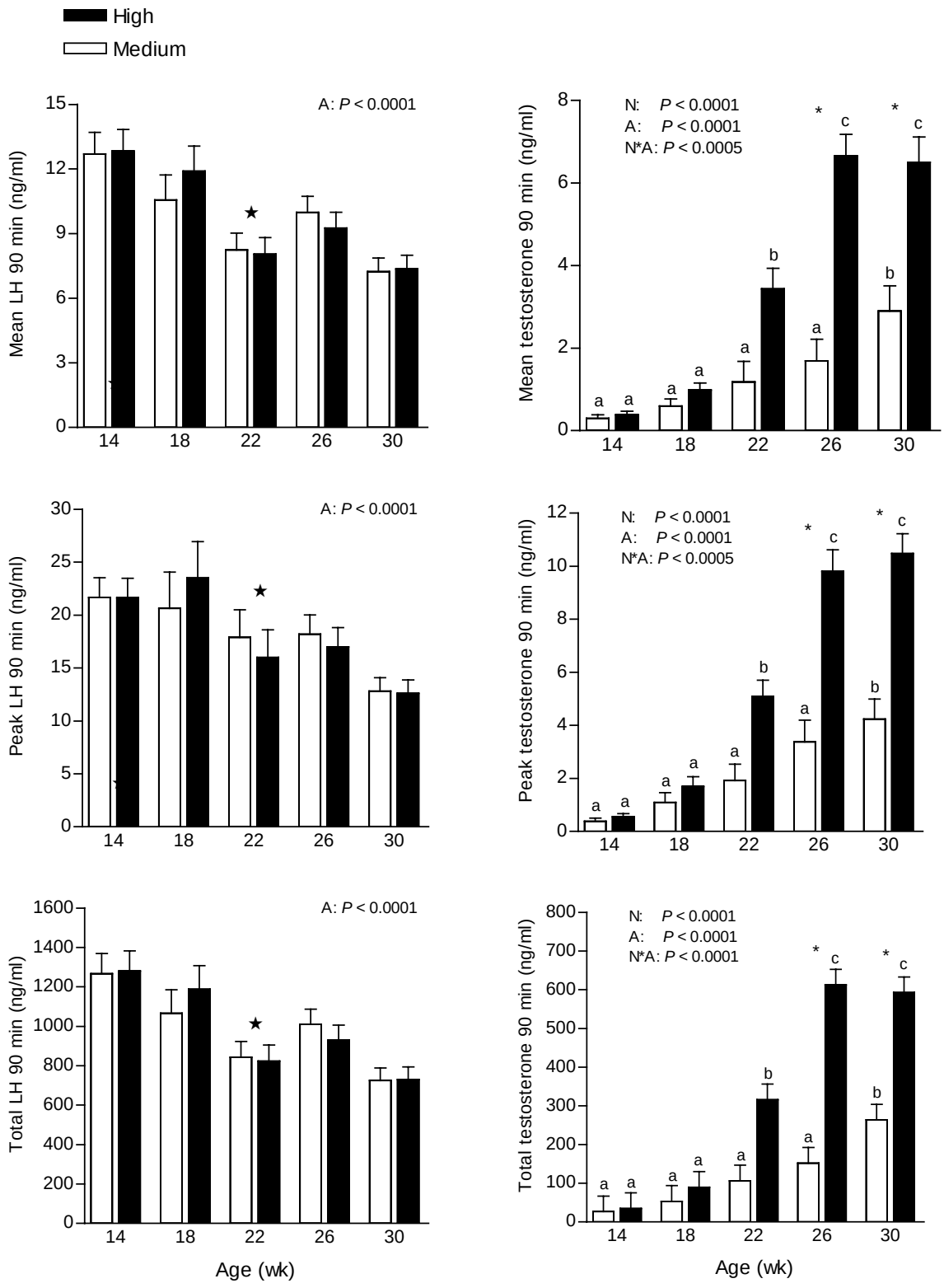

High
Medium
A: P < 0.0001
N*A: P < 0.05
LH (ng/ml)
FSH (ng/ml)
A: P < 0.0001
N: P = 0.08
A: P < 0.0001
N*A: P = 0.07
Testosterone (ng/ml)
Age (wk)

Figure 6.7. Mean (± SEM) serum LH and testosterone mean and peak concentrations, and total secretion after a GnRH challenge (0.04 μg/kg, IV) in bulls receiving high or medium nutrition (n = 8/group) from 8 to 30 wk of age and medium nutrition from 31 to 74 wk of age. N: nutrition effect; A: age effect; N*A: nutrition-by-age interaction effect. [a,b]Superscripts indicate differences ($P < 0.05$) among ages within group. ★Superscripts indicate differences ($P < 0.05$) between groups within age. *Superscripts indicate overall differences ($P < 0.05$) among ages.


High
Medium
Mean LH 90 min (ng/ml)
A: P < 0.0001
Mean testosterone 90 min (ng/ml)
N: P < 0.0001
A: P < 0.0001
N*A: P < 0.0005
Peak LH 90 min (ng/ml)
A: P < 0.0001
Peak testosterone 90 min (ng/ml)
N: P < 0.0001
A: P < 0.0001
N*A: P < 0.0005
Total LH 90 min (ng/ml)
A: P < 0.0001
Total testosterone 90 min (ng/ml)
N: P < 0.0001
A: P < 0.0001
N*A: P < 0.0001
Age (wk)
Age (wk)

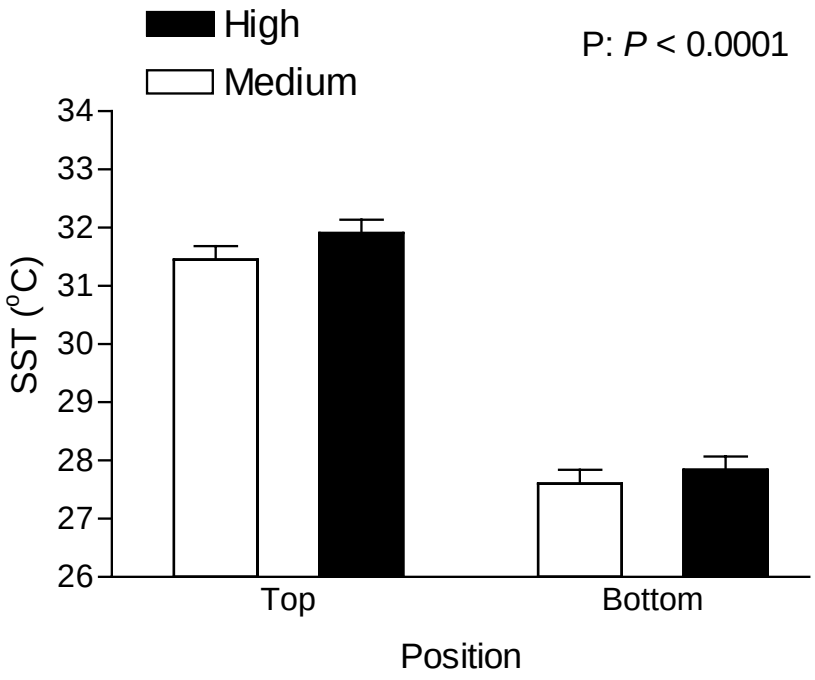


Figure 6.8. Mean (± SEM) top and bottom scrotal surface temperature (SST) at 70 wk of age in bulls receiving high (n = 17) or medium nutrition (n = 16) from 8 to 30 wk of age and medium nutrition from 31 to 74 wk of age.. P: position effect.

Table 6.1.
Mean (± SEM) age at puberty and maturity, and testicular characteristics, daily sperm production (DSP), epididymal sperm reserves (ESR), and proportion of normal sperm at 74 wk of age in bulls receiving medium or high nutrition from 10 to 30 wk of age.

	Medium (n = 16)	High (n = 17)
Age at puberty (d)	326.9 ± 9.3	314.1 ± 8.3
Age at maturity (d)	381.5 ± 9.2	380.6 ± 14.9
Paired testes weight (g)	531.2 ± 18.4[a]	610.5 ± 27.9[b]
DSP/g (x 10^6 sperm/g)	14.1± 0.5	14.9 ± 0.6
Total DSP (x 10^9 sperm)	7.4 ± 0.4[a]	9.5 ± 0.7[b]
ESR (x 10^9 sperm)	13.1± 1.1	15.6 ± 1.4
Normal sperm (%)	74.8 ± 3.5	74.3 ± 4.6

Columns with different superscripts differ ($P < 0.05$).

6.2. Discussion

Feed supplementation during calfhood hastened body development and increased testicular mass and sperm production at 16 mo of age; the beneficial effects on feed supplementation during calfhood extended beyond the period of supplementation.

The effects of supplemental feed on testicular development were probably related to the effects on LH and testosterone secretion. Increased circulating LH concentrations after exogenous treatments during calfhood hastened testicular development in bulls (Chandolia et al., 1997b). In the present study, bulls receiving supplemental feed during calfhood feed had a greater and more sustained increase in LH pulse frequency during the period of the early gonadotropin rise, indicating that nutrition affected the GnRH pulse generator in the hypothalamus. Metabolic hormones and other gut hormones (ghrelin, neuromedin U, and amylin) are involved in signaling nutritional status to the brain. Within the brain, these signals are translated into orexins, neuropeptide Y, and other neurotransmitters to regulate the function of GnRH neurons. This neural apparatus designed to monitor body metabolism and energy (the "metabolic sensor") is a central link between nutrition and reproduction and probably involves a number of interactions among several hormones and neuropeptides that are also involved in controlling appetite and metabolism (Blache et al., 2002; Blache et al., 2003; Jobst et al., 2004; Schneider, 2004).

Circulating insulin and IGF-I concentrations were greater in bulls receiving supplemental feed during calfhood; these hormones may have been associated with the

differences in LH secretion. Plasma and cerebrospinal fluid insulin concentrations in rams increased concomitantly with increased LH pulse frequency after increases in nutrition plane and intra-cerebral infusion of insulin increased LH pulse frequency (Blache et al., 2002; Blache et al., 2000; Martin and Walkden-Brown, 1995). Receptors for IGF-I have been identified on GnRH-secreting cells (GT-17 cells), and in the hypothalamus in rodents (Bohannon et al., 1988; Daftary and Gore, 2004; Miller and Gore, 2001; Olson et al., 1995). GnRH-secreting neurons expressed IGF-I receptors and expression changed with age, indicating a possible direct role for IGF-I on GnRH release by the hypothalamus (Daftary and Gore, 2004), although the regulatory role of circulating IGF-I on GnRH secretion has been questioned in rams, since IGF-I concentrations did not increase in the cerebrospinal fluid after improved nutrition, despite increased plasma concentrations. Furthermore, IGF-I infusion into the third ventricle did not affect GnRH secretion (Blache et al., 2000; Martin and Walkden-Brown, 1995; Miller et al., 1998).

After the early gonadotropin rise, testicular growth continues, regulated by GnRH-independent mechanisms (Blache et al., 2002; Blache et al., 2000). GnRH-independent testicular growth during the peripubertal period may be regulated by metabolic hormones like insulin and IGF-I. In the present study, greater serum insulin and IGF-I concentrations in bulls receiving supplemental feed during calfhood were associated with greater physiological and GnRH-induced testosterone secretion, testicular mass and sperm production, suggesting a direct effect of these metabolic hormones on the testes. Insulin and IGF-I receptors have been identified in Leydig and Sertoli cells in several species and these hormones might be involved in non-germinal testicular cells

proliferation, differentiation, and function (Abele et al., 1986; Bellve and Zheng, 1989; Borland et al., 1984; Oonk et al., 1989; Rouiller-Fabre et al., 1998). Studies with IGF-I-null mice indicated that IGF-I is an essential complement to LH for promoting Leydig cell development (Wang and Hardy, 2004; Wang et al., 2003). Moreover, IGF-I up-regulated Leydig cell steroidogenesis by up-regulating the number of LH receptors (Lin, 1995; Spiteri-Grech and Nieschlag, 1992). Since testosterone up-regulated IGF-I production and IGF-I receptor expression by Leydig and Sertoli cells (Cailleau et al., 1990), the establishment of a positive feedback loop between IGF-I secretion and testosterone production may be important for testicular development.

In conclusion, nutrition regulated the hypothalamus-pituitary-testes axis through effects on the GnRH pulse generator in the hypothalamus and effects on the testes. Supplemental feed prolonged the period of increased LH pulse frequency during the early gonadotropin rise. Temporal associations suggested that circulating insulin and IGF-I might be involved in regulating the duration of the early gonadotropin rise in bulls. A hastened peripubertal increase in testosterone concentration was associated with greater circulating insulin and IGF-I concentrations, suggesting a role for these hormones in regulating Leydig cell function. Moreover, supplemental feed during calfhood resulted in greater testicular mass and sperm production in mature bulls, indicating that a more prolonged LH pulse frequency during calfhood and increased serum insulin and IGF-I concentrations were associated with improved testicular cell proliferation and function.

7. GENERAL DISCUSSION

The results presented in this thesis clearly demonstrated that nutrition during calfhood affected LH secretion during the early gonadotropin rise with consequent effects on sexual development. The effects of nutrition during the peripubertal period seemed to have a much smaller effect on sexual development, although a definitive conclusion in that regard could not be made, since different nutrition did not result in consistent effects on body weight and backfat in Experiment I. These results present a paradigm to bull producers; beef bull calves are usually nursing during calfhood and very little attention is paid to their nutrition, whereas nutrition offered to dairy bull calves is often sub-optimal. It is clear that nutritional management practices during calfhood will have greater beneficial effects on reproductive function than management practices during the peripubertal period in bulls.

These results of the present studies demonstrated that in bulls, as in other species and in females (I'Anson et al., 1991; Schneider, 2004; Williams, 1998), nutrition is involved in regulating GnRH secretion. Moreover, the present results confirmed the crucial role of LH secretion pattern during the early gonadotropin rise on sexual development in bulls (Aravindakshan et al., 2000; Evans et al., 1995). Although no inference could be made about the mechanisms controlling the initiation of the early gonadotropin rise, since increased gonadotropin secretion was already apparent at the beginning of the

present experiments, high nutrition during calfhood resulted in a more sustained increase in LH pulse frequency during the early gonadotropin rise and greater testicular development at maturity, indicating that LH secretion during calfhood may somehow "prime" testicular development and determine maximum adult testicular size. This conclusion was also supported by the increased paired testes weight observed at 54 wk of age in bulls receiving multiple, exogenous GnRH treatments during calfhood (Chandolia et al., 1997b).

On the other hand, restricted nutrition during calfhood suppressed LH secretion during the early gonadotropin rise and delayed puberty and reduced testicular development at maturity. Similar effects have been produced by supressing LH secretion with prolonged GnRH-agonist treatments during calfhood (early gonadotropin rise) in bulls (Chandolia et al., 1997a). Furthermore, reduced nutrition could be associated with smaller SC in bulls raised by heifers. Although an *in utero* effect could be argued, Bagu et al. (personal communication) observed similar differences in LH secretion pattern between calves raised by heifers or by cows to those observed in the present study between calves receiving low or medium nutrition. Therefore, decreased testicular development in bulls raised by heifers was likely due to smaller milk production (low nutrition) and reduced LH secretion during calfhood.

It was interesting that the suppression of LH secretion in Experiment III was more pronounced than in Experiment II. In Experiment II, low nutrition during calfhood reduced LH pulse frequency, but mean and peak LH concentrations and total LH secretion were not affected as in Experiment III. Moreover, in contrast to Experiment II,

GnRH-stimulated LH secretion was reduced in bulls receiving low nutrition in Experiment III, indicating an effect on pituitary function in the latter experiment. A possible explanation for these differences is the difference in design between the two studies. In Experiment II, the group of bulls receiving low nutrition received a diet of plain forage (no concentrate), but intake was not limited. That differs from Experiment III, during which intake was limited in the low nutrition group. Perhaps LH secretion is regulated not only the availability of nutrients, but also by the central center responsible for the sensations of hunger and satiety located in the hypothalamus (Baile and McLaughlin, 1987). The inhibitory effects of limited availability of nutrients on LH secretion appeared to be exerted only on the hypothalamus (Experiment II), whereas the combination of limited availability of nutrients with the hunger sensation experienced by bulls with restricted intake in Experiment III affected both hypothalamic and pituitary function, producing a much more severe inhibition of LH secretion.

Circulating IGF-I concentrations increased constantly during calfhood and the peripubertal period, and only reached a plateau (or decreased slightly) when sexual development was completed (i.e. after maturity and maximal testicular growth), indicating that IGF-I may be involved in regulating sexual development. Increased GnRH/LH secretion associated with high nutrition was associated with increased IGF-I concentrations, whereas reduced GnRH/LH secretion associated with low nutrition was associated with decreased IGF-I concentrations. These temporal associations strongly argue for a regulatory role of IGF-I on GnRH secretion; however, more studies should be conducted to determine if IGF-I can indeed promote GnRH secretion in bulls.

Nutrition also affected testicular steroidogenesis (testosterone concentrations), reflecting effects on Leydig cells number, function, or both. The increase in physiological and GnRH-stimulated circulating testosterone concentrations observed with age was hastened in bulls receiving high nutrition and delayed in bulls receiving low nutrition. Since LH and IGF-I have crucial, complementary roles in promoting Leydig cells proliferation, differentiation, and testosterone secretion (Wang and Hardy, 2004; Wang et al., 2003), the effects of nutrition on testicular steroidogenesis were probably mediated by both LH secretion and IGF-I concentrations.

There was some variation in circulating leptin concentrations changes during sexual development among experiments. While leptin concentration increased at approximately 30 to 34 wk of age in Experiments I and II, considerable increases in leptin concentrations were observed only after 50 wk of age in Experiment III. A possible explanation for these differences is the difference in RIA procedures among experiments, i.e. a multi-species commercial kit was used in Experiments I and II and a bovine-specific procedure was used in Experiment III; however, there did not seem to be great differences in absolute concentrations among studies. There was great variation in the changes in circulating insulin concentrations; a considerable increase in insulin concentrations was observed after approximately 34 wk in Experiments II and III, after 44 wk in Experiment I, and after 58 wk in Experiment IV. In general, GH concentrations decreased during sexual development in bulls, concomitant with the increase in IGF-I concentrations, indicating that there may be important sources of circulating IGF-I other than the liver (perhaps the testes) in developing bulls.

A consistent observation was that leptin, insulin, and GH concentrations did not differ among groups during the early gonadotropin rise and therefore could not be involved in the differences in LH secretion produced by different nutrition. This indicates that the role of these hormones, if any, in regulating GnRH secretion is permissive. However, leptin and insulin had moderate to good correlations with SC and paired testes volume in Experiments I and II, indicating that these hormones may have beneficially affect testicular development.

Changes in testes echotexture during sexual development were obseved, although differences among groups of bulls with different ages at puberty were not always clear. Surprisingly, high nutrition did not have a significant effect on testicular vascular cone fat cover. However, high nutrition during calfhood and the peripubertal period (Experiment II) resulted in increased scrotal temperature with possible adverse consequences in future sperm production and semen quality.

In conclusion, reproductive function in bulls could be maximized by providing high nutrition during calfhood (preweaning period) and adequate nutrition during the peripubertal period (postweaning period). Based on the results presented in this thesis, it is suggested that target average daily gain during calfhood should be > 1.2 kg/d. Future research that would contribute to the understanding of the physiological mechanisms linking nutrition and reproduction in bulls should include direct evaluation of the effects of circulating IGF-I on GnRH/LH secretion. Nutritional and pharmacological manipulation of the early gonadotropin rise during calfhood also warrant investigation.

8. SUMMARY

- Nutrition is involved in regulating GnRH secretion in bulls.

- Increased nutrition during calfhood resulted in a more sustained increase in LH pulse frequency during the early gonadotropin rise and greater testicular development at maturity.

- Low nutrition during calfhood suppresses LH secretion during the early gonadotropin rise and results in delayed puberty and reduced testicular development at maturity.

- Restricted intake during calfhood results in compromised hypothalamic and pituitary function with more severe effects on LH secretion than reduced availability of nutrients.

- IGF-I is a possible signal to the central “metabolic sensor” involved in translating body nutritional status to the GnRH pulse generator.

- Effects of nutrition on testicular steroidogenesis (Leydig cells number and function) are probably mediated by both LH secretion and IGF-I concentrations.

- Leptin and insulin may have only permissive roles on GnRH secretion, but may be positively involved in testicular development.

- GH concentrations decrease concomitantly with increasing IGF-I concentrations during sexual development in bulls, suggesting that perhaps the testes could contribute with considerable amounts of circulating IGF-I.

- Management strategies to optimize reproductive function in bulls should focus on increasing nutrition during calfhood.

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