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Plane of nutrition before and after 6 months of age in Holstein-Friesian bulls: II. Effects on metabolic and reproductive endocrinology and identification of physiological markers of puberty and sexual maturation

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ABSTRACT

The aim of this study was (1) to examine the effect of plane of nutrition during the first and second 6 mo of life on systemic concentrations of reproductive hormones and metabolites in Holstein-Friesian dairy bulls, and (2) to establish relationships with age at puberty and postpubertal semen production potential. Holstein-Friesian bull calves (n = 83) with a mean (standard deviation) age and body weight of 17 (4.4) d and 52 (6.2) kg, respectively, were assigned to a high or low plane of nutrition for the first 6 mo of life. At 24 wk of age, bulls were reassigned, within treatment, either to remain on the same diet or to switch to the opposite diet until puberty, resulting in 4 treatment groups: high-high, high-low, low-low, and low-high. Monthly blood samples were analyzed for metabolites (albumin, urea, total protein, β-hydroxybutyrate, glucose, non-esterified fatty acid, triglycerides and creatinine), insulin, insulin-like growth factor-1, leptin, adiponectin, FSH, and testosterone. A GnRH challenge was carried out at 16 and 32 wk of age (n = 9 bulls per treatment). Blood was collected at 15-min intervals for 165 min, with GnRH administered (0.05 mg/kg of body weight, i.v.) immediately after the third blood sample. Blood samples were subsequently analyzed for LH, FSH, and testosterone. Stepwise regression was used to detect growth and blood measurements to identify putative predictors of age at puberty and subsequent semen quality traits. Metabolic hormones and metabolites, in general, reflected metabolic status of bulls. Although FSH was unaffected by diet, it decreased with age both in monthly samples and following GnRH administration. Testosterone was greater in bulls on the high diet before and after 6 mo of age. Testosterone concentrations increased dramatically after 6 mo of age. Luteinizing hormone was unaffected by diet following GnRH administration but basal serum LH was greater in bulls on a high diet before 6 mo of age. In conclusion, the plane of nutrition offered before 6 mo of age influenced metabolic profiles, which are important for promoting GnRH pulsatility, in young bulls.
Key words: gonadotropin, follicle-stimulating hormone, luteinizing hormone, testosterone

INTRODUCTION

Based on the published literature, the average age at puberty across *Bos taurus* beef and dairy-bred bulls is approximately 320 d, ranging between 287 and 369 d of age (Brito et al., 2007b; Dance et al., 2015; Harstine et al., 2015). The onset of puberty relies on the initiation of the GnRH pulse generator, which signals the anterior pituitary to secrete FSH and LH (Duittoz et al., 2016). These hormones are also important for Leydig and Sertoli cell proliferation and differentiation in the testes. The timing and intensity of the early transient LH rise, which typically occurs from approximately 8 to 20 wk of age, is an important determinant of age at puberty in the bull (Evans et al., 1995).

Secretion of GnRH is regulated by metabolic hormones in response to the metabolic status of the animal (Schneider, 2004; Clarke and Arbabi, 2016). Metabolites such as glucose have been shown to play an important role within the arcuate nucleus region of the hypothalamus by exciting pro-opiomelanocortin neurons and inhibiting appetite-promoting neuropeptide-Y, thereby promoting GnRH secretion (Burdakov et al., 2005). Insulin-like growth factor-1 and insulin are important for signaling metabolic status to the hypothalamus, and

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an increase in LH secretion between 12 and 18 wk of age has been reported in bulls, associated with a concomitant increase in IGF-1 (Brito et al., 2007a; Dance et al., 2015). Although neither of the aforementioned studies reported an effect of nutrition on systemic leptin before 31 wk of age, kisspeptin, a mediator of metabolic status, has neurons that are anatomically linked to pro-opiomelanocortin and neuropeptide-Y neurons (De Bond and Smith, 2014). These kisspeptin neurons have been shown to have leptin receptors, suggesting that kisspeptin may mediate metabolic signaling to the hypothalamus (Sanchez-Garrido and Tena-Sempere, 2013). Pulsatility of LH is not detected in bull calves before 8 wk of age (Rodriguez and Wise, 1989); thus, if appreciable increases are to be attained in LH secretion, nutritional programming should be commenced before and continued beyond 8 wk of age.

In a companion study (Byrne et al., 2018), we reported that age at puberty in Holstein-Friesian bulls is regulated by diet pre-6 mo of age, irrespective of the diet post-6 mo of age. Although characteristic changes in gonadotropins (LH and FSH) in response to age and diet have been reported in prepubertal dairy bulls (Dance et al., 2015), prepubertal metabolites have not been characterized in the same way. In prepubertal Holstein-Friesian bulls, the metabolic and endocrinological response to an increase or decrease in plane of nutrition in later calfhood remains unclear. Characterization of how nutrition and nutritional changes in the prepubertal period affect age at puberty and subsequent semen production in bulls is necessary.

We hypothesized that a high plane of nutrition before 6 mo of age would increase blood concentrations of gonadotropins and metabolites consistent with an improved metabolic status, thus promoting earlier sexual and testicular development. The specific objective of the study was to examine the effect of growth rate (consistent vs. interrupted) on key reproductive and metabolic hormones that regulate reproductive and sexual development and to identify physiological markers indicative of age at puberty and subsequent sexual maturation and semen quality in Holstein-Friesian bulls.

MATERIALS AND METHODS

All animal procedures performed were conducted under experimental license from the Irish Department of Health and Children (license number B100/4516). Protocols were developed in accordance with the *Cruelty to Animals Act* (Ireland 1876, as amended by European Communities regulations 2002 and 2005) and the European Community Directive 86/609/EC.

Animals and Management

The experimental design and animals used in the current study have been described previously (Byrne et al., 2018). Briefly, autumn-born Holstein-Friesian bull calves (n = 83) with a mean (±SD) age and BW of 17 (4.4) d and 52 (6.2) kg, respectively, were blocked by age, BW, sire, and farm of origin and assigned to a high (**HPN**) or low (**LPN**) plane of nutrition for the first 6 mo of life. Bulls assigned to HPN (n = 37) and LPN (n = 46) received 1,200 and 450 g of milk replacer, respectively. Bulls on HPN were fed concentrate ad libitum, whereas LPN bulls received a maximum of 1 kg of concentrates daily. All bulls were offered hay as a source of roughage and had ad libitum access to water. Bulls were individually fed using an electronic feeding system (Vario, Forster-Technik, Engen, Germany) before weaning and thereafter were penned according to treatment until turnout to pasture at 24 wk of age. Bulls were rotationally grazed in their respective treatment groups until the onset of puberty, at which time they were rehoused in slatted-floor pens. Bulls were offered these diets for a minimum of 56 d and weaned once they were consuming 1 kg of concentrates for 3 consecutive days. After weaning, HPN bulls were offered ad libitum concentrate and LPN bulls received 1 kg of concentrate daily; both groups were offered hay to appetite. At 24 wk of age, bulls were reassigned, within treatment, either to remain on the same diet or to change to the opposite diet until onset of puberty. This resulted in 4 groups: HPN-HPN (**Hi-Hi**), HPN-LPN (**Hi-Lo**), LPN-LPN (**Lo-Lo**), and LPN-HPN (**Lo-Hi**), with n = 19, 18, 22, and 24 bulls, respectively. Bulls were turned out to pasture at 26 wk of age, where Hi-Hi and Lo-Hi bulls received grass and concentrate ad libitum, and Lo-Lo and Hi-Lo bulls received grass to appetite plus 0.5 kg of concentrate daily. Puberty was deemed to have been reached when an ejaculate containing a minimum of 50×10^6 sperm with >10% progressive linear motility (**PLM**) was collected (Wolf et al., 1965). After puberty, semen was collected monthly. Sexual maturity was deemed to have been reached when a bull produced an ejaculate containing ≥30% PLM and ≥70% normal morphology (Brito et al., 2004). All semen was collected via electro-ejaculation.

Blood Sampling

Monthly blood samples were collected via jugular venipuncture, until puberty was attained. On each occasion, blood was collected into a 9-mL evacuated tube containing lithium heparin (Greiner Vacuette; Cruinn Diagnostics, Dublin, Ireland) and subsequently