

The effect of nutrition on sexual development of bulls

A.D. Barth^{*}, L.F.C. Brito, J.P. Kastelic

*Department of Large Animal Clinical Sciences, Western College of Veterinary Medicine,
University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 5B4*

Abstract

Most bulls that are managed for sale as yearlings are fed high-energy diets in the post-weaning period to maximize rates of gain in body weight. High-energy diets with adequate protein, vitamins and minerals result in a larger scrotal circumference at 1 y of age, however, part of this increase in size is likely due to scrotal fat. It is unclear whether testis size and spermatogenesis is significantly affected by nutritional intake in the post-weaning period.

There are indications of an effect of calthood nutrition on age at puberty and testis size. Scrotal circumference was smaller in yearling bulls raised by first-parity dams, compared to those raised by older dams. This may have been due to lower milk production by first-parity dams, an in utero effect, or both. The effect of reduced calthood nutrition may be mediated through gonadotropin secretion.

Calves destined to become later maturing bulls with smaller testes had lower amounts of LH secretion during the period of the early gonadotropin rise (8–16 wk of age). Furthermore, augmenting circulating LH concentrations at this time by treating calves with GnRH hastened pubertal development. In addition, FSH treatments in calthood also increased scrotal circumference and hastened spermatogenesis. In that regard, FSH has been considered a main driver of Sertoli cell proliferation in prepubertal animals. Since Sertoli cell multiplication ceases at 20–25 wk of age in bulls, final testis size in bulls is likely determined in calthood.

Four experiments were done to investigate the effects of calthood nutrition on pubertal development. These studies confirmed that superior calthood nutrition augmented gonadotropin secretion (which is probably mediated by metabolic hormones); this resulted in larger testes at 1 y of age and an earlier onset of spermatogenesis.

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1. Introduction

In the past 10–15 y, it has become common to use yearling *Bos Taurus* bulls as primary breeders. In a 10-y study conducted in a western Canadian research herd, yearling bulls used at bull to female ratios of 1:25 achieved fertility comparable to that of 2-y-old bulls [1].

The use of bulls at an early age reduces production costs, shortens the generation interval and may increase genetic gains. However, the variability of onset of puberty (among and within breeds) has resulted in great variability in reproductive performance of young bulls in producer herds. Poor reproductive performance by yearling bulls may be due partly to inadequacy in mating ability; however, semen quality is a more important factor. Age of puberty, and subsequently maturity, is the main factor involved in semen quality in yearling bulls. Therefore, the successful use of yearling bulls will depend on production systems that hasten maturity.

^{*} Corresponding author. Tel.: +1 306 966 7151;
fax: +1 306 966 7159.

E-mail address: albert.barth@usask.ca (A.D. Barth).

2. Effect of post-weaning nutrition

Most bulls that are managed for sale as yearlings are fed high-energy diets in the post-weaning period to maximize rates of gain in body weight. High-energy diets with adequate protein, vitamins and minerals may hasten the onset of puberty in bulls [2]. Different levels of nutrition after weaning appear to affect the rate of testicular growth; however, it is not clear whether age of onset of puberty is also affected [3–7]. In one study [3], beef bulls were fed a high-concentrate diet (high energy) or a hay +1/2 concentrate (low energy) diet from weaning (6 mo of age) for a 168-d test period. The test period was divided into two periods of 77 d, separated by a 14-d adjustment period. Four dietary regimens were fed during the test periods resulting in energy levels of high–high, high–low, low–high or low–low. Those on the highest plane of nutrition had the largest scrotal circumference (SC) which may indicate accelerated pubertal development (Table 1). However, this may also have been due to increased scrotal fat, with no difference in age at puberty.

The high–high energy diet appeared to have a detrimental effect on semen quality. However, the bulls were only 11.5 and 13.5 mo of age when semen was examined, a time when most bulls are still undergoing pubertal changes in semen quality. Therefore, it was not possible to separate the effect of immaturity from the effects of diet on semen quality.

Pruitt et al. [4] reported that high-energy intake in the post-weaning period, up to approximately 12 mo of age in beef bulls, did not impair future semen quality, provided that rations from 1 to 2 y of age did not result in excessive fattening. Ohl et al. [5] examined the effects of rate of gain on SC and testicular histology in 23 half-sibling beef bulls. The bulls were fed high- or low-gain rations from 11.6 to 15.3 mo of age. At the end of the test period, the mean SC was 34.0 and 31.7 for high- and low-gain rations, respectively. However, sperm morphology was not different between groups on Days 50 and 111. Seidel et al. [6] fed two groups of Angus bulls for 154 d, from 7 to 11 mo of age, on a diet containing 133 and 95% of requirements for total

digestible nutrient (TDN). At the end of the feeding period, there was a larger SC in bulls on the high-energy ration. Interestingly, the weight of the testes recovered at slaughter was larger for the high-energy group, but not significantly larger, and the bulls on the high plane of nutrition had heavier scrotal weights. Based on these studies, we inferred that larger SC measurements at 1 y of age in bulls fed for maximal growth were partly due to hastened testis development and partly due to excess fat deposition in the scrotum. In these studies, high-energy rations in the post-weaning period did not hasten the age at which good-quality semen was produced. In another study [7], Angus and Hereford bulls fed a high-energy diet (80% grain and 20% forage) from weaning to 15 mo of age had a greater average SC at 12 mo, but not at 15 mo, than bulls fed a medium-energy diet. Bulls on the high-energy diet also had significantly lower sperm outputs at 15 mo than bulls on a medium-energy diet. Therefore, high-energy rations continued past 12 mo of age could have a detrimental effect on semen quality, perhaps due to an increase in scrotal fat and impaired testicular thermoregulation.

Restricted dietary protein has deleterious effects on sexual function. In a series of experiments [8,9], 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 84–170 d. Testes, epididymis, and seminal glands weights were markedly reduced in bulls fed protein-deficient rations, and seminiferous tubule diameter and seminiferous epithelium thickness were smaller in bulls with a restricted protein intake. Sperm morphology and motility were not adversely affected until CP was reduced to 1.35%. It was noteworthy that protein restriction was so severe in these studies that some bulls died or were slaughtered as they were nearly moribund; these bulls had lost approximately 40% of their initial body weight [8,9]. In another study, body weight, SC, total sperm in the ejaculate, and sperm motility were greater after 12–14 mo of age in bulls receiving a high protein diet

Table 1

Effect of level of dietary energy in bulls, from 6 to 11.5 mo of age, on scrotal circumference and semen quality at the end of the test period [3]

	Diet			
	High–high	High–low	Low–high	Low–low
Number of bulls	21	26	25	20
Scrotal circumference (cm)	38.3	36.4	36.4	35.3
Abnormal sperm (%)	24.2	15.2	17.1	16.4

(14.5%) than in bulls receiving low protein (8.5%) from 6 to 21 mo of age [10].

Based on these studies, the nutritional level after weaning affected SC, however, it is not clear whether the earlier attainment of larger testis size in bulls on higher nutritional level resulted in earlier production of good-quality semen, i.e., maturity. It appears that high-energy intakes from weaning to 12 mo of age will not impair semen quality in bulls at maturity; however, there is evidence that excessive energy intake in young bulls may result in abnormal foot growth due to laminitis [11], as well as abnormal bone and cartilage growth, resulting in stiffness and lameness. In addition high-energy diets increase the risk of rumen inflammation and liver abscesses, which may lead to the development of infection of the vesicular glands [1].

3. Effect of calfhood nutrition

There is very little information regarding the effect of calfhood nutrition on sexual development. In a study by Bratton et al. [2], 30 Holstein bull calves were raised on three levels of energy from 1 to 80 wk of age. The low, medium- and high-energy rations, were expected to provide 60–75, 100, and 140–160% of normal requirements, respectively. Semen was collected at 14-d intervals. The results are shown (Table 2).

In this study, restriction of feed intake in calves from 1 to 80 wk of age had a tremendous effect on pubertal development. In another study, bull calves raised to weaning age by first-parity dams (calving at 2 y of age) had smaller testes at a year of age than those raised by older cows [12]. Perhaps puberty is delayed by nutritional restriction in calfhood, regardless of adequate post-weaning nutrition.

In studies of the endocrinology of sexual development in bulls, gonadotropin concentrations in the prepubertal period were related to age of onset of puberty. Sexual development can be divided into the infantile, prepubertal, and pubertal periods, according

to changes in gonadotropins and testosterone concentrations. The infantile period is characterized by low gonadotropin and testosterone secretion, and extends from birth to approximately 8 wk of age. A transient increase in gonadotropin secretion occurs from approximately 8–20 wk of age; this has been called the ‘early gonadotropin rise’ and characterizes the prepubertal period. Testosterone concentrations begin to rise during the prepubertal period. The pubertal period corresponds to the period of accelerated reproductive development after 20 wk of age through puberty; during this period, gonadotropin secretions decrease, although testosterone secretion continues to increase [13–19].

There is evidence that calves destined to become later maturing bulls with smaller testes have lower amounts of LH secretion during the early gonadotropin rise [20,21]. Furthermore, augmenting LH production just before the early gonadotropin rise by giving calves exogenous GnRH hastened pubertal development [22]. Bull calves were treated with GnRH every 2 h for 2 wk from 4 to 6 wk of age. In treated calves, mean serum testosterone and LH pulse amplitude were increased at 24 wk of age, and at 52 wk of age, treated calves had enhanced testicular growth and spermatogenesis and a greater number of Sertoli cells per tubule. Conversely, when LH, FSH and testosterone production were suppressed by treatment with Leuprolide at 6, 10 and 14 wk of age, testis weight was reduced, and there were lower numbers of spermatids and spermatocytes at 50 wk of age [23].

Sertoli cells are able to support a finite number of germinal cells [24]. A key element in seminiferous tubule size, and therefore testis size, is the number of Sertoli cells. In bulls, Sertoli cell multiplication ceases at 20–25 wk of age [25]; thus final testis size would be determined before weaning. It is noteworthy that FSH has been considered a main driver of Sertoli cell proliferation in prepubertal animals (rats) [26]. Therefore, calves that achieve greater FSH output in calfhood would be expected to develop larger testes and possibly an earlier age of puberty. In a study by Bagu et al. [25], bull calves were treated with 3 mg LH, or 4 mg FSH once every 2 d from 4 to 8 wk after birth. The age at which scrotal circumference first reached ≥ 28 cm occurred earlier in FSH-treated calves compared to saline-treated (control) calves. Based on testicular histology at 56 wk of age, treatment with FSH increased numbers of Sertoli cells, elongated spermatids and spermatocytes per seminiferous tubule. These observations were consistent with those of Bame et al. [27] in which calves immunized against inhibin in calfhood

Table 2
Effect of level of nutrition on age of onset of puberty in Holstein bulls [2]

	Level of feed intake		
	Low	Medium	High
Age at puberty (wk)	57	49	43
Weight at puberty (kg)	255	288	330
Number of ejaculates up to 80 wk	12	14	19
Sperm per ejaculate ($\times 10^9$)	2.3	3.8	3.7
60–90 d pregnancy rates	74.1	72.9	74.2

had increased mean serum FSH concentrations and augmented spermatogenesis. Treatment with LH also appeared to have stimulatory effects on testis development; however, in this experiment, endpoints were not significantly different than those of saline-treated controls.

Overall, the magnitude of the early gonadotropin rise between 6 and 20 wk of age appeared to be a critical factor in the initiation and timing of pubertal development in bulls.

The level of nutritional intake during the early gonadotropin rise of development could have important effects on age of sexual maturity by affecting Sertoli cell multiplication. It is not clear whether the level of nutrition intake after 20 wk of age has an additional effect on age at onset of puberty or age at maturity (as measured by semen quality) in yearling bulls. There is clinical evidence of an effect of calthood nutrition on age at puberty and testis size. In two separate reports [12,25], scrotal circumference was smaller in yearling bulls that were raised by first-parity dams compared to those raised by older dams. Presumably, first-parity dams produce less milk thus affecting pubertal development, however, an in utero effect was also possible.

4. The relationship of nutrition and hormonal mechanisms

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 called the “metabolic sensor”. This sensor translates signals provided by circulating concentrations of specific hormones into neuronal signals that ultimately regulate the GnRH pulse generator and control reproduction. 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–gonad axis and affect sexual development [28–33].

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 [34,35]. In female cattle, acute fasting

decreased circulating leptin and LH concentrations, but LH secretion was restored by leptin treatments [35,36].

Insulin receptors have been demonstrated in the hypothalamus in mice; those with disrupted insulin receptors had reduced circulating LH concentrations and impaired spermatogenesis [37]. Insulin receptors were also observed in the hypothalamus in rams; increased GnRH/LH secretion promoted by improved nutrition was accompanied by increased insulin concentrations in blood and cerebrospinal fluid [38].

Growth hormone and IGF-I concentrations increased during sexual development in humans and have been suggested to be involved in regulating GnRH secretion [39].

Insulin and IGF-I receptors have been identified in Leydig cells in several species [40–42] and IGF-I receptors have also been identified in rat Sertoli cells [43]. 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. Insulin and IGF-I increased in vitro basal and stimulated testosterone secretion in a dose-dependent manner [41,44,45]. These metabolic hormones, among others, might be involved in the continued development of the testis after the decline of gonadotropin concentrations in the peripubertal period.

A series of experiments were designed to test the hypothesis that calthood nutrition affects sexual development in bulls. The objective of these experiments was to evaluate the effects of nutrition on endogenous metabolic hormones, gonadotropins and testosterone concentrations, sexual development, sperm production, and semen quality in bulls. These experiments were reported in full in the PhD thesis of Leonardo Brito, University of Saskatchewan, 2006, and were the first to document the temporal relationships among metabolic hormones (leptin, insulin, growth hormone, and IGF-I), gonadotropins, and testosterone during the entire period of sexual development in bulls. The following paragraphs summarize the highlights of this thesis, which is available on-line (<http://library.usask.ca/theses/available/etd-04012006-184638/>).

5. Materials and methods

Angus and Angus × Charolais bull calves from first-parity dams were weaned at 8 wk of age and were used in four experiments to investigate the effects of nutrition. Although calves were actually weaned at 8 wk of age for purposes of this experiment, the common age of weaning in producer herds is at

approximately 26 wk of age. Therefore, for ease of discussion, for the remainder of this paper, the term post-weaning will refer to calves that are older than 26 wk of age, whereas the term calfhood will refer to the period prior to 26 wk.

The four experiments were as follows:

Experiment I, the post-weaning period (27–31 to 70 wk of age).

Experiment II, calfhood (10 to 26–30 wk of age) throughout the post-weaning period.

Experiment III, feed restriction during calfhood.

Experiment IV, feed supplementation during calfhood.

Diets were mainly composed of barley silage, rolled barley grain and canola meal, and were balanced for minerals and vitamins. The low-nutrition diets contained no concentrate and total digestible nutrients were adjusted in medium- and high-nutrition diets by addition of concentrates.

In Experiment I, 40 calves received the same medium-nutrition diet *ad libitum* from 8 to 26 wk of age. After 26 wk, they received low-, medium-, or high-nutrition diets *ad libitum* until 70 wk of age (proportion of concentrate; low 0%, medium 6.6%, and high 37%).

In Experiment II, 37 calves received low-, medium-, or high-nutrition diets *ad libitum* from 10 to 70 wk of age.

In Experiment III, 44 calves received low- or medium-nutrition diets from 10 to 26 wk of age. Bulls in the medium-nutrition group were fed *ad libitum*, whereas 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), whereas the diet was changed to the same as that fed to the medium-nutrition group (low/medium) or to high nutrition (low/high) for bulls previously receiving low nutrition. Bulls were fed *ad libitum* after 27 wk of age.

In Experiment IV, 33 calves received medium or high nutrition *ad libitum* from 10 to 30 wk of age. From 31 to 74 wk of age, all bulls received the same medium nutrition.

The bulls were examined every 4 wk during the experimental period. Body weight, backfat and scrotal circumference were measured. Once SC reached 26 cm, semen collection (electroejaculation) was attempted every 2 wk. Puberty was defined as the first time that an ejaculate contained $\geq 50 \times 10^6$ spermatozoa with $\geq 10\%$ motile spermatozoa [46]. Following confirma-

tion of puberty, ejaculates were collected once every 4 wk and sperm morphology was determined in eosin–nigrosin stained smears. Sexual maturity was characterized by an ejaculate containing $\geq 70\%$ morphologically normal spermatozoa [47]. Bulls were sent to slaughter at the end of the experimental period (approximately 16 mo of age) and the testes were recovered and weighed.

Blood samples were collected from seven or eight bulls from each treatment 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 sampling, every 15 min for 10 h, was done every 4 wk from 10 to 26 wk of age, and at 44 and 48 wk of age. Gonadotropin-releasing hormone (GnRH) was administered after the 10 h intensive sampling and blood samples were collected every 15 min for 90 min. Additionally, single blood samples were collected every 2 or 4 wk during periods when intensive samplings were not performed. In Experiment III, intensive blood sampling, followed by GnRH challenge, was done every 4 wk from 14 to 34 wk of age and single blood samples were collected every 4 wk during the period when intensive sampling was not done. In Experiment IV, intensive blood sampling followed by GnRH challenge was done every 4 wk from 14 to 30 wk of age, and single blood samples were collected every 4 wk during the period when intensive sampling was not done. In Experiments II, III, and IV, LH concentrations were determined in serum samples obtained during the entire intensive sampling period, and after GnRH treatment. Concentrations of leptin, insulin, GH, IGF-I, FSH, and testosterone 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.

6. Results

6.1. Experiment 1: effect of post-weaning nutrition

The effect of nutrition on body development was unexpected. Bulls on low nutrition were lighter than bulls on medium nutrition, but did not differ significantly from bulls on high nutrition. It is difficult to explain the outcome in body weight gains; however,

Table 3

Mean ($\pm$ S.E.M.) age at puberty and maturity, testes weight, and proportion of normal sperm at 70 wk of age of age in beef bulls receiving low, medium, and high nutrition from 26 to 70 wk of age (Experiment 1)

	Low ($n = 14$)	Medium ($n = 13$)	High ($n = 13$)
Age at puberty (d)	301.6 $\pm$ 7.7 ^a	328.4 $\pm$ 6.2 ^b	299.3 $\pm$ 8.0 ^a
Age at maturity (d)	364.5 $\pm$ 11.0	373.7 $\pm$ 11.4	360.8 $\pm$ 14.0
Paired testes weight (g)	618.7 $\pm$ 27.4	573.6 $\pm$ 12.7	610.8 $\pm$ 17.7
Normal sperm (%)	80.2 $\pm$ 6.7	80.2 $\pm$ 5.9	80.7 $\pm$ 9.4

^{a,b}Numbers in rows with different superscripts differ ($P < 0.05$).

Table 4

Mean ($\pm$ S.E.M.) age at puberty and maturity, testes weight, and proportion of normal sperm at 70 wk of age in beef bulls receiving low, medium, and high nutrition from 10 to 70 wk of age (Experiment 2)

	Low ($n = 13$)	Medium ($n = 12$)	High ($n = 12$)
Age at puberty (d)	326.9 $\pm$ 5.5	304.7 $\pm$ 7.4	292.3 $\pm$ 4.6
Age at maturity (d)	390.5 $\pm$ 7.9	384.3 $\pm$ 6.8	397.3 $\pm$ 11.4
Paired testes weight (g)	523.9 $\pm$ 25.8 ^a	552.4 $\pm$ 21.1 ^a	655.2 $\pm$ 21.2 ^b
Normal sperm (%)	76.3 $\pm$ 6.0	77.5 $\pm$ 5.5	72.4 $\pm$ 6.0

^{a,b}Numbers in rows with different superscripts differ ($P < 0.05$).

since dietary intake was ad libitum, bulls on low nutrition might have compensated by higher feed intake. Bulls on medium nutrition were older at puberty, despite being generally heavier (Table 3).

6.2. Experiment 2: effect of nutrition during calfhood and throughout post-weaning

Bulls on low nutrition were lighter, and had smaller testes and delayed puberty when compared with bulls on medium or high nutrition (Table 4). 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.

Table 5

Mean ($\pm$ S.E.M.) age at puberty and maturity, testes weight, and proportion of normal sperm at 70 wk of age in beef bulls receiving low or medium nutrition from 10 to 26 wk of age, and medium or high nutrition from 27 to 70 wk of age (Experiment 3)

	Medium/medium ($n = 15$)	Low/high ($n = 14$)	Low/medium ($n = 15$)
Age at puberty (d)	293.0 $\pm$ 8.0 ^a	333.7 $\pm$ 12.1 ^b	334.0 $\pm$ 8.5 ^b
Age at maturity (d)	366.4 $\pm$ 12.8	392.6 $\pm$ 10.6	388.7 $\pm$ 10.8
Paired testes weight (g)	597.4 $\pm$ 11.2 ^a	547.6 $\pm$ 18.6 ^{ab}	503.1 $\pm$ 22.0 ^b
Normal sperm (%)	78.1 $\pm$ 3.5	77.3 $\pm$ 5.9	78.1 $\pm$ 3.5

^{a,b}Numbers with superscripts not in common differ ($P < 0.05$).

6.3. Experiment 3: feed restriction during calfhood, with or without supplementation post-weaning

Bulls with restricted nutrition during calfhood had a lower early gonadotropin rise; however, there were no differences in LH secretion among groups when rations were changed to medium or high nutrition in the post-weaning period. Therefore, nutrition affected the GnRH pulse generator in the hypothalamus, during the period of the early gonadotropin rise in bulls. Low nutrition during calfhood delayed body and testicular development, even after feed supplementation during the post-weaning period. Bulls on low nutrition during calfhood were older at puberty and had smaller testes at 16 mo of age (Table 5). Therefore, nutrition during calfhood had long-term effects on sexual development, regardless of the nutrition offered during the peripubertal period.

6.4. Experiment 4: effect of feed supplementation during calfhood

In this study, bulls receiving supplemental feed during calfhood had a greater and more sustained

Table 6

Mean ($\pm$ S.E.M.) age at puberty and maturity, testes weight, and proportion of normal sperm at 74 wk of age in beef bulls receiving medium or high nutrition from 10 to 30 wk of age and medium nutrition from 31 to 74 wk of age (Experiment 4)

	Medium ($n = 16$)	High ($n = 17$)
Age at puberty (d)	326.9 $\pm$ 9.3	314.1 $\pm$ 8.3
Age at maturity (d)	381.5 $\pm$ 9.2	380.6 $\pm$ 14.9
Paired testes weight (g)	531.2 $\pm$ 18.4 ^a	610.5 $\pm$ 27.9 ^b
Normal sperm (%)	74.8 $\pm$ 3.5	74.3 $\pm$ 4.6

^{a,b}Numbers in rows with different superscripts differ ($P < 0.05$).

increase in LH pulse frequency during the period of the early gonadotropin rise. Feed supplementation above normally recommended levels during calthood hastened body development and increased testicular mass and sperm production at 16 mo of age (Table 6). The beneficial effects of feed supplementation during calthood extended beyond the period of supplementation.

Scrotal circumference increased continuously during the experimental period in bulls in the medium-nutrition group, whereas SC in the high-nutrition group increased until 66 wk of age. Although SC measurements did not increase in the high-nutrition group at 70 and 74 wk of age, they remained higher at those times than SC measurements in the medium-nutrition group. Paired testes volume increased ($P < 0.05$) until 66 wk of age in both groups.

7. Discussion

In Experiment 1, post-weaning nutrition did not result in significant weight differences, probably because bulls receiving low and medium nutrition compensated with greater intake. The lack in weight differences was reflected in the lack of differences in age at onset of puberty, SC at both 12 and 16 mo, and testis weight at 16 mo. Most data in the literature deal with post-weaning nutrition; there are contradictory results regarding the effect of post-weaning nutrition on age at onset of puberty. However, a constant observation was that higher energy diets in the post-weaning period result in greater SC and increased testis weight at 12–15 mo of age. High-energy intakes in the post-weaning period most likely result in achievement of maximum testes size earlier, whereas low post-weaning nutrition results in slower development of the testes. However, within a reasonable range, post-weaning nutrition likely does not influence testis size of mature bulls. The effect of nutrition on age at maturity, as determined by the first production of $\geq 70\%$ normal sperm, has so far not been

clearly elucidated by any published studies; however, it could be surmised that earlier onset of puberty should lead to earlier production of good-quality semen.

These experiments clearly demonstrated that nutrition during calthood affected LH secretion during the early gonadotropin rise, with consequent effects on sexual development. The effects of nutrition during the post-weaning 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 in Experiment I. Beef bull calves are usually nursing during calthood 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 calthood will have greater beneficial effects on reproductive function than management practices during the post-weaning period in bulls.

These results demonstrated that in bulls, as in other species and in females [29,31,32], nutrition is involved in regulating GnRH secretion, with consequent effects on sexual development. Gonadotropin secretion after GnRH challenge was not consistently affected by nutrition, indicating that the effects of nutrition may not involve the pituitary. However, during the period of the early gonadotropin rise in bulls, nutrition regulated the hypothalamus–pituitary–testis axis by modulating the GnRH pulse generator in the hypothalamus. High nutrition during calthood resulted in a more sustained increase in LH pulse frequency during the early gonadotropin rise and greater testicular development at maturity; therefore, LH secretion during calthood 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 calthood [22].

Conversely, restricted nutrition during calthood suppressed LH secretion during the early gonadotropin rise and delayed puberty and reduced testicular development at maturity. Similar effects have been produced by suppressing LH secretion with prolonged GnRH-agonist treatments during calthood in bulls [23]. Reduced nutrition could be associated with a smaller SC in bulls raised by first-parity dams. Bagu et al. (personal communication) observed reduced LH secretion at 8–20 wk of age in calves raised by first-parity dams compared to calves raised by older cows. However, an in utero effect on calf growth and development might also be involved in reduced testes size and delayed puberty in calves raised by first-parity dams.

GnRH-stimulated LH secretion was reduced in bulls receiving restricted calthood 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 forage only (no concentrate), but intake was not limited. In contrast, intake was limited in the low-nutrition group in Experiment III. Perhaps LH secretion is regulated not only by the availability of nutrients, but also by the central center responsible for the sensations of hunger and satiety located in the hypothalamus [48]. 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.

There were elevated FSH concentrations during the early gonadotropin rise in these experiments. This rise in FSH is likely also involved in earlier onset of puberty, as treatment of calves with bFSH from 4 to 8 wk of age hastened testicular growth, resulted in greater numbers of Sertoli cells, and hastened spermatogenesis [25].

Circulating IGF-I concentrations increased constantly during calthood and the peripubertal period, and only reached a plateau (or decreased slightly) when sexual development was completed (semen quality similar to mature bulls), 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 cell 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 cell proliferation, differentiation, and testosterone secretion [49,50], the effects of nutrition on testicular steroidogenesis were probably mediated by both LH secretion

and IGF-I concentrations. 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.

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. Therefore, 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 promote testicular development.

In conclusion, reproductive function in bulls could be maximized by providing high nutrition during calthood and adequate nutrition in the post-weaning period. The results of these experiments suggest that a target for average daily gain during calthood 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 calthood also warrant investigation.

References

- [1] Farid A, Makarechian M, Price MA, Berg RT. Repeatability of reproductive performance of beef bulls as yearlings and 2-year-olds at pasture. *Anim Reprod Sci* 1987;14:21–9.
- [2] Bratton RW, Musgrave SD, Dunn HO, Foote RH, Henderson CR. Semen production and fertility of young bulls raised on three different energy levels of feed intake. *J Anim Sci* 1956;15:1296–7 (Abstr).
- [3] Mwansa PB, Makarechian M. The effect of post weaning level of dietary energy on sex drive and semen quality of young beef bulls. *Theriogenology* 1991;35:1169–78.
- [4] Pruitt RJ, Corah LR, Stevenson JS, Kiracofe GH. Effect of energy intake after weaning on the sexual development of beef bulls. II Age of first mating, age at puberty, testosterone and scrotal circumference. *J Anim Sci* 1986;63:579–85.
- [5] Ohl MW, Ott RS, Faulkner DB, Hornbuckle T, Hess RA, Cmarik GF, et al. Effects of rate of gain on scrotal circumference and histopathologic features of the testes of half-sibling yearling beef bulls. *Am J Vet Res* 1996;57:844–7.
- [6] Seidel Jr GE, Pickett BW, Wilsey CO, Seidel SM. Effect of high level of nutrition on reproductive characteristics of Angus bulls. In: 9th Internat Cong Anim Reprod Artific Insem. III. Symposia (Free communications). 1980. p. 359 (Abstr.).
- [7] Coulter GH, Carruthers TD, Amann RP, Kozub GC. Testicular development, daily sperm production and epididymal sperm reserves in 15-mo-old Angus and Hereford bulls: effects of bull strain plus dietary energy. *J Anim Sci* 1987;64:254–60.

- [8] Meacham TN, Cunha TJ, Warnick AC, Hentges Jr JF, Hargrove DD. Influence of low protein rations on growth and semen characteristics of young beef bulls. *J Anim Sci* 1963;22:115–20.
- [9] Meacham TN, Warnick AC, Cunha TJ, Hentges Jr JF, Shirley RL. Hematological and histological changes in young beef bulls fed low protein rations. *J Anim Sci* 1964;23:380–4.
- [10] Rekwot PI, Oyedipe EO, Akerejola OO, Kumi-Diaka J. The effect of protein intake on body weight, scrotal circumference and semen products of Bunaji bulls and their Friesian crosses in Nigeria. *Anim Reprod Sci* 1988;16:1–9.
- [11] Greenough PR, Vermunt JJ, McKinnon JJ, Fathy FA, Berg PA, Cohen RDH. Laminitis-like changes in the claws of feedlot cattle. *Can Vet J* 1990;31:202–8.
- [12] Lunstra DD, Gregory KE, Cundiff LV. Heritability estimates and adjustment factors for the effects of bull age and age of dam on yearling testicular size in breeds of bulls. *Theriogenology* 1988;30:127–36.
- [13] Amann RP. Endocrine changes associated with onset of spermatogenesis in Holstein bulls. *J Dairy Sci* 1983;66:2606–22.
- [14] Amann RP, Walker OA. Changes in the pituitary–gonadal axis associated with puberty in Holstein bulls. *J Anim Sci* 1983;57:433–42.
- [15] Amann RP, Wise ME, Glass JD, Nett TM. Prepubertal changes in the hypothalamic–pituitary axis of Holstein bulls. *Biol Reprod* 1986;34:71–80.
- [16] Evans A, Pierson R, Garcia A, McDougall L, Hrudka F, Rawlings N. Changes in circulating hormone concentrations, testes histology and testes ultrasonography during sexual maturation in beef bulls. *Theriogenology* 1996;46:345–57.
- [17] Lacroix A, Pelletier J. Short-term variations in plasma LH and testosterone in bull calves from birth to 1 year of age. *J Reprod Fertil* 1979;55:81–5.
- [18] McCarthy MS, Convey EM, Hafs HD. Serum hormonal changes and testicular response to LH during puberty in bulls. *Biol Reprod* 1979;20:1221–7.
- [19] McCarthy MS, Hafs HD, Convey EM. Serum hormone patterns associated with growth and sexual development in bulls. *J Anim Sci* 1979;49:1012–20.
- [20] Aravindakshan JP, Honaramooz A, Bartlewski PM, Beard AP, Pierson RA, Rawlings NC. Pattern of gonadotropin secretion and ultrasonographic evaluation of developmental changes in the testis of early and late maturing bull calves. *Theriogenology* 2000;54:339–54.
- [21] Evans A, Davies F, Nasser L, Bowman P, Rawlings N. Differences in early patterns of gonadotrophin secretion between early and late maturing bulls, changes in semen characteristics at puberty. *Theriogenology* 1995;43:569–78.
- [22] Chandolia RK, Honaramooz A, Bartlewski PM, Beard AP, Rawlings NC. Effects of treatment with LH releasing hormone before the early increase in LH secretion on endocrine and reproductive development in bull calves. *J Reprod Fertil* 1997;111:41–50.
- [23] Chandolia RK, Evans AC, Rawlings NC. Effect of inhibition of increased gonadotrophin secretion before 20 weeks of age in bull calves on testicular development. *J Reprod Fertil* 1997;109:65–71.
- [24] Orth JM. The role of follicle stimulating hormone in controlling Sertoli cell proliferation in testes of fetal rats. *Endocrinology* 1984;115:1248–55.
- [25] Bagu ET, Madgwick S, Duggavathi R, Bartlewski PM, Barrett DMW, Huchkowsky S, et al. Effects of treatment with LH or FSH from 4 to 8 weeks of age on the attainment of puberty in bull calves. *Theriogenology* 2004;62:861–73.
- [26] Singh J, Handelsman DJ. Neonatal administration of FSH increases Sertoli cell numbers and spermatogenesis in gonadotropin deficient (*hpg*) mice. *J Endocrinol* 1996;151:37–48.
- [27] Bame JH, Dalton JC, Degelos SD, Good TEM, Ireland JLH, Jimenez KF, et al. Effect of long term immunization against inhibin on sperm output in bulls. *Biol Reprod* 1999;60:1360–6.
- [28] Blache D, Zhang S, Martin GB. Fertility in male sheep: modulators of the acute effects of nutrition on the reproductive axis of male sheep. *Reproduction Suppl* 2003;61:387–402.
- [29] l'Anson H, Foster DL, Foxcroft GR, Booth PJ. Nutrition and reproduction. In: Milligan SR, editor. Oxford reviews of reproduction. Oxford: Oxford University Press; 1991. p. 239–311.
- [30] Martin GB, Walkden-Brown SW. Nutritional influences on reproduction in mature male sheep and goats. *J Reprod Fertil Suppl* 1995;49:437–49.
- [31] Schneider JE. Energy balance and reproduction. *Physiol Behav* 2004;81:289–317.
- [32] Williams GL. Nutrition factors and reproduction. In: Knobil E, Neil JD, editors. Encyclopedia of reproduction. San Diego: Academic Press; 1998. p. 412–22.
- [33] Barb CR, Kraeling R. Role of leptin in the regulation of gonadotropin secretion in farm animals. *Anim Reprod Sci* 2004;82–83:155–67.
- [34] Zieba DA, Amstalden M, Williams GL. Regulatory roles of leptin in reproduction and metabolism: a comparative review. *Domest Anim Endocrinol* 2005;29:166–85.
- [35] Amstalden M, Garcia MR, Stanko RL, Nizielski SE, Morrison CD, Keisler DH, et al. Central infusion of recombinant ovine leptin normalizes plasma insulin and stimulates a novel hypersecretion of luteinizing hormone after short-term fasting in mature beef cows. *Biol Reprod* 2002;66:1555–61.
- [36] Maciel MN, Zieba DA, Amstalden M, Keisler DH, Neves JP, Williams GL. Leptin prevents fasting-mediated reductions in pulsatile secretion of luteinizing hormone and enhances its gonadotropin-releasing hormone-mediated release in heifers. *Biol Reprod* 2004;70:229–35.
- [37] Bruning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, et al. Role of brain insulin receptor in control of body weight and reproduction. *Science* 2000;289:2122–5.
- [38] Blache D, Chagas LM, Blackberry MA, Vercoe PE, Martin GB. Metabolic factors affecting the reproductive axis in male sheep. *J Reprod Fertil* 2000;120:1–11.
- [39] Wilson ME. The impact of the GH-IGF-I axis on gonadotropin secretion: inferences from animal models. *J Pediatr Endocrinol Metab* 2001;14:115–40.
- [40] Abele V, Pelletier G, Tremblay RR. Radioautographic localization and regulation of the insulin receptors in rat testis. *J Recept Res* 1986;6:461–73.
- [41] Lin T. Regulation of Leydig cell function by insulin-like growth factor-I and binding proteins. *J Androl* 1995;16:193–6.
- [42] Rouiller-Fabre V, Lecref L, Gautier C, Saez JM, Habert R. Expression and effect of insulin-like growth factor I on rat fetal Leydig cell function and differentiation. *Endocrinology* 1998;139:2926–34.
- [43] Borland K, Mita M, Oppenheimer CL, Blinderman LA, Massague J, Hall PF, et al. The actions of insulin-like growth factors I and II on cultured Sertoli cells. *Endocrinology* 1984;114:240–6.
- [44] Bernier M, Chatelain P, Mather JP, Saez JM. Regulation of gonadotropin receptors, gonadotropin responsiveness, and cell multiplication by somatomedin-C and insulin in cultured pig Leydig cells. *J Cell Physiol* 1986;129:257–63.

- [45] Spiteri-Grech J, Nieschlag E. The role of growth hormone and insulin-like growth factor I in the regulation of male reproductive function. *Horm Res* 1992;38(Suppl. 1):22–7.
- [46] Wolf FR, Almquist JO, Hale EB. Prepuberal behavior and puberal characteristics of beef bulls on high nutrient allowance. *J Anim Sci* 1965;24:761–5.
- [47] Brito LF, Silva AE, Unanian MM, Dode MA, Barbosa RT, Kastelic JP. Sexual development in early- and late-maturing *Bos indicus* and *Bos indicus* × *Bos taurus* crossbred bulls in Brazil. *Theriogenology* 2004;62:1198–217.
- [48] Baile CA, McLaughlin CL. Mechanisms controlling feed intake in ruminants: a review. *J Anim Sci* 1987;64:915–22.
- [49] Wang G, Hardy MP. Development of Leydig cells in the insulin-like growth factor-I (IGF-I) knockout mouse: effects of IGF-I replacement and gonadotropic stimulation. *Biol Reprod* 2004;70:632–9.
- [50] Wang GM, O'Shaughnessy PJ, Chubb C, Robaire B, Hardy MP. Effects of insulin-like growth factor I on steroidogenic enzyme expression levels in mouse Leydig cells. *Endocrinology* 2003;144:5058–64.