

# Review: The effect of nutrition on timing of pubertal onset and subsequent fertility in the bull

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*The advent of genomic selection has led to increased interest within the cattle breeding industry to market semen from young bulls as early as possible. However, both the quantity and quality of such semen is dictated by the age at which these animals reach puberty. Enhancing early life plane of nutrition of the bull stimulates a complex biochemical interplay involving metabolic and neuroendocrine signalling and culminating in enhanced testicular growth and development and earlier onset of sexual maturation. Recent evidence suggests that an enhanced plane of nutrition leads to an advancement of testicular development in bulls at 18 weeks of age. However, as of yet, much of the neuronal mechanisms regulating these developmental processes remain to be elucidated in the bull. While early life nutrition clearly affects the sexual maturation process in bulls, there is little evidence for latent effects on semen traits post-puberty. Equally the influence of prevailing nutritional status on the fertility of mature bulls is unclear though management practices that result in clinical or even subclinical metabolic disease can undoubtedly impact upon normal sexual function. Dietary supplements enriched with various polyunsaturated fatty acids or fortified with trace elements do not consistently affect reproductive function in the bull, certainly where animals are already adequately nourished. Further insight on how nutrition mediates the biochemical interaction between neuroendocrine and testicular processes will facilitate optimisation of nutritional regimens to optimise sexual maturation and subsequent semen production in bulls.*

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**Keywords:** calf-hood nutrition, puberty, neuroendocrine, testes

## Implications

This review highlights the importance of enhancing early life nutrition of bulls in order to ensure early onset of puberty and sexual maturation. We also highlight the lack of evidence for a substantial effect of dietary augmentation on advancement of sexual maturity, once calves have reached 6 months of age, or indeed an appreciable effect of diet *per se* on semen quality in well managed post-pubertal bulls. Therefore, cattle producers and breeding companies must ensure that young bulls receive preferential nutritional and health management from birth in order to maximise lifetime fertility and return on their investment.

## Introduction

The reproductive performance of the bull contributes to half of overall herd fertility (Perry and Patterson, 2001). While the incidence of infertility in bulls is generally thought to be low

(3% to 5%), sub-fertility has been reported in ~25% of bulls undergoing standard bull breeding soundness evaluations (BBSE) (Kennedy *et al.*, 2002). In an era where cattle breeding programmes are dominated by genomically assisted selection approaches, the generation interval becomes an important limiting factor in dictating the rate of genetic gain (Kasinathan *et al.*, 2015). Genomic selection has made it possible to identify potential elite sires within weeks of their birth, long before they are pubertal or even capable of producing spermatozoa. As a result, cattle breeding companies are now focusing on pre-pubertal management regimes that will hasten the onset of puberty and subsequent production of adequate quantities of high quality semen. In the immediate post-pubertal period, ejaculates from young bulls will typically only yield between 50 and 150 saleable doses of frozen semen. Indeed, during their first season at a breeding centre, a young bull may only have 35% to 50% of the semen production capacity of a mature sire (Amann and DeJarnette, 2012). Within the context of a seasonal calving management system, this situation can result in a large imbalance between supply and demand of semen from genetically elite

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young sires thus accentuating the requirement for appropriate management regimen to consistently advance the availability of adequate supplies.

### Endocrine control of sexual maturation

It is now widely accepted that the early rise in LH, typically occurring between 10 and 20 weeks of age, is one of the most important factors in determining the age at onset of puberty in bulls (Rawlings and Evans, 1995). Hypophyseal portal vein cannulation has shown that GnRH pulsatile secretion occurs as early as 2 weeks of age. However, LH secretion is not evident until 8 weeks of age (Rodriguez and Wise, 1989), indicating the cessation of the infantile period and beginning of the pre-pubertal period. The same study also demonstrated that the frequency of pulsatile release of GnRH, within a 10 h period, increases twofold between 2 and 12 weeks of age. The secretion of FSH is typically high post-natally and remains elevated until ~20 weeks of age, after which it declines, reaching a nadir at ~25 weeks of age (Brito, 2014). This raises the question as to why GnRH secretion exclusively promotes the production and secretion of FSH in the immediate post-natal period. One possible reason is due to the role that FSH plays in Sertoli cell differentiation during early life and possibility pre-natally (Brito, 2014). For example, a combination of IGF-1 and FSH positively influenced the proliferation of Sertoli cells, cultured *in vitro* from testicular tissue of 8-week-old calves (Dance *et al.*, 2017). These data also demonstrate a lack of effect from FSH alone, suggesting that the metabolic status of the bull calf could impact on the number of Sertoli cells that are available to produce spermatozoa in later life.

Systemic concentrations of testosterone (TT) recorded in bull calves before 24 weeks of age are normally low with a marked rise thereafter coinciding with rapid testicular growth (Rawlings *et al.*, 2008; Byrne *et al.*, 2018a). This initial rise in LH secretion is necessary for differentiation and maturation of testicular Leydig cells, leading to the production and secretion of TT. As Leydig cells begin to increase steroidogenesis, negative feedback to the hypothalamus limits further gonadotropin production and secretion. This androgen negative feedback loop in bulls has been reviewed in detail by Rawlings and Evans (1995). The importance of TT for testicular growth has been shown in Angus bull calves where TT concentrations >1 ng/ml, at 20 weeks of age, was subsequently correlated ( $r=0.67$ ) with larger testes and a greater Sertoli cell number at 54 weeks of age (Moura *et al.*, 2011).

### Metabolic influence on sexual development

Metabolic hormones play an important role in sexual development of the bull through hypothalamus targeted signalling processes which in turn regulates GnRH pulsatility. The expected systemic profiles of IGF-1, insulin, leptin and growth hormone in beef bulls receiving adequate nutrition from 10 to 70 weeks of age have been reviewed by Brito (2014).

In both beef (Brito *et al.*, 2007a, 2007d and 2007c) and dairy bulls (Dance *et al.*, 2015; Byrne *et al.*, 2018b), studies have shown that offering pre-pubertal bulls an enhanced plane of nutrition leads to an improved metabolic state which in turn initiates an increase in gonadotropin secretion and advanced onset of puberty and sexual maturation. Temporal secretion patterns of IGF-1 and LH were reported in Holstein-Friesian bulls (Dance *et al.*, 2015), highlighting the role of IGF-1 in the regulation of the early gonadotropin rise and subsequent reproductive development. GnRH neurons express mRNA for IGF-1 and IGF-1R in an age-dependent manner, indicating the existence of an autocrine regulatory mechanism of the IGF-1 system in neuronal GnRH secretion. Moreover, treatment with IGF-1 also increases LH secretion in castrated rams *in vivo* (Adam *et al.*, 1998). In addition, exogenous insulin administration suppressed the release of neuro peptide-Y (NPY) in the paraventricular nucleus in the hypothalamus leading to reduced circulating concentrations of LH (60% reduction in males and 90% reduction in females). Reductions in LH secretion have also been reported following disruption of hypothalamus insulin receptors in mice (Bruning *et al.*, 2000). This in turn was associated with impaired spermatogenesis and ovarian follicle maturation. In ruminants, improving the nutrition of mature rams led to an increase in circulating and cerebrospinal fluid insulin concentrations resulting in increased GnRH/LH secretion (Blache *et al.*, 2000).

The role of leptin in the regulation of gonadotrophin secretion in livestock has been reviewed (Barb and Kraeling, 2004), with the hormone playing an important role in energy intake and satiety. Leptin secretion increases proportionally in line with accumulation of body fat (Landry *et al.*, 2013). The primary location of leptin receptors in the brain is in the hypothalamus, and was thought to be in areas associated with appetite control and reproduction. However, a lack of leptin receptors in GnRH neurons has been confirmed in rodents (Quennell *et al.*, 2009); suggesting that leptin may play an indirect rather than a direct role in reproduction. Indeed, the regulatory role of leptin on GnRH pulsatility is now thought to be mediated via an afferent neuronal network comprising kisspeptin neurons (Pinilla *et al.*, 2012); however, this has yet to be substantiated in the bull.

When referring to the role of leptin in reproduction, it is important to consider the metabolic status of the animals, as it has a major influence on the responsiveness to leptin. In ruminants, exogenous leptin will only evoke a significant gonadotropin response if the animal has been fasted or subjected to chronic negative energy balance (Amstalden *et al.*, 2005). Numerous studies examining the effect of plane of nutrition in bulls before 6 months of age report no difference in blood leptin concentration (Brito *et al.*, 2007a; Dance *et al.*, 2015; Byrne *et al.*, 2018b); with this lack of difference most likely due to the relatively small amounts of adipose tissue present at this age. Despite this, our own recent work shows that that leptin concentrations are greater in bull calves offered a high plane of nutrition post 6 months of age (Byrne *et al.*, 2018b) consistent with increased

ultrasonically measured back fat in these animals. Adiponectin, another adipokine, is found at greater systemic concentrations in animals with a higher fat deposition (Cominos *et al.*, 2014). Increased adiponectin concentrations are associated with disturbances in GnRH pulsatility in men apparently mediated via downregulation of the *KISS1* gene in the hypothalamus (Wen *et al.*, 2008), indicating that adiponectin has an opposing effect to that of leptin on reproduction. Adiponectin receptors; *AdipoR1* and *AdipoR2*, are expressed in both the human anterior pituitary and in the hypothalamus (Wen *et al.*, 2008). We recently reported that serum concentrations are reduced at 12 weeks of age in pre-pubertal bull calves offered a high v. a low plane of nutrition (Byrne *et al.*, 2017b). Adiponectin and its receptors has also been found in spermatozoa of Holstein-Friesian bulls (Kasimanickam *et al.*, 2013). Studies by the same group have found that serum concentration of AdipoQ ( $R^2 = 0.80$ ) and spermatozoal mRNA abundances for *AdipoR1* ( $R^2 = 0.80$ ) and *AdipoR2* ( $R^2 = 0.90$ ) were positively related, to sire conception rate.

Resistin is a secretory protein, produced by both white and brown adipose tissue, but has also been identified in other peripheral tissues. The role of resistin in reproductive function in both male and female humans and rodents has been reviewed (Rak *et al.*, 2017); the authors concluded that resistin is present and active in the hypothalamo-pituitary-gonadal axis and many *in vitro* studies report that adipokines such as resistin can regulate gonadal steroidogenesis and gametogenesis. While there are no data for pre-pubertal bulls, dairy cows offered divergent planes of nutrition displayed no difference in apelin (Weber *et al.*, 2016); however, it should be noted that body condition score was also unaffected, indicating that the diets may not have evoked sufficiently different metabolic status in the population of cows under investigation. Apelin is an adipokine, up-regulated by insulin and has been shown to increase glucose uptake by adipose tissue in mice (Dray *et al.*, 2008). Intra-cerebral infusion of apelin decreases testosterone release by suppressing LH secretion in rats (Sandal *et al.*, 2015). As both resistin and apelin are implicated in signalling metabolic status and the hypothalamus has receptors for interpreting nutrient availability; their influence on hypothalamic function warrants further investigation and the role of resistin and apelin in the hypothalamus and pituitary remains to be determined, in cattle.

### Neuroendocrine regulation of puberty

While there are many factors which influence the onset of puberty and subsequent reproductive function in bulls all are mediated through the hypothalamic-pituitary-axis. There has been a large volume of research into the role of neuroendocrinology in ovarian function and female fertility and has been reviewed by Duittoz *et al.* (2016) but research on the neuroendocrinological control on testicular function and semen production is limited (Plant, 2015). In the case of bulls this lack of knowledge is further accentuated and many of

the hypotheses proposed for bulls are based on results that have been extrapolated from data generated by studies in heifers or cows. The arcuate nucleus (ARC) of the hypothalamus is among the most important with regard to reproduction due to its role in interpreting metabolic signals, such as those discussed above, to nuclei that are responsible for GnRH release (Hill *et al.*, 2008). Therefore, the ARC manipulates the timing of sexual development in bulls via regulation of gonadotropin-releasing hormone (GnRH) secretion (Brito *et al.*, 2007c). Kisspeptin (KP), pro-opiomelanocortin (POMC) and NPY are some of the better known neuropeptides that are involved in the initiation of pulsatile LH release, required for puberty to occur (Amstalden *et al.*, 2014). Neuropeptides known to decrease (i.e. POMC) and increase (i.e. NPY) feed intake have been located in the ARC and pre-optic area (POA) in close proximity to kisspeptin neurons in sheep (Backholer *et al.* 2010). These latter authors (Backholer *et al.* (2010) also reported a possible interaction between these neuronal pathways when they observed that intra-cerebroventricular injection of KP increased *NPY* gene expression and resulted in a decrease in *POMC* gene expression in the ARC. They also demonstrated the close contact between POMC fibres and kisspeptin cells which is supported by Cardoso *et al.* (2015) who reported an increase in *POMC* mRNA in the ARC of heifers on an elevated plane of nutrition. While not the only neuropeptide that promotes GnRH secretion, nesfatin, a post-translational processing product derived from nucleobindin2 (NUCB2), has been identified as a potent, anorexigenic agent (García-Galiano *et al.*, 2010). Moreover, the same authors also report that nesfatin plays an important role in gonadotropin secretion during the peri-pubertal period in female rats; however, nutrient restriction results in a reduction in nesfatin and thus GnRH.

Recently, we offered Holstein-Friesian calves a high or a low plane of nutrition from 2 to 18 weeks of age at the end of which we harvested hypothalamic and anterior pituitary tissue. Following a targeted qPCR approach a down regulation in ghrelin receptor was observed in the ARC of the hypothalamus and the anterior pituitary tissue of bulls offered a high plane of nutrition (English *et al.*, unpublished data), consistent with the inhibitory effect of ghrelin on GnRH pulsatility in heifers (Chouzouris *et al.*, 2016). Conversely, RNA sequencing data showed no effect of plane of nutrition on gene expression in the ARC nucleus at 18 weeks of age (English *et al.*, unpublished data). In the anterior pituitary, of the same calves genes relating to cell cycle processes such as mitotic roles of polo-like kinase and cell cycle: G2/M DNA damage checkpoint regulation were down regulated in the low relative to the high plane of nutrition groups; however, there was no evidence for differential expression of genes with a known function in reproductive processes in the anterior pituitary. In the testes, genes involved in cholesterol and androgen biosynthesis were downregulated in bull calves on the low compared with the high plane of nutrition. In agreement with this finding, the calves on the high plane of nutrition also displayed

characteristics of greater testicular development such as heavier testes, greater Sertoli cell number and volume density, seminiferous tubule diameter and more mature spermatogenic cells; indicating that these calves were at a more advanced physiological stage of sexual maturity. It is clear that these neuronal pathways influence the early onset of puberty; however, clarity is required to determine the exact mechanisms mediating the effect of elevated nutritional regimes on initiation of the pubertal process (Amstalden *et al.*, 2014). In addition, the influence of the more recently discovered adipokines, amongst other signalling proteins, on the hypothalamic–pituitary–testicular axis in bulls remains to be elucidated.

### Early life nutrition (birth to 6 months)

On commencing this review, we intended to conduct a meta-analysis of studies which have examined the effect of plane of nutrition on age at onset of puberty in bulls. However, on reviewing the published literature, we were unable to find sufficient studies to provide a meaningful analysis (Table 1). Moreover, commonality in design between these studies was almost non-existent. A summary of Table 1 suggests that offering a high plane of nutrition to bull calves during the putative window of increased gonadotrophin secretion (8 to 20 weeks of age) advances onset of puberty in both beef (Brito *et al.*, 2007a, 2007b and 2007c) and dairy bulls (Dance *et al.*, 2015; Byrne *et al.*, 2018a). However, results are not wholly consistent for dairy bulls. For example, Harstine *et al.* (2015) offered divergent planes of nutrition from 10 until 31 weeks of age to Holstein-Friesian dairy bulls and reported no difference in age at attainment of puberty. Also, in Holstein-Friesian bulls, divergent planes of nutrition offered during the infantile period (birth to 12 weeks of age) did not result in differences in age at attainment of puberty (Bollwein *et al.*, 2016 and 2017). In the latter study, it is possible that the early gonadotropin rise was missed due to premature cessation of the nutritional regimen. From this work it is obvious that there is a necessity for further research in order to identify the specific window of opportunity for nutritional modulation of HPT function and the specific biochemical pathways involved.

### Six months to puberty

Similar to overall body growth, the testes of pre-pubertal bulls follow a sigmoidal growth trajectory (Rawlings *et al.*, 2008); with the most rapid period of scrotal occurring from ~25 weeks of age onwards. Despite incremental scrotal growth being greater after 6 months of age, studies in beef bulls (Brito *et al.*, 2007c) together with our own work in dairy bulls (Byrne *et al.*, 2018a) have shown that there is no effect of prevailing plane nutrition during this period on the age at which puberty is reached. For example, Brito *et al.* (2007c) using Angus and Angus × Charolais bulls, offered either a control or restricted plane of nutrition from 10 until 26 weeks of age. Following this the control bulls were maintained

on the same plane of nutrition, whereas their previously restricted contemporaries were offered either the control or an enhanced plane of nutrition. Likewise, using Holstein-Friesian bulls, Byrne *et al.* (2018a) offered a high or a low plane of nutrition from 2 until 24 weeks of age; thereafter, bulls were re-assigned within their original dietary group to either remain on their diet or move to the opposite diet, until puberty. In both studies, bulls offered an improved plane of nutrition after reaching 6 months of age displayed BW and scrotal growth similar to that of bulls that were afforded the consistently unrestricted dietary regimen. However, despite achieving a similar growth rate post 6 months of age, bulls restricted in early life were 25 days older at puberty than their counterparts, unrestricted during this period, thus showing an inability to 'compensate' for their earlier dietary restriction/poorer metabolic status. The conclusion of both studies is that the plane of nutrition offered before 6 months of age is the most important determinant of age at puberty in bulls and attempts to mitigate against early life nutritional restriction by offering an enhanced plane of nutrition thereafter, will be in earnest. In addition, at least in the case of Holstein-Friesian bulls, the advantages of an unrestricted plane of nutrition, in terms of influencing age at puberty are not reversed by moderate dietary restriction post 6 months of age (Byrne *et al.*, 2018a) again highlighting the central importance of early life management.

### Effect of nutrition on post-pubertal semen characteristics

#### *Pre-pubertal nutrition*

Based on our own findings (Byrne *et al.*, 2018a) and that of Dance *et al.* (2016) with Holstein-Friesian bulls, sexual maturation occurs, on average, ~35 days after puberty, again highlighting the importance of early life nutrition. Notwithstanding this, while an improved nutritional status during early life advances sexual maturity, latent effects, *per se* on semen production thereafter, seem limited. Dance *et al.* (2016) reported that enhanced nutrition up to 31 weeks of age increased the number of harvestable spermatozoa by ~30% in bulls, post-puberty. Characteristics affecting fertility, such as post-thawing motility, *in vitro* fertilisation (IVF) ability, live/dead ratios and spermatozoal proteins were unaffected by early life nutrition. In contrast, both our own findings (Byrne *et al.* 2018a) and those of Harstine *et al.* (2015) show that pre-pubertal plane of nutrition has no effect on the number of harvestable sperm post-puberty. All three studies are unanimous in finding no evidence for latent effects of pre-pubertal nutrition on the quality of post-pubertal semen production. In addition to the findings of Dance *et al.* (2016), recent data from our group indicate that there is no effect of pre-pubertal plane of nutrition on oocyte fertilisation or subsequent blastocyst rate when post-pubertal frozen-thawed semen was used under IVF conditions (Byrne *et al.*, unpublished). Notwithstanding this, older studies have reported that a high plane of nutrition; in particular, high cereal-based diet can negatively impact

**Table 1** Effects of plane of nutrition on pubertal and sexual development in bulls

	Breed	Start age (days)	Diets		n	ADG (kg/day)	Age at puberty (days) <sup>1</sup>	Age at maturity (days) <sup>2</sup>	Paired testes weight (g)	Experiment end age (days)
Brito <i>et al.</i> (2007b)	Angus and Angus × Charolais	56	Pre 26 weeks of age Control: 13.2% CP, <i>ad libitum</i> Restricted: 75% of control consumption	Post 26 weeks of age Control/control: <i>ad libitum</i> Restricted/control Restricted/high: 14.4% CP	24	–	293 <sup>a</sup> 331 <sup>b</sup> 313 <sup>ab</sup>	–	600 <sup>a</sup> 528 <sup>b</sup> 553 <sup>ab</sup>	490
Brito <i>et al.</i> (2007c)	Angus and Angus × Charolais	70	Pre 30 weeks Control: 13.5% CP High: 15.1% CP until 19 weeks of age, 21.3% CP thereafter	Post 30 weeks All received control	33	–	327 314	–	531 <sup>a</sup> 611 <sup>b</sup>	518
Brito <i>et al.</i> (2007d)	Angus and Angus × Charolais	70	Low: 12.3% CP Medium: 13.1% to 16.3% CP, diet changed after 30 weeks of age High: 20.4% CP		23	–	321 <sup>a</sup> 299 <sup>b</sup> 288 <sup>b</sup>	–	520 <sup>a</sup> 549 <sup>a</sup> 655 <sup>b</sup>	490
Bollwein <i>et al.</i> (2016)	Holstein-Friesian	2	Pre 5 weeks of age Restricted: 4 l MR <i>Ad libitum</i> -low: <6 l MR <i>Ad libitum</i> -high: >12 l MR	Post 5 weeks of age All received a conventional finishing ration	24	0.38 <sup>a</sup> 1.28 <sup>b</sup>	275 274 278	–	–	~448
Byrne <i>et al.</i> (2017)	Holstein-Friesian and Jersey	21	Pre 10 weeks of age Low HF: 4 l MR, JE: 3.5 l MR, all 1 kg concentrate High HF: 8 l MR, JE: 6 l MR, all <i>ab libitum</i> concentrate	8 to 16 weeks of age Low HF: 1.7 kg, JE 1.4 kg concentrate High: <i>ad libitum</i>	34	0.99 <sup>a</sup> 0.76 <sup>b</sup> 0.63 <sup>c</sup> 0.44 <sup>d</sup>	<sup>3</sup> HHF: 37 <sup>a</sup> HJE: 34 <sup>a</sup> LHF: 43 <sup>b</sup> LJE: -	–	–	343
Byrne <i>et al.</i> (unpublished)	Holstein-Friesian	14	Pre 10 weeks of age as HF above	10 to 24 weeks of age Low: 1 kg concentrate High: <i>ad libitum</i> concentrate	83	0.57 <sup>a</sup> 0.84 <sup>b</sup> 0.96 <sup>b</sup> 1.24 <sup>c</sup>	319 <sup>a</sup> 283 <sup>b</sup> 323 <sup>a</sup> 298 <sup>b</sup>	343 <sup>a</sup> 314 <sup>b</sup> 352 <sup>a</sup> 331 <sup>b</sup>	626 <sup>a</sup> 658 <sup>b</sup> 594 <sup>a</sup> 660 <sup>b</sup>	504
Dance <i>et al.</i> (2015)	Holstein-Friesian	3	Pre 31 weeks of age Low: 12.2% CP Medium: 17.0% CP High: 20% CP	Post 31 weeks of age All received medium	26	–	369 <sup>a</sup> 327 <sup>ab</sup> 324 <sup>b</sup>	<sup>4</sup> 385 391 366	562 <sup>a</sup> 611 <sup>ab</sup> 727 <sup>b</sup>	504
Harstine <i>et al.</i> (2015)	Holstein-Friesian	58	Pre 32 weeks of age Control: 0.92 Mcal/kg High: 1.24 Mcal/kg Diets were isonitrogenous (18.2% CP)	Post 32 weeks of age All received control	15	1.00 <sup>a</sup> 1.51 <sup>b</sup>	302 323	–	268 <sup>a</sup> 318 <sup>b</sup>	569

<sup>a,b,c,d</sup>Values within study with different superscripts differ significantly ( $P < 0.05$ ).

<sup>1</sup>Based on ability to produce an ejaculate containing  $\geq 50$  million sperm with  $\geq 10\%$  progressive linear motility (Wolf *et al.*, 1965).

<sup>2</sup>Based on ability to produce a pubertal ejaculate with  $\geq 70\%$  morphologically normal sperm and  $\geq 30\%$  progressive linear motility (Brito *et al.*, 2004).

<sup>3</sup>Based on attainment of scrotal circumference of 28 cm (Lunstra *et al.*, 1978).

<sup>4</sup>Taken from Dance *et al.* (2016).

progressive motility and sperm morphology in beef bulls (Coulter *et al.*, 1997). These observations coincided with an associated increase in scrotal temperature in bulls offered an 80% cereal compared with a 100% forage diet from ~6 to 12 months of age. In contrast, data from our research group report that offering a barley-based concentrate on an *ad libitum* basis during the pre-pubertal period alters scrotal temperature; has no negative effects on semen quality (Byrne *et al.*, 2018a).

#### *Post-pubertal nutrition and semen production*

All over the world potential breeding bulls are commonly offered an excessively high level of energy dense concentrate feed before being offered for sale. While the early sections of this review have highlighted the importance of a high plane of nutrition for hastening the age at puberty; there is evidence that increasing the amount of concentrates in a bull's diet can have a negative impact on semen quality (Coulter *et al.*, 1997). It is widely accepted that testicular temperature of the bull must be maintained at 2°C to 6°C lower than body temperature (Kastelic, 2014) in order for normal spermatogenesis to occur. Infrared thermography has been used to assess scrotal temperature gradient in bulls (Kastelic *et al.*, 1996); with a smaller gradient (difference in temperature between testicular vascular cone and bottom of scrotum) associated with increased proportions of damaged sperm cells. An increase in dietary energy intake has been associated with a concomitant increase in scrotal fatness and temperature and decreases in the percentage of morphologically normal and progressively linear motile sperm in bulls (Coulter *et al.*, 1997). In contrast to these findings, we have shown that offering *ad libitum* access to high energy, grain-based diets for an extended period during pre-pubertal and early post-pubertal stages of development does not negatively impact semen quality of Holstein-Friesian bulls, despite elevating scrotal fatness and surface temperature (Byrne *et al.*, 2018a). Bulls on a high plane of nutrition in our study, had an ADG of 1.6 kg/day for 3 to 5 months before puberty, which is similar to that of the Angus and Angus × Charolais bulls reported by Brito *et al.* (2012), where no negative effects on semen production were also reported.

Rapid introduction of concentrate-based diets, rich in readily rumen fermentable carbohydrate to cattle results in a possible reduction in ruminal pH (Owens *et al.*, 1998), leading to sub-acute ruminal (SARA) or even acute acidosis. Animals suffering from SARA may not display overt symptoms and remain untreated. In mature Saint Gertrudis bulls, SARA was induced by oral administration of highly soluble oligo-fructose and compared with non-acidotic control animals which were administered water orally (Callaghan *et al.*, 2016). Bulls challenged with oligo-fructose experienced a drop in ruminal pH to 5.7 within 8 h of administration and ruminal pH did not return to levels comparable with the control animal until 24 h after dosing. The transient induction of SARA in that study led to a reduction in the percentage of morphologically normal sperm with large increases in proximal droplets, knobbed acrosome and vacuoles also reported

60 days after oligo-fructose administration. It is also worth noting that these increases in abnormal sperm morphology had not decreased by the end of the trial (90 days post oligo-fructose administration).

Feeding cattle high concentrate-based diets also leads to an increased incidence of laminitis. The role of dietary protein in hoof health is less clear with reports suggesting both putatively positive and negative effects of rumen degradable protein on the incidence of laminitis (Lean *et al.*, 2013). Conversely, an inadequate supply of the sulphur-containing amino acids, methionine and cysteine to the corium may increase incidence of lameness as a result of the formation of soft horn. In our studies we failed to observe any negative effect on locomotion score when the aforementioned young Holstein-Friesian bulls were offered *ad libitum* access to concentrate from ~2 weeks to 18 months of age (Byrne *et al.*, unpublished). Whether such chronic exposure to high grain diets during this key developmental phase could have a latent impact on joint health warrants further investigation. Indeed in a study of bulls culled for infertility in Sweden, Persson *et al.* (2007) reported that despite observing no clinical signs of lameness, 67% of the 34 infertile bulls had moderate or severe lesions associated with osteoarthritis compared with none in a contemporary group of 11 control (normal fertility) bulls. In addition, assessment of caudal sperm from infertile bulls showed that sperm morphology was not sufficiently poor to explain complete infertility, and the author suggested that weakness in the hind limbs as a consequence of the observed joint lesions may have been the primary cause of infertility. The Swedish study highlights another issue around bull fertility, which is libido. Such bulls will very likely pass a BBSE when EE is used to collect semen for evaluation. Despite the importance of normal expression of sexual impetus, there is limited research in the area in recent years. Wierzbowski (1978) reported that offering bulls a high compared with a low plane of nutrition reduced libido as a consequence of inducing greater BW with knock on negative effects for locomotory ability. Libido appears to be influenced by a range of factors, as reviewed by Petherick (2005) making treatment low libido bulls particularly challenging.

#### *Dietary restriction and weight loss*

In a series of experiments, Angus, Hereford and Angus × Hereford bulls were offered either protein-deficient diets (8, 5 or ~1.35% CP) diets *ad libitum* or a diet with adequate CP concentration (14%) at a quantity equivalent to 2.25% of BW, adjusted every 14 days between 8 and 12 months of age (Meacham *et al.*, 1963). Testes, epididymis, and seminal gland weights were markedly reduced in bulls fed protein-deficient rations. In addition, seminiferous tubule diameter and seminiferous epithelium thickness were lower in bulls with a restricted protein intake; however, this had no negative effects on semen production. Interestingly, in that study, semen characteristics were not affected until CP was reduced to 1.35%; semen volume and total spermatozoa in the ejaculate were decreased, but sperm morphology and

motility remained the same as for the other dietary treatments. Indeed the apparent robustness of the spermatogenic process was evident from the fact that, protein restriction was so severe in these studies that half of the protein restricted (1.35%) bulls died or were slaughtered before imminent death after losing ~40% of their initial BW. In rams, planes of nutrition meeting either 110% of maintenance led to a reduction in testis mass and spermatogenesis associated with impaired basic Sertoli cell function but did not alter the number of Sertoli cells compared with a plane of nutrition meeting 90% of maintenance requirements. This reduction in Sertoli cell function led to DNA damage in sperm cells and reduced sperm velocity in rams also (Guan *et al.*, 2014).

#### *Dietary supplements and semen quality/bull fertility*

In the preceding sections we examined the impact of quantity of feed offered on aspects of semen quality and bull fertility. There are also a limited number of studies that have examined the potential of certain dietary supplements to augment reproductive potential in ruminants (Gholami *et al.*, 2010; Fair *et al.*, 2014). Animals cannot synthesise omega-3 or omega-6 poly-unsaturated fatty acids (PUFA) *de novo* as they lack the appropriate fatty acid de-saturase enzymes and need to obtain these or their precursors from dietary sources. These PUFA are important components of animal cell membranes, and play a crucial role in oocyte fertilisation (Wathes *et al.*, 2007). Indeed omega-3 and omega-6 PUFA cumulatively make up 30% to 40% of the lipid content in bovine spermatozoa cells (Byrne *et al.*, 2017a).

Offering Holstein-Friesian bulls a DHA-enriched nutraceutical for 9 weeks resulted in no difference in semen volume, concentration per ml or total spermatozoa number (Gholami *et al.*, 2010). A subjective examination of motility in that study found a greater number of motile spermatozoa when bulls were fed the DHA-enriched diet; however, this was not substantiated when the same samples were analysed using computer-assisted semen analysis (CASA). The DHA-enriched diet led to a higher percentage of bulls displaying a positive hypo-osmotic swell test, suggesting an improvement in sperm cell membrane integrity in these animals. Unfortunately, cell FA compositional changes were not analysed in that study so it is impossible to gauge the level of cellular incorporation required to elicit the recorded response. In another study in bulls, employing alpha-linoleic acid (ALA) and palmitic acid (PA) supplements (Gürler *et al.*, 2015), no difference in preliminary semen characteristics (volume, concentration, motility) was observed which agrees with the findings of Gholami *et al.* (2010). However, Bulls offered both the ALA and PA supplements had increased levels of plasma membrane and acrosome-intact cells post-thawing. Interestingly, while there was no difference in semen lipid peroxidation (LPO) levels, measured by BODIPY581/591, between treatments post-thawing; LPO was higher after a 3-h post-thaw incubation period in bulls offered the ALA supplement. While the quantity of spermatozoa produced has not been altered in bulls, dietary supplementation of rams with fish oil extract led to a higher

semen concentration per ml; however, there was no difference between diets on any of the other semen quality parameters including semen volume, wave motion, progressive linear motility, ability to penetrate artificial mucus, or ability to resist lipid peroxidation in either fresh or liquid stored semen (Fair *et al.*, 2014). Recent data from our research group (Byrne *et al.*, 2017a) indicate that supplementing young post-pubertal bulls (14 months) for 12 weeks with either an omega-6 (safflower oil) or an omega-3 (distilled fish oil) enriched diet altered the PUFA composition of spermatozoal cells and seminal plasma but did not lead to any appreciable improvements to the quantity or quality of fresh semen. Many of the reported improvements as a result of dietary PUFA supplementation are linked with post-thaw spermatozoa suggesting that PUFA are important for ensuring spermatozoa survive cryopreservation. Despite this, in our study we failed to observe any improvements in frozen-thawed semen analysed for a range of CASA motility or flow cytometry-based parameters (Byrne *et al.*, 2017a).

#### *Mineral supplementation and bull fertility*

The dietary mineral requirements of cattle have been reviewed by (Ledoux and Shannon, 2005) and for most processes a relatively large dietary range is evident. Under normal circumstances unrestricted forage availability will meet minimal trace element requirements for bulls. In domestic farm species, zinc has been reported to play an important role in maintenance of testosterone production (Martin *et al.*, 1994) and also synthesis of RNA and DNA polymerases, necessary for sperm function (Hidiroglou and Knipfel, 1984). Following copper and zinc supplementation to pre-pubertal beef bulls, Geary *et al.* (2016) reported that although a greater percentage of supplemented bulls reached puberty earlier; no effect on semen quality was evident.

The most commonly used supplements are derived from inorganic (rock-based) minerals mainly as a function of cost. However, inorganic sources of minerals are not as easily absorbed as organic mineral compounds (Ledoux and Shannon, 2005). In this regard, when 4 to 9-year-old Angus and Gelbvieh × Angus bulls were supplemented with organic compared with inorganic forms of zinc, copper, cobalt and manganese, in one compound, the organic mineral compound resulted in 9% to 10% higher overall semen motility and progressive linear motility (Rowe *et al.*, 2014). Using a large number ( $n = 167$ ) of yearling Angus bulls, Arthington *et al.* (2002) found that increasing the inclusion level (60 v. 40 ppm) of a combination of organic and inorganic minerals in a dietary supplement at a high (60 ppm) led to a reduction in the number of bulls failing pre-sale BBSE. Most improvements in semen quality following dietary mineral supplementation are likely observed where the base forage offered is of poor nutritional composition.

## Conclusions

Enhancing the plane of nutrition of bull calves during the first 6 months of life will increase gonadotropin secretion and

testicular development, resulting in earlier onset of puberty. Recent evidence shows that this is likely mediated through the signalling activity of peripherally derived metabolites and metabolic hormones to neuroendocrine centres within the brain and mediated by the actions of specialised neuropeptides. This leads to enhanced gonadotrophin synthesis and secretion which in turn controls testicular development and function. Improvement of our knowledge on these complex biochemical interactions will be important for designing future nutritional regimes to hasten the onset of puberty, particularly for genetically elite young bulls. Post-pubertal nutrition also plays a role in maintenance of normal semen production; however, most of these improvements are often only observed in situations where animals are already deficient in the nutrient under investigation.

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### Declaration of interest

The authors have no conflicts of interest to disclose.

### Ethics statement

The work described in this manuscript was carried out in accordance with local and national animal ethics recommendations.

### Software and data repository resources

The data and models presented in this manuscript are not deposited in an official repository.

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