

Nutritional Control of Puberty in Beef Heifers

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Introduction

Management of beef heifers for reproductive success, both before and after puberty, plays a major role in determining the efficiency of any beef production system. A general understanding of the processes that underlie pubertal development in heifers can aid in understanding the basis of established management protocols. The prepubertal period is characterized, at least in part, by a state of anestrus or the absence of estrous cycles. The hormonal and physiological changes preceding first ovulation occur as a result of maturational changes within the central nervous system, which ultimately trigger normal ovarian function, the onset of regular estrous cycles, and the potential to become pregnant. The purpose of this communication is to review the physiological processes that control puberty in heifers and the genetic, nutritional, and managerial factors that influence it.

Physiology and Endocrinology of Puberty in the Heifer

Maturation of the Central Reproductive Axis

Puberty in heifers is defined as the attainment of a developmental state that supports normal ovarian cyclicity (follicular development and ovulation) and the ability to become pregnant. Activation of the central reproductive axis is a major event preceding the onset of ovarian cycles in all mammalian females, including the heifer. As puberty approaches, an increase in the release of a key hormone from the hypothalamus (lower brain) occurs. The hormone, gonadotropin-releasing hormone (**GnRH**), is the master regulator of reproductive function. It is secreted locally in discrete pulses into the portal circulation supplying the anterior pituitary. An increase in secretion of GnRH preceding puberty results in a concomitant increase in production and release of the pituitary hormone luteinizing hormone (**LH**) (Foster and Jackson, 2006). Each biologically significant GnRH pulse results in a pulse of LH and in an overall increase in the concentration of LH in the general circulation. This elevation in LH is the signal that drives final maturation of ovarian follicles and the production of steroid hormones within the follicle (e.g., estrogens). Thus, a major limiting factor for the onset of puberty is the lack of high-frequency pulses of GnRH and LH (Wildt et al., 1980). The relative inactivity of the central reproductive axis during prepuberty is created primarily by a negative feedback system involving estradiol-17 β (**E2**), the most physiologically-relevant estrogen produced by the ovarian follicle. As puberty approaches, the hypothalamus becomes less sensitive to the negative feedback effect of E2 on GnRH secretion (Foster and Ryan 1979; Day et al., 1984). As a result, GnRH pulsatile release from the

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hypothalamus increases which in turn stimulates increased circulating LH (**Figure 1**). The development and maturation of a large, estrogen-active follicle that follows these events represents a “switch” from a negative to a positive feedback effect on both the hypothalamus and pituitary. Increased release of E2 by the maturing follicle also causes the expression of behavioral estrus (heat) and is responsible for triggering a surge release of LH. This results in the first ovulation and the formation of a corpus luteum (**CL**). The CL produces progesterone, which regulates the length of the estrous cycle. The duration of the estrous cycle of cattle is 17 to 21 days, with the average slightly less in virgin heifers than in mature cows. If the female does not become pregnant, the CL regresses near the end of the cycle, a new follicle matures, and a new cycle ensues. However, if the heifer is bred and becomes pregnant, the CL does not regress and high circulating concentrations of progesterone necessary for the maintenance of pregnancy persist.

Although it is known that developmental changes within the hypothalamus controlling negative feedback sensitivity to estradiol and secretion of GnRH drive the onset of puberty, changes within the brain that underlie the functional beginning of sexual maturation and the neural pathways controlling this phenomenon remain unclear. Recent studies in our laboratories indicate that changes in expression of specific signaling peptides (i.e., neuropeptide Y, **NPY**; and proopiomelanocortin, **POMC**) in a metabolic-sensing region of the hypothalamus, may serve as developmental focal points for modifications that precede activation of the GnRH pulse generator (Allen et al., 2012; Cardoso et al., 2018). In addition, the recent discovery of a new family of neuropeptides, the RF-amides, has revolutionized our understanding of the regulation of GnRH neurons. In particular, the hormone, kisspeptin has been shown to stimulate GnRH secretion (Messenger et al., 2005), may communicate both the positive and negative effects of estradiol on GnRH/LH release (Smith et al., 2007), and is absolutely critical for pubertal development (Seminara et al., 2003). Mutations in the genes encoding kisspeptin (Lapatto et al., 2007) and kisspeptin receptor (Seminara et al., 2003) disrupt sexual maturation. Kiss1, the gene encoding kisspeptin, is expressed in specific areas of the hypothalamus (preoptic area and arcuate nucleus) that are critical for gonadal steroid hormone (estradiol and progesterone) control of reproduction (Pielecka-Fortuna et al., 2008). Kisspeptin is a potent stimulator of LH release in mammals, including cattle (Kadokawa et al., 2008) and this effect is believed to occur by direct actions on GnRH neurons (Smith et al., 2008). Our studies investigating the involvement of the kisspeptin system during pubertal development suggest that kisspeptin is a key neuropeptide mediating the nutritional acceleration of puberty in beef heifers (Cardoso et al., 2015). These results indicate that activation of the kisspeptin system is a major limiting factor, and perhaps the final neuroendocrine bridge, for the establishment of puberty.

Reproductive Tract Development

As the central reproductive axis becomes active nearing puberty, the production of ovarian hormones contributes to the development of secondary reproductive traits, including mammary and reproductive tract development. In the prepubertal heifer, the uterine horns tend to be rather flaccid and underdeveloped. However, as puberty

approaches, and in response to increased stimulation by ovarian estrogens, the uterus and cervix begin to grow larger and to exhibit more smooth muscle tone when palpated manually. Anderson et al., (1991) developed a procedure for estimating pubertal status using palpation of the reproductive tract. The bottom portion of **Figure 1** demonstrates the changes in reproductive tract score (**RTS**) that occur as the heifer develops from early prepubertal to pubertal. The complete reproductive tract scoring system is shown in **Table 1**. The scores are subjective estimates of sexual maturity, based on ovarian follicular development and palpable size of the uterus. A RTS of 1 represents an infantile or relatively undeveloped tract. In this case, the uterine horns are small and have very little tone, and the ovaries are small and lack significant structures. Heifers with a RTS of 1 are likely the furthest from puberty at the time of examination. As the heifer develops and approaches puberty, the RTS increases due to larger uterine horns and ovaries. Heifers given a RTS of 3 have significant uterine tone and large follicles, and are estimated to be very near the first pubertal ovulation. Heifers scoring a 4 are assumed to be pubertal and therefore exhibiting regular estrous cycles. These heifers will have greater size and tone of uterine horns compared to less developed heifers scored lower, the horns will exhibit coiling, and a large preovulatory size follicle (e.g., $\geq 10\text{mm}$) may be present. Heifers assigned a score of 5 have a palpable CL indicating that ovulation has already occurred.

Interaction of Genetics and Nutrition in Regulating Age at Puberty

Lifetime productivity of beef heifers is heavily-dependent upon their ability to reach sexual maturity, to conceive early in the initial breeding season, and to calve the first time as 2 year-olds (Lesmeister et al., 1973). Importantly, early conception is positively influenced by the number of estrous cycles occurring before targeted first breeding (Byerly et al., 1987). However, a significant proportion of heifers either does not reach puberty, or become pubertal too late, to conceive during their first breeding season as yearlings. This is particularly true for later-maturing breeds, even those that have reached an apparent body size capable of delivering a calf safely. Nutritional management involving continuous, high rates of gain is one option for promoting the timely onset of sexual maturation using the concept of “targeted body weight”. This approach sets a target of 60 to 65% of mature body weight (**BW**) as a practical rule of thumb for individual heifers to have reached puberty. The physiological precept of this principle is that heifers are genetically programmed to reach puberty at a predetermined size (Lamond, 1970; Taylor and Fitzhugh, 1971).

More recent evidence indicates that such targets also represent a minimum level of adiposity and a threshold circulating level of the adipose-derived hormone, leptin (Williams et al., 2002). Therefore, it is clear that genetic composition will have a major impact on what 65% of mature BW represents. Heifers that ultimately will have a large frame size at maturity (e.g., frame score 9 and mature BW > 1400 lb or 420 kg) will need to reach a much heavier BW to reach puberty compared to those with an expected mature frame size of 1 and mature BW of < 900 lb (Fox et al., 1988). However, in all cases, puberty can be accelerated or delayed by nutrition both before and after weaning. Early work by Wiltbank et al. (1966) indicated that BW gain during the pre-

weaning period may have a greater influence on age at puberty than post-weaning BW gain. This falls in line with the concept of precocious puberty (puberty reached at 10 months of age or less) occurring as a result of early-age (4 to 7 months of age) exposure to high energy, high-gain diets (Gasser et al. 2006 a,b,c). However, although there is opportunity for manipulating early calfhood nutrition to accelerate puberty, this can result in excessive fattening, impairment of mammary development, and risk of unwanted, ill-timed pregnancies.

Therefore, our research team is currently exploring the development of novel strategies that address these issues. Our primary goal is to develop strategies to nutritionally program heifers in a manner that promotes sound development and consistent, timely onset (12-14 months of age) of puberty, while avoiding the negative effects that can occur with excessive fattening. In this regard, our recent studies (Cardoso et al., 2014) in *Bos indicus*-influenced beef heifers demonstrate that metabolic programming of processes underlying puberty can be shifted temporally through the use of a stair-step, compensatory growth model such that puberty is optimally-timed to occur at approximately 12 months of age (**Figure 2**). More specifically, our studies suggest that feeding heifers a high-concentrate diet during critical windows of development (4 to 9 months of age) results in changes in the metabolic endocrine status, characterized by elevated circulating concentrations of leptin, insulin, and IGF-1, which in turn can program the onset of puberty that occurs months later, allowing optimal timing of sexual maturation in replacement beef heifers. Importantly, we found that heifers that gained body weight at high rates between 4 and 6.5 months of age, and were subsequently subjected to a marked feed restriction between 6.5 and 9 months of age, still attained early puberty (puberty <12 months of age) at rates comparable to heifers fed a high-concentrate diet continuously (Cardoso et al., 2014). Similarly, *Bos taurus* heifers that were fed to gain body weight at high rates between 126 and 196 days of age exhibited a high incidence of precocious puberty (Gasser et al., 2006 a,b) However, puberty was not advanced to the same extent when heifers were fed a similar diet later during juvenile development. Collectively, these results indicate that during early development, plausibly between 4 and 9 months of age, heifers are more sensitive to the programming effects of nutrition in advancing puberty.

Conclusions

Approximately 4 million replacement beef heifers enter the U.S. cow herd annually. Very few of these heifers are “programmed” nutritionally or otherwise to optimize lifetime reproductive performance, even though pre-breeding growth and nutrition are major contributors to fertility and lifetime reproductive performance. Effective managerial and technological practices to program replacement heifers for optimal reproductive performance are available, yet adoption of these practices in the US beef production industry has been limited. Our studies in *Bos indicus*-influenced beef heifers clearly demonstrate that metabolic programming of processes underlying puberty can be shifted temporally through the use of a stair-step, compensatory growth model such that puberty is optimally-timed to occur at approximately 12 months of age.

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Table 1. Reproductive tract scores (RTS)^a

RTS	Uterine horns	Ovarian length (mm)	Ovarian height (mm)	Ovarian width (mm)	Ovarian structures
1	Immature, < 20 mm diameter, no tone	15	10	8	No palpable follicles
2	20-25 mm diameter, no tone	18	12	10	8 mm follicles
3	20-25 mm diameter, slight tone	22	15	10	8-10 mm follicles
4	30 mm diameter, good tone	30	16	12	10 mm follicles, CL possible
5	> 30 mm diameter, coiled (?)	> 32	20	15	CL present

^a From Anderson et al. (1991).

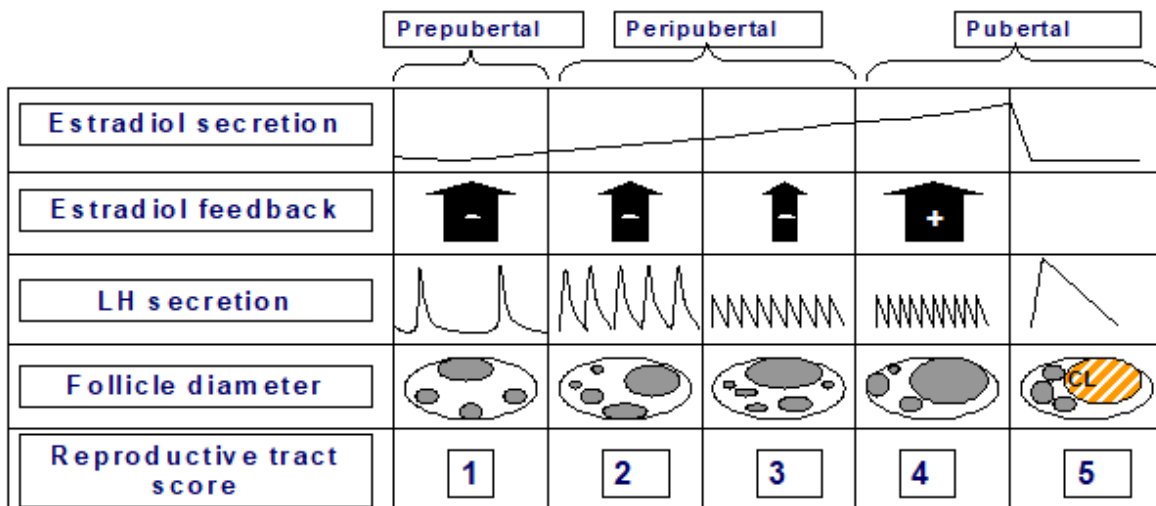


Figure 1. Endocrine and ovarian changes associated with puberty onset in the heifer and associated reproductive tract score (adapted from Day and Anderson, 1998, Anderson et al., 1991, and Patterson et al., 2005).

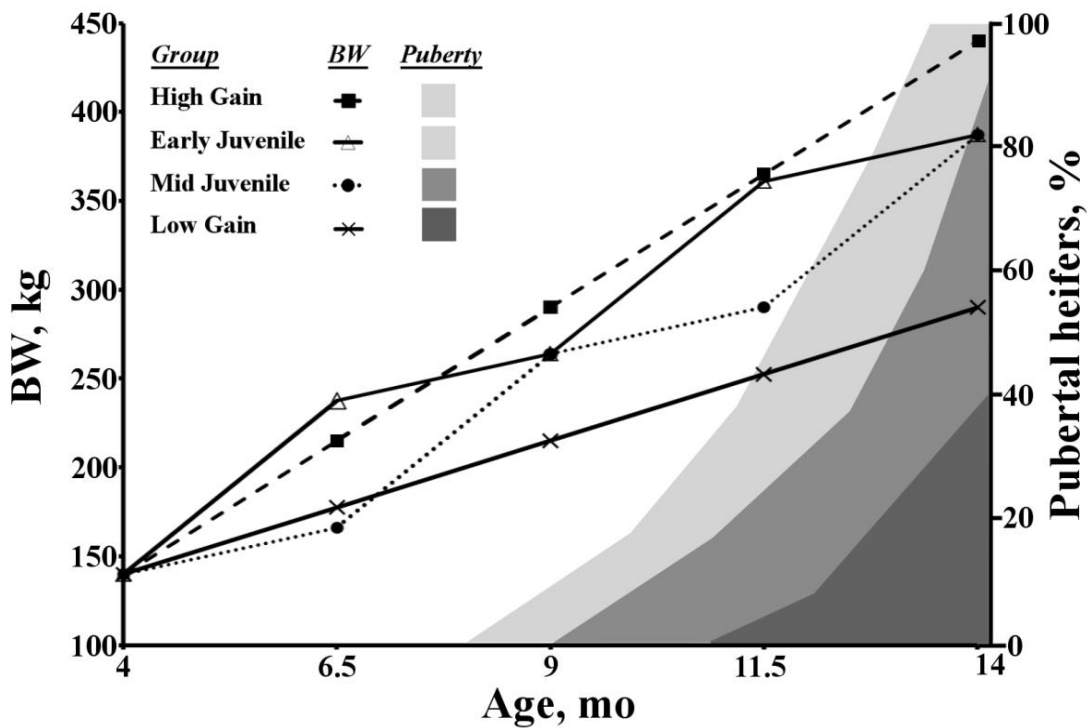


Figure 2. Model for a stair-step nutritional regimen applied to heifers during targeted periods of juvenile development to optimize growth, and promote genomic, biochemical and structural alterations in the hypothalamus that are permissive for early onset of puberty. Elevated body weight (BW) gain during the early- and mid-juvenile periods programs neuroendocrine functions and accelerates pubertal development in heifers. Heifers that are fed ad libitum a high-concentrate diet beginning at 4 months of age (Early Juvenile) become pubertal at a similar time as heifers gaining BW at high rates (High Gain) throughout the prepubertal period, even though feed restriction is applied during the mid-juvenile period. Most heifers that are restricted during the early juvenile period, but fed ad libitum between 6.5 and 9 months of age (Mid Juvenile) becomes pubertal by 14 months of age, whereas only a small proportion of heifers gaining BW at low rates (Low Gain) becomes pubertal by 14 months of age. Adapted from Cardoso et al. (2014).

SESSION NOTES