ABSTRACT

Holstein bull calves often reach artificial insemination centers in suboptimal body condition. Early-life nutrition is reported to increase reproductive performance in beef bulls. The objective was to determine whether early-life nutrition in Holstein bulls had effects similar to those reported in beef bulls. Twenty-six Holstein bull calves were randomly allocated into 3 groups at approximately 1 wk of age to receive a low-, medium-, or high-nutrition diet, based on levels of energy and protein, from 2 to 31 wk of age. Calves were on their respective diets until 31 wk of age, after which they were all fed a medium-nutrition diet. To evaluate secretion profiles and concentrations of blood hormones, a subset of bulls was subjected to intensive blood sampling every 4 wk from 11 to 31 wk of age. Testes of all bulls were measured once a month; once scrotal circumference reached 26 cm, semen collection was attempted (by electroejaculation) every 2 wk to confirm puberty. Bulls were maintained until approximately 72 wk of age and then slaughtered at a local abattoir. Testes were recovered and weighed. Bulls fed the high-nutrition diet were younger at puberty (high = 324.3 d, low = 369.3 d) and had larger testes for the entire experimental period than bulls fed the low-nutrition diet. Bulls fed the high-nutrition diet also had an earlier and more substantial early rise in LH than those fed the low-nutrition diet and had increased concentrations of insulin-like growth factor-I (IGF-I) earlier than the bulls fed the low-nutrition diet. Furthermore, we detected a temporal association between increased IGF-I concentrations and an early LH rise in Holstein bulls. Overall, these results support our hypothesis that Holstein bull calves fed a high-nutrition diet reach puberty earlier and have larger testes than those fed a low-nutrition diet, and they provide clear evidence that nutritional modulation of Holstein bull calves during early life has profound effects on reproductive development.

Key words: Holstein bull, nutrition, puberty, testes

INTRODUCTION

Most bulls in Canadian AI centers are Holstein. Future AI sires are typically born and raised on commercial dairy farms and moved to an AI center at approximately 8 mo of age. As soon as postthaw semen quality is acceptable, semen is collected, cryopreserved, and used in AI programs for progeny testing. Bulls that reach puberty faster (facilitating progeny testing) or produce more doses of semen (greater profitability), or both, are clearly desirable. Regardless, many future potential AI sires arrive at the AI center in suboptimal body condition.

The effects of nutrition on the onset of puberty in beef bulls have been studied previously (Wolf et al., 1965; Brito et al., 2007a,b,c). Beef bull calves fed a high level of nutrition (both protein and energy) during early life have increased (~20–25%) testicular weight and sperm production by 74 wk of age. Therefore, determining the effects of early-life nutrition on puberty and sperm production in Holstein bulls could be of great benefit to the AI industry.

Reproductive development of the bull can be divided into 3 periods: infantile, prepubertal, and pubertal. The infantile period (0–8 wk of age) is characterized by low secretions of both gonadotropins and testosterone (Amann et al., 1986; Rawlings et al., 2008). Thereafter, during the prepubertal period (8 to 20 wk), there is a transient increase in blood gonadotropin concentrations (early gonadotropin rise) and a concurrent increase in testosterone secretion (Amann and Walker, 1983; Barth et al., 2008; Rawlings et al., 2008). Peripheral LH concentrations start to increase at 4 to 5 wk, peak
at 12 to 16 wk, and then decline, reaching a baseline at 25 wk (Amann and Walker, 1983; Barth et al., 2008). It is noteworthy that LH concentrations during the prepubertal period affect sexual development and are inversely related to age at puberty (Amann and Walker, 1983; Evans et al., 1995). Blood FSH concentrations generally increase during the early increase (less marked than corresponding increases in LH) and decrease to baseline by approximately 25 wk (Amann and Walker, 1983; Evans et al., 1995).

The early gonadotropin increase may play a pivotal role in sexual development and maturation of the bull calf (Rawlings et al., 2008). Specifically, the magnitude of prepubertal gonadotropin secretion may be critical in the initiation and extent of testicular development in bulls (Barth et al., 2008). Before 25 wk of age, testicular growth occurs slowly. Thereafter (pubertal period), there is rapid testicular development through puberty, despite low serum gonadotropin concentrations, suggesting that gonadotropin-independent mechanisms regulate testicular development in bulls. Ultimately, decreased sensitivity of the hypothalamus to testosterone and estrogen, with a concomitant increase in concentrations of GnRH, LH, FSH, and testosterone, culminates in puberty.

Experimental evidence indicates that GnRH neurons communicate with a neural system (so-called metabolic sensor) that relays nutritional status to GnRH neurons by detecting concentrations of metabolic hormones (e.g., leptin, IGF-I, insulin, and growth hormone) and nutrients (Blache et al., 2000). Apparently, this communication enables GnRH neurons to overcome the negative feedback effects of testosterone and estradiol, and initiate pulsatile release of GnRH. Determining what metabolic hormones influence GnRH concentrations is important in order to elucidate the effects of nutritional status on reproduction. For example, concentrations of IGF-I are higher during the early gonadotropin rise in beef bulls (Brito et al., 2007a); indeed, because IGF-I concentrations are directly related to LH concentrations, it is thought that IGF-I may be involved in regulating the early gonadotropin rise in bulls (Brito et al., 2007b).

Most of the previous research on the effects of early-life nutrition on reproductive development in bulls was done in beef cattle. The overall objective of this study was to determine whether early-life nutrition in Holstein bulls had effects similar to those reported in beef bulls. We hypothesized that the Holstein bull calves on a high nutrition diet will have greater LH concentrations during the prepubertal phase, reach puberty earlier, and have larger testes than those maintained on a low-nutrition diet.

MATERIALS AND METHODS

Bulls and Treatments

Twenty-six Holstein bull calves were randomly allocated into 3 groups at approximately 1 wk of age to receive either a low-, medium-, or high-nutrition diet from 2 to 31 wk of age. Typically, calves raised in intensive systems are fed milk (twice daily) to 10% of the calves’ BW (approximately 6 L of milk per day; Jasper and Weary, 2002). Therefore, in the present study, calves were fed milk (4, 6, and 8 L/d in the low, medium and high groups, respectively) from 2 to 8 wk of age and thereafter transitioned onto diets based on barley silage (the forage source for all diets). All diets contained 1.6% vitamin-mineral premix (as fed). The low-nutrition diet (n = 8) was barley silage (plus premix, but no concentrate) and had 12.2% CP and 62.9% TDN (note that for this and all other diets, CP and TDN are reported on a DM basis). The medium-nutrition diet (n = 9) contained 4.8% rolled barley, 4.8% rolled corn, 3.8% canola meal, and 3.8% soybean meal (overall, 17.0% CP and 66.0% TDN). The high-nutrition diet (n = 9) consisted of 49.7% rolled barley, 9.7% rolled corn, 7.6% canola meal, and 7.6% soybean meal (20.0% CP and 67.9% TDN). These diets were created and classified as low, medium, and high levels of nutrition based on diets used in a previous study (Brito et al., 2007a,b), and roughly on National Research Council requirements (NRC, 2001). The high-nutrition group was fed ad libitum and, based on their intake, the same amount of feed (on an as-fed basis) was offered to the low- and medium-nutrition groups. Calves were on their respective diets until 31 wk of age, after which they were all fed the medium-nutrition diet. This experiment was conducted in accordance with the guidelines of the Canadian Council on Animal Care and was reviewed and approved by the Lethbridge Research Centre institutional animal care committee.

Sexual Development

Testicular Characteristics. Once monthly, from 8 to 71 wk of age, all bulls were weighed, and scrotal circumference (SC) was determined with a Coulter Scrotal Tape (Trueman Manufacturing, Edmonton, AB, Canada). In addition, the length and width of each testis were measured with calipers, and paired testes volume (PTV) was calculated using the following formula: PTV = 0.5236 × length × width² (Bailey et al., 1998). The PTV as a percentage of overall BW was also calculated for each bull. Concurrent with determination of testicular size, testes were examined by conventional
diagnostic ultrasonography, as described (Brito et al., 2012). Briefly, 2 images (testicular parenchyma and testicular vascular cone) from each testis of each bull were electronically frozen and then recorded. For 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 frozen. Testicular pixel intensity (TPI) was determined on a scale of 1 (white) to 255 (black); therefore, reduced TPI corresponded to increased tissue echodensity (brightness). The area for pixel analysis was selected by drawing a rectangle 0.5 to 1.0 cm deep into the parenchyma, where it appeared homogeneous; the area above the mediastinum was selected and edges of the image were avoided (Gábor et al., 1998). For the testicular vascular cone, the transducer was held horizontally and a cross-sectional image of each cone was recorded. The average diameter of the testicular vascular cones was also determined.

**Blood Samples and Hormone Analyses.** To evaluate blood hormone concentrations and release profiles, a subset of bulls (n = 6 per group) were subjected to intensive blood sampling every 4 wk (the same bulls were used for each sampling period) from 11 to 31 wk of age. Indwelling catheters were placed (into a jugular vein) one afternoon and, starting very early the following morning, blood was collected every 15 min for 11.5 h. At 10 h after the start of blood collection, GnRH (0.04 μg/kg i.v.; Fertagyl 2, Merck Animal Health, Intervet Canada Corp., Kirkland, QC, Canada) was given to stimulate the release of LH and testosterone; thereafter, blood sampling was continued for another 1.5 h. All blood samples were maintained at room temperature overnight to allow them to clot; the following morning, clots were removed and the tubes centrifuged (3,480 × g for 20 min at 20°C). Serum from every sample was removed with a pipette, placed separately in a storage tube, frozen, and stored until analyzed. Every sample was assayed to determine serum LH concentrations, whereas a pooled sample from the first 10 h for each bull was used to evaluate serum concentrations of FSH, testosterone, insulin, IGF-I, and leptin. All samples collected after the GnRH challenge (6 samples per bull) were individually assayed for concentrations of testosterone, LH, and FSH.

Serum concentrations of LH, FSH, and leptin were determined by double-antibody RIA, whereas serum concentrations of testosterone and insulin were determined by solid-phase RIA. Serum IGF-I concentrations were determined with a solid-phase, enzyme-labeled, chemiluminescent immunometric assay on a Siemens Immulite 1000 analyzer (Siemens AG, Erlangen, Germany). Intra- and interassay coefficients of variation were <10% for all hormones. Leptin concentrations were determined using a multi-species leptin RIA kit (Linco Research, St. Charles, MO); the sensitivity of the assay was 1.0 ng/mL. Testosterone and insulin concentrations were determined using solid-phase RIA kits (Siemens, Los Angeles, CA). Testosterone standards were prepared with purified hormone (Sigma Chemical Co., St. Louis, MO) diluted in charcoal-stripped bovine serum; the sensitivity of the testosterone assay was 0.04 ng/mL. The sensitivity of the insulin assay was 1.2 μIU/mL. Gonadotropin concentrations were determined as described (Evans et al., 1995). Luteinizing hormone NIH-bLH-B4 [National Hormone and Peptide Program (NHPP), Torrance, CA] was used for preparation of standards and AFP 11743B bLH (NHPP) was used for tracer labeling. Luteinizing hormone concentrations were determined using rabbit bovine-LH antiserum and the sensitivity of the assay was 0.05 ng/mL. Follicle-stimulating hormone AFP5318C (NHPP) was used for preparation of standards and NIDDK-oFSH-I-2 (NHPP) was used for tracer labeling. Follicle-stimulating hormone concentrations were determined using rabbit ovine-FSH antiserum (anti-oFSH-1; AFPC5288113; NHPP) and the sensitivity of the assay was 0.1 ng/mL.

**Age at Puberty**

Once scrotal circumference reached 26 cm, semen collection was attempted (electroejaculation) every 2 wk to confirm puberty. Puberty was defined as >50 × 10⁶ sperm in the ejaculate with >10% motility (Wolf et al., 1965); the age at which each bull was confirmed to have reached puberty was recorded. In addition, the age at which each bull first achieved a scrotal circumference of 28 cm was considered a proxy for puberty, as described (Rawlings et al., 2008).

Bulls were maintained until approximately 72 wk of age and then slaughtered at a local abattoir. Testes were recovered and weighed. Daily sperm production (DSP) per gram of testicular parenchyma (DSP/g) and epididymal sperm reserves (ESR) were estimated from one testis and one epididymis from each bull, as reported previously (Brito et al., 2007c). In brief, testicular parenchyma samples (approximately 20 g), and separately the entire epididymis, were thoroughly homogenized and diluted in 0.05% Triton X-100 solution. Cells were counted using a hemocytometer and phase-contrast microscopy (400× 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.
**Statistical Analyses**

Characteristics of LH secretion (including pulse frequency and pulse amplitude) were determined using PC-Pulsar software (Gitzen and Ramirez, University of Illinois, Chicago, IL). Statistical analyses were conducted using JMP (version 7; SAS Institute Inc., Cary, NC). We used a multivariate ANOVA response model to determine and locate effects of nutrition (low, medium, high) and age, and the nutrition × age interaction, on BW, SC, PTV, TPI, diameter of the testicular vascular cone, and serum hormone concentrations. If either the main effect or the interaction were significant (Wilks’ lambda), differences were located using Tukey’s HSD (Honestly Significant Difference) test. In addition, one-way ANOVA (with Tukey’s HSD) was used to determine and locate diet effects on age at puberty, epididymis weight, DSP/g, ESR, age at SC >28 cm, paired testes weight, and total DSP. Percentage data were arc-sine transformed before analysis (nontransformed data are reported).

**RESULTS**

We detected nutrition, age, and nutrition × age interaction effects ($P < 0.0001$) on BW. Bulls fed the high-nutrition diet were heavier than those fed the low-nutrition diet from 15 to 71 wk and heavier than bulls fed the medium-nutrition diet from 19 to 51 wk (Figure 1). For SC, we detected effects of nutrition, age ($P < 0.001$ for each), and a nutrition × age interaction ($P < 0.006$). Bulls fed the high-nutrition diet had larger SC than those fed the low- and medium-nutrition diets from 11 to 71 wk and from 23 to 39 wk, respectively (Figure 1). We detected effects of nutrition ($P < 0.006$), age ($P < 0.001$), and a nutrition × age interaction ($P < 0.001$) on PTV. Bulls fed the high-nutrition diet had a greater PTV than those fed the low-nutrition diet from 15 to 71 wk; furthermore, their PTV exceeded that of the medium group at 27 and 39 wk (Figure 1). In addition, bulls fed the high-nutrition diet had significantly greater PTV as a percentage of overall BW than bulls fed the low-nutrition diet until 39 wk (Figure 2); this trend continued until 71 wk of age (although we found no significant difference from the low or medium diets after 39 wk).

We observed effects of age ($P < 0.0013$) and nutrition × age interaction ($P < 0.05$) on TPI. Bulls fed the high-nutrition diet had significantly greater TPI than those fed the low-nutrition diet at 27 and 31 wk, and significantly lower TPI than those fed the low-nutrition diet at 63 wk (Figure 2). There were effects of nutrition ($P < 0.05$), age ($P < 0.0001$), and nutrition × age interaction ($P < 0.002$) on mean testicular vascular cone diameter. Bulls fed the high-nutrition diet had larger testicular vascular cone diameters at 11, 27 to 43, and 63 wk compared with bulls fed the low-nutrition diet (Figure 2).

Age ($P < 0.05$) and a nutrition × age interaction ($P < 0.05$) affected serum LH secretion. All bulls, regardless of nutrition group, had an early increase in LH. However, in bulls fed the high-nutrition diet, this early rise was advanced by 8 wk (11 vs. 19 wk) and mean LH concentration was greater compared with that of the other 2 groups (Figure 3). The LH pulse frequency had a similar pattern. We observed an age ($P < 0.05$) effect, with bulls fed the high-nutrition diet having more frequent LH pulses between 11 and 15 wk (Figure 3). The peak in LH pulse frequency occurred earlier in the bulls fed the high-nutrition diet compared with the other 2 groups, and peaked at a higher magnitude than in the bulls fed the low-nutrition diet. We found no significant effects of nutrition or age on LH pulse amplitude (Figure 3).

Following the GnRH challenge, we detected effects of age on mean LH and total LH secretion ($P < 0.005$). In all groups, LH secretion increased following the GnRH challenge at 19 versus 15 wk; this LH secretion peaked from 19 to 23 wk and appeared to plateau at 27 wk (Figure 4).

Effects of age, nutrition, and nutrition × age interaction were not significant for basal FSH concentrations or for FSH concentrations after GnRH (Figure 5). We detected effects of nutrition ($P < 0.05$), age ($P < 0.05$), and a nutrition × age interaction ($P < 0.001$) on basal serum testosterone concentrations. In all groups, testosterone concentrations increased after 11 wk; bulls fed the high-nutrition diet had greater testosterone concentrations than those fed the low-nutrition diet from 11 to 27 wk (Figure 6). There were effects of nutrition ($P < 0.05$) and age ($P < 0.001$), and a nutrition × age interaction ($P < 0.05$) on mean testosterone concentration after GnRH. Bulls fed the high-nutrition diet had a greater response to GnRH (in terms of total testosterone secretion) compared with bulls fed the low-nutrition diet at 15, 19, 23, and 27 wk (Figure 6).

We observed nutrition, age, and nutrition × age interaction effects ($P < 0.0001$) on serum IGF-I concentrations; bulls fed the high-nutrition diet had greater IGF-I concentrations than bulls fed either the medium- or low-nutrition diets throughout the entire blood-sampling period (Figure 7). However, no nutrition, age, or nutrition × age interaction effects were detected for insulin or leptin (Figure 7).

Bulls fed the high-nutrition diet were younger ($P < 0.05$) at puberty and when they reached a SC of 28...
Figure 1. Mean (±SEM) for BW, scrotal circumference, and paired testes volume in Holstein bulls fed low-nutrition (n = 8), medium-nutrition (Med; n = 9), or high-nutrition (n = 9) diets from 2 to 31 wk of age. N = nutrition effect, A = age effect, N × A = nutrition × age interaction effect. Asterisks indicate differences (P < 0.05) among groups within age (*all groups differed; **low differed from high).
Figure 2. Mean (±SEM) paired testes volume (PTV) as a percentage of total BW, testicular pixel intensity, and testicular vascular cone diameter in Holstein bulls fed low-nutrition (n = 8), medium-nutrition (Med; n = 9), or high-nutrition (n = 9) diets from 2 to 31 wk of age. N = nutrition effect, A = age effect, N × A = nutrition × age interaction effect. Asterisks indicate differences (P < 0.05) among groups within age (*all groups differed; **low differed from high).
cm than bulls fed the low-nutrition diet. At 72 wk, bulls fed the high-nutrition diet had greater ($P < 0.05$) paired testes weight, epididymis weight, and ESR than the bulls fed the low-nutrition diet. However, nutrition had no significant effects on age at maturity, DSP/g, or total DSP (Table 1).

**DISCUSSION**

In the present study, bulls fed the high-nutrition diet consistently had larger testes than those fed the low-nutrition diet throughout the entire experimental period. It is noteworthy that these differences in testes size were maintained beyond the differential feeding period, as evident from SC, PTV, and paired testes weight (at slaughter). Furthermore, bulls fed the high-nutrition diet were younger at puberty (~45 d) than those fed the low-nutrition diet. An even greater difference (~80 d) between the high and low groups was observed for the age at which SC was >28 cm (a proxy for puberty; Lunstra et al., 1978). The latter variable is probably a more accurate representation of age at puberty than characteristics of the ejaculate. In that regard, for some bulls, there was a series of ejaculates with no or few sperm, followed by an ejaculate that greatly exceeded the minimum standards; therefore, we inferred that those bulls had previously passed puberty, although we lacked the semen sample to reach the classical definition of puberty (Wolf et al., 1965). Puberty has been reported to occur in Holstein bulls between 39 and 41 wk (Amann, 1983). Consistent with this report, bulls fed the high-nutrition diet in the current attained puberty at a similar age. Earlier puberty, even by 45 d, would be welcomed by the dairy industry, as this could hasten collection of semen needed for progeny testing, and thus allow bulls to be proof-tested and into production earlier. Overall, these results supported our hypothesis that Holstein bull calves on a high-nutrition diet reach puberty earlier and have larger testes than those on a low-nutrition diet.

Regarding gonadotropin concentrations, we did not characterize the entire prepubertal period, as this has already been done in Holstein and beef bulls (Rawlings et al., 1978; Amann and Walker, 1983; Evans et al., 1995, 1996; Brito et al., 2007a). Notwithstanding, we evaluated temporal relationships among gonadotropins, testosterone, and metabolic hormones during the differential feeding period; in that regard, basic hormone profiles were consistent with the aforementioned reports.

The LH profiles of these Holstein bull calves had the typical early gonadotropin rise, including characteristic increases in basal concentrations and pulse frequency. However, increased LH secretions apparently occurred.
earlier than in our previous nutrition study in beef bulls (23 wk in Holstein bulls vs. 26 wk in beef bulls; Brito et al., 2007b,c). This was consistent with a previous report that, on average, Holstein bulls reach puberty earlier than beef bulls (Wolf et al., 1965).

Bulls fed the high-nutrition diet had an earlier and more substantial early increase in LH than those fed the low-nutrition diet, as reported in our beef bull study (Brito et al., 2007a). Consistent with that study, we detected no significant differences in the secretion of gonadotropins following the GnRH challenge, suggesting that diet did not affect the ability of bulls to respond to GnRH. However, there was clear evidence that nutrition influenced the hypothalamus-pituitary-testes axis by modulating the GnRH pulse generator during the early gonadotropin rise (Brito et al., 2007c).

In the present study, bulls fed the high-nutrition diet had increased IGF-I concentrations earlier than the bulls fed the low-nutrition diet. Furthermore, we detected a temporal association between increased IGF-I concentrations and an early LH rise in the bulls fed the high-nutrition diet. Therefore, we inferred that IGF-I had a role in regulating the early gonadotropin rise (in particular, LH) and thus reproductive development of Holstein bulls. Similarly, in our previous studies in beef bulls, IGF-I was identified as a possible mediator of the

Figure 4. Mean (±SEM) serum LH concentrations and total LH during the 90 min after a GnRH challenge in Holstein bulls fed low-nutrition (n = 8), medium-nutrition (Med; n = 9), or high-nutrition (n = 9) diets from 2 to 31 wk of age. N = nutrition effect, A = age effect, N × A = nutrition × age interaction effect.

Figure 5. Mean (±SEM) FSH secretion during 10-h intensive blood sampling (every 15 min) and FSH secretion during the 90 min after a GnRH challenge in Holstein bulls fed low-nutrition (n = 8), medium-nutrition (Med; n = 9), or high-nutrition (n = 9) diets from 2 to 31 wk of age. N = nutrition effect, A = age effect, N × A = nutrition × age interaction effect.
nutritional effect of reproduction on the hypothalamus-pituitary-testes axis (Brito et al., 2007c). In that study, there was a strong association between IGF-I concentrations and BW, scrotal circumference, and PTV. In addition, IGF-I concentrations were also the single best predictor of BW, back fat, SC, and PTV (Brito et al., 2007a). Therefore, IGF-I may be potentiating its effect in 2 ways: by acting as a messenger of nutritional status to the hypothalamus and by directly affecting somatic cells of the testes. There are IGF-I receptors in GnRH neurons in the hypothalamus, and their numbers increase during sexual development (Daftary and Gore, 2004). In addition to the systemic role of IGF-I, it also has important autocrine and paracrine roles at the cellular level, regulating mitosis, apoptosis, and cellular differentiation. The testes are clearly a site of IGF-I biosynthesis and action (Wang and Hardy, 2004); LH stimulates IGF-I secretion in rodent testes (Cailleau et al., 1990) and both IGF-I and its receptors have been detected in Leydig cells, Sertoli cells, and spermatocytes in various species (Hansson et al., 1989; Villalpando et al., 2008). Wang and Hardy (2004) evaluated a target gene deletion of IGF-I in the testes and concluded that both IGF-I and LH are critical factors in determining Leydig cell numbers and their steroidogenic capacity (Wang and Hardy, 2004). Furthermore, LH appears to not be a direct mitogenic factor for Leydig cells, but instead, it acts through IGF-I. In addition, IGF-I has a critical autocrine/paracrine role in the control of adult Leydig cell numbers and functions by promoting both proliferation and differentiation of precursors in the Leydig cell lineage (Wang and Hardy, 2004). This further substantiated our data, suggesting that both LH and IGF-I affected the testicular environment by promoting testicular development, perhaps as a consequence of an increasing somatic cell population; namely, promoting proliferation, differentiation, or both, of these somatic cells. Furthermore, in another study, IGF-I treatment increased “gonadal sensitivity to gonadotropins and hastened puberty in female monkeys” (Wilson, 1998). In rats, exogenous IGF-I significantly reduces age at puberty and stimulates GnRH release from the hypothalamus (Hiney et al., 1996). In the present study, bulls fed the high-nutrition diet had more LH, and IGF-I was increased earlier; based on the reports cited above, these differences presumably contributed to their larger testes. Clearly, more work needs to be done to elucidate the effects of earlier increases in LH and IGF-I on the testes.

It was noteworthy that IGF-I increases testosterone secretion from Leydig cell cultures (Lin et al., 1986) and upregulates secretion of testosterone from Leydig cells, indicative of a positive feedback. In that regard, IGF-I initially upregulates testosterone production from Leydig cells, which upregulates both the IGF-I receptor and IGF-I production by Leydig cells. In the present study, testosterone concentrations increased steadily from 11 to 31 wk, consistent with previous reports (Amann and Walker, 1983). That testosterone was also affected by nutrition modulation supports the assertion that the higher level of nutrition hastened reproductive development in the bulls fed the high-nutrition diet. These testosterone data were consistent with the conclusions that nutritional modulation affected LH and IGF-I concentrations and thus testicular somatic cells. In a previous study, effects of IGF-I deletion on develop-

Figure 6. Mean (±SEM) serum testosterone concentrations during 10-h intensive blood sampling (every 15 min) and during the 90 min after a GnRH challenge in Holstein bulls fed low-nutrition (n = 8), medium-nutrition (Med; n = 9), or high-nutrition (n = 9) diets from 2 to 31 wk of age. N = nutrition effect, A = age effect, N × A = nutrition × age interaction effect. Within an age, groups without a common letter differed (P < 0.05).
Development of the adult Leydig cell population were assessed by evaluating the effects on Leydig cell precursor cells in mice (Hu et al., 2010). In that study, decreased concentrations of testicular testosterone in IGF null mice from postnatal d 21 to 90 were attributed to the absence of IGF-I.

In the present study, TPI increased from 23 to 39 wk, with TPI increasing earliest in bulls fed the high-nutrition diet. This was consistent with previous reports, based on increased testicular echogenicity (Brito et al., 2012), which is apparently associated with maturation of the testes, including formation of the blood testes barrier, Sertoli cell differentiation, and ultimately initiation of spermatogenesis (Curtis and Amann, 1981; Wrobel, 1990). In the current study, however, there was a slightly different pattern of TPI; following the characteristic increase, there was a much larger decrease in TPI in Holstein bulls compared with beef bulls (Brito et al., 2012). Although TPI is useful for determining puberty and maturation status, TPI has inconsistent associations with sperm production, seminiferous tubule, epithelium area, and sperm morphology following bull maturation (Brito et al., 2012). Therefore, TPI may be of less value in postpubertal bulls.

The values of DSP/g were lower in the bulls fed the high-nutrition diet versus bulls fed the low-nutrition diet; consequently, we found no significant difference in total DSP among the 3 diets, which was unexpected. Notwithstanding, we observed a trend for bulls fed the high-nutrition diet to have a greater total DSP. In the previous beef bull study, the DSP/g values were higher in the bulls fed the high-nutrition diet, although not significantly so, and total DSP was significantly higher in the bulls fed the high-nutrition diet compared with those fed the low-nutrition diet (Brito et al., 2007b). We may have failed to detect this significance due to breed differences. The ESR for bulls fed the high-nutrition diet exceeded those of bulls fed the low-nutrition diet and high-nutrition bulls also had greater epididymis weights at slaughter, consistent with their larger testes.

Overall, the present data were consistent with our previous work in beef bulls and provided clear evidence that nutritional modulation of Holstein bull calves during early life had profound effects on reproductive development. It was unclear, however, whether nutritional modulation is required for the entire 31 wk to obtain these effects on reproductive development or whether a shorter interval would be as effective. Furthermore, the present findings support the conclusion that because the beneficial effects of enhanced nutrition extend beyond the differential feeding period and into adulthood, these effects are probably related to increased LH concentrations during early calfhood (Brito et al., 2007b). Overall, feeding Holstein bull calves diets higher than

Figure 7. Mean (±SEM) serum concentrations of IGF-I, insulin, and leptin during 10-h intensive blood sampling (every 15 min) in Holstein bulls fed low-nutrition (n = 8), medium-nutrition (Med; n = 9), or high-nutrition (n = 9) diets from 2 to 31 wk of age. N = nutrition effect, A = age effect, N × A = nutrition × age interaction effect. Within an age, groups without a common letter differed (P < 0.05).
Table 1. Mean (±SEM) for various reproductive end points in bulls fed low-nutrition (n = 8), medium-nutrition (n = 9), or high-nutrition (n = 9) diets from 2 to 31 wk of age.

<table>
<thead>
<tr>
<th>Item</th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at puberty (d)</td>
<td>369.3 ± 14.1a</td>
<td>327.4 ± 9.5ab</td>
<td>324.3 ± 11.7b</td>
</tr>
<tr>
<td>Age at SC &gt;28 cm (d)</td>
<td>371.0 ± 5.3b</td>
<td>335.2 ± 15.3a</td>
<td>290.5 ± 12.2c</td>
</tr>
<tr>
<td>Paired testes weight (g)</td>
<td>561.6 ± 23.1b</td>
<td>611.1 ± 59.1ab</td>
<td>727 ± 33.0a</td>
</tr>
<tr>
<td>Epididymis weight (g)</td>
<td>27.7 ± 1.5b</td>
<td>30.5 ± 1.6ab</td>
<td>33.2 ± 1.2a</td>
</tr>
<tr>
<td>Total DSP (×109 sperm)</td>
<td>6.6 ± 0.9</td>
<td>7.3 ± 0.8</td>
<td>7.4 ± 0.5</td>
</tr>
<tr>
<td>DSP/g (×106 sperm/g)</td>
<td>11.6 ± 1.4</td>
<td>12.1 ± 1.0</td>
<td>10.1 ± 0.7</td>
</tr>
<tr>
<td>ESR (×106 sperm)</td>
<td>12.7 ± 1.4b</td>
<td>16.7 ± 1.8ab</td>
<td>20.1 ± 1.7a</td>
</tr>
</tbody>
</table>

a,bWithin an end point, means without a common superscripts differed (P < 0.05).
SC = scrotal circumference; DSP = daily sperm production; ESR = epididymal sperm reserves.

acknowledgments

This work was supported by the Canadian Agriculture Adaptation Program (Ottawa, ON, Canada) and L’Alliance Boviteq Inc. (Saint-Hyacinthe, QC, Canada).

References


