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-17β (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 et al., 1979; Day et al., 1984). As a result, GnRH pulsatile release from the 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 17-21 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.

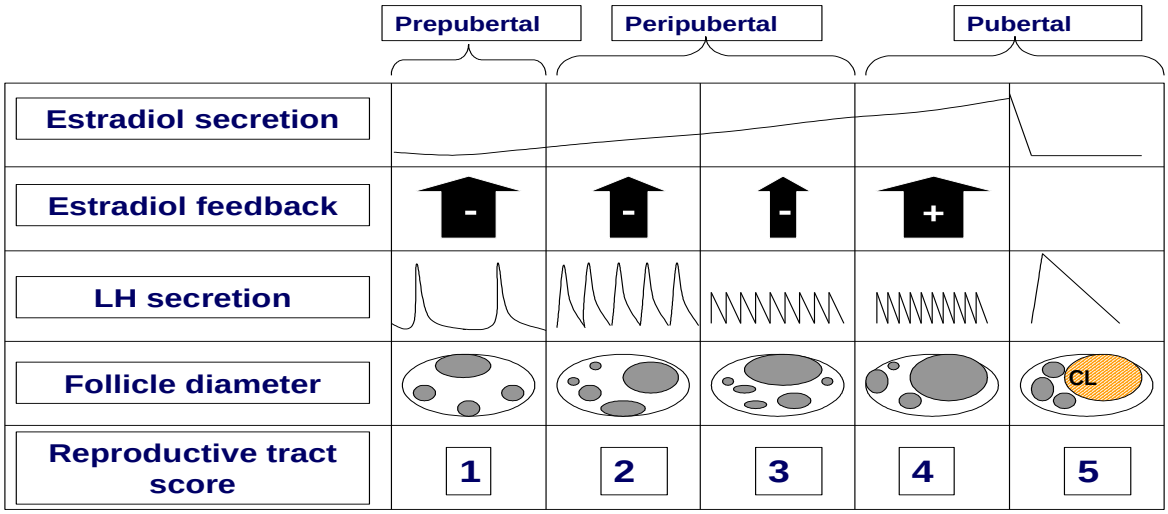

Figure 1. Endocrine and ovarian changes associated with puberty onset in the heifer and associated reproductive tract score (adapted from Day and Anderson, 1998, Anderson et al., 1991, and Patterson et al., 2005).

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 (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 (Messager 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 **Fig. 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., ≥ 10mm) may be present. Heifers assigned a score of 5 have a palpable CL indicating that ovulation has already occurred.

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.

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 60-65% 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) 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 mo of age or less) occurring as a result of early-age (4-7 mo of age) exposure to high energy, high-gain diets (Gasser et al. 2006 a,b,c). However, while 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 mo 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 mo 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.

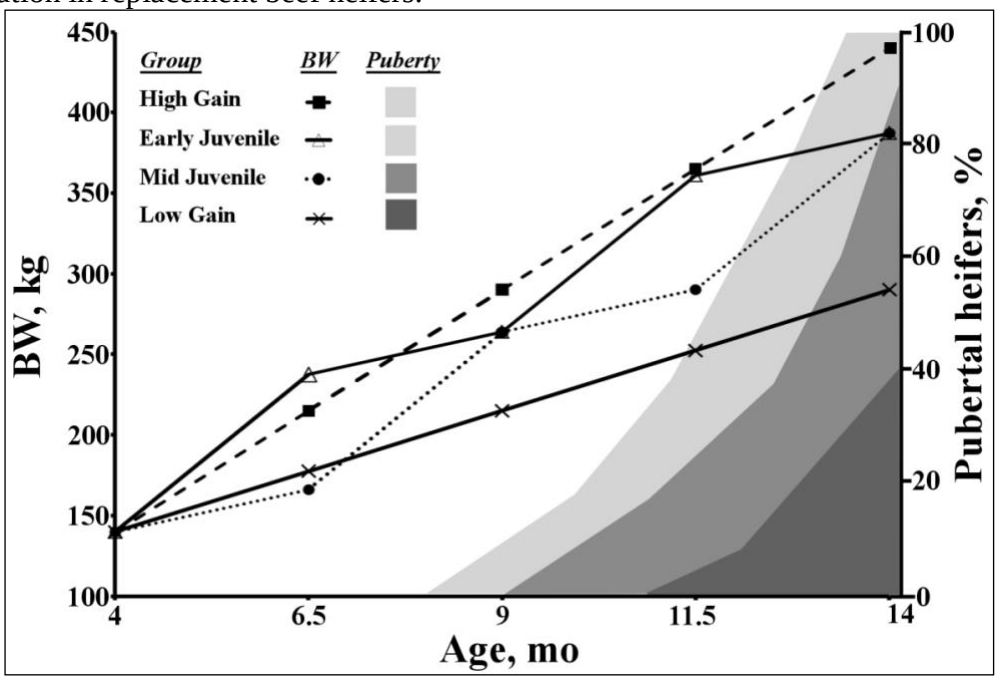

Figure 2. Model for a stair-step nutritional regimen applied to heifers during targeted periods of juvenile development to optimize growth, and promote genomic, biochemical and structural alterations in the hypothalamus that are permissive for early onset of puberty. Elevated BW gain during the early- 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 mo 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 mo of age (Mid Juvenile) becomes pubertal by 14 mo of age, whereas only as small proportion of heifers gaining BW at low rates (Low Gain) becomes pubertal by 14 mo of age. Adapted from Cardoso et al., 2014.

Summary and 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 mo of age.

Literature Cited

Allen CC, X. Li, L. O. Tedeschi, H. Zhou, J.A. Paschal, T.E. Spencer, U.M. Braga-Neto, D.H. Keisler, M. Amstalden, and G.L.Williams. 2009. Dietary treatments that facilitate early onset of puberty in heifers alter gene expression in the arcuate nucleus. Proc. Soc. Study Reprod. (Abstract 489).

Anderson, K.J., D.G. Lefever, J.S. Brinks, and K.G. Odde. 1991. The use of reproductive tract scoring in beef heifers. Agri-Practice. 12(4) 123-128.

Byerly D.J., R.B. Staigmiller, J.G. Berardinelli, and R.E. Short. 1987. Pregnancy rates of beef heifers bred either on puberal or third estrus. J. Anim. Sci. 65:645-650.

Cardoso, R. C., B. R. C. Alves, L. D. Prezotto, J. F. Thorson, L. O. Tedeschi, D. H. Keisler, C. S. Park, M. Amstalden, and G. L. Williams. 2014. Use of a stair-step compensatory gain nutritional regimen to program the onset of puberty in beef heifers. Journal of Animal Science 92: 2942-2949.

Cardoso, R. C., Alves, B. R. C., Sharpton, S. M., Williams, G. L., & Amstalden, M. (2015). Nutritional programming of accelerated puberty in heifers: involvement of pro-opiomelanocortin neurones in the arcuate nucleus. Journal of neuroendocrinology, 27(8), 647-657.

Cardoso, R. C., Alves, B. R., & Williams, G. L. (2018). Neuroendocrine signaling pathways and the nutritional control of puberty in heifers. Animal Reproduction (AR), 15(1), 868-878.

Day M.L., K. Imakawa, M. Garcia-Winder, D.D. Zalesky, B.D. Schanbacher, R.J. Kittok, and J.E. Kinder. 1984. Endocrine mechanisms of puberty in heifers: estradiol negative feedback regulation of luteinizing hormone secretion. Biol. Reprod. 31:332-341.

Day, M. L., and L. H. Anderson. 1998. Current concepts on the control of puberty in cattle. J. Anim. Sci. 76(Suppl. 3):1-15.

Foster D.L. and L.M. Jackson. 2006. Puberty in the sheep. In Knobil and Neill's Physiology of Reproduction (Neill Ed.). Elsevier 2127-2176.

Foster DL and K.D. Ryan 1979. Endocrine mechanisms governing transition into adulthood: a marked decrease in inhibitory feedback action of estradiol on tonic secretion of luteinizing hormone in the lamb during puberty. Endocrinology 105:896-904.

Fox, D. G., Sniffen, C. J., & O'connor, J. D. (1988). Adjusting nutrient requirements of beef cattle for animal and environmental variations. Journal of Animal Science, 66(6), 1475-1495.

Gasser C.L., D.E. Grum, M.L. Mussard, F.L. Fluharty, J.E. Kinder, and M.L. Day. 2006a. Induction of precocious puberty in heifers I: enhanced secretion of luteinizing hormone. J. Anim. Sci. 84:2035-2041.

Gasser C.L., C.R. Burke, M.L. Mussard, E.J. Behlke, D. E. Grum, J.E. Kinder, and M.L. Day. 2006b. Induction of precocious puberty in heifers II: advanced ovarian follicular development. J. Anim. Sci. 84:2042-2049.

Gasser C.L., G.A. Bridges, M.L. Mussard, D.E. Grum, J.E. Kinder, and M.L. Day. 2006. Induction of precocious puberty in heifers III: hastened reduction of estradiol negative feedback on secretion of luteinizing hormone. J. Anim. Sci. 84:2050-2056.

Kadokawa H, M. Matsui, K. Hayashi, N. Matsunaga, C. Kawashima, T. Shimizu, K. Kida, and A. Miyamoto. 2008. Peripheral administration of kisspeptin-10 increases plasma concentrations of GH as well as LH in prepubertal Holstein heifers. J. Endocrinol. 196:331-334.

Lamond, D. R. 1970. The influence of undernutrition on reproduction in the cow. Anim.

Breed. Abstr. 38:359-372.

Lapatto R. , J.C. Pallais, D. Zhang , Y.M. Chan, A. Mahan, F. Cerrato, W.W. Le, G.E. G.E. Hoffman, and S.B. Seminara. 2007. Kiss1-/- mice exhibit more variable hypogonadism than Gpr54-/- mice. Endocrinology 148:4927-4936.

Lesmeister JL, P.J. Burfening , and R.L. Blackwell. 1973. Date of first calving in beef cows and subsequent calf production. J Anim Sci 36:1-6.

Messager S, E.E. Chatzidaki, D. Ma, A.G. Hendrick, D. Zahn, J. Dixon, R.R. Thresher, I. Malinge, D. Lomet, M.B. Carlton, W.H. Colledge, A. Caraty, and S.A. Aparicio. 2005. Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. PNAS USA; 102:1761-1766.

Patterson, D.J., S. L. Wood, and R. F. Randle. 2005. Procedures that support reproductive management of replacement heifers. Proc. Applied Reproductive Strategies in Beef Cattle, Texas A&M University, College Station, pp 271-292.

Pielecka-Fortuna J, Z. Chu, and S.M. Moenter. 2008. Kisspeptin acts directly and indirectly to increase gonadotropin-releasing hormone neuron activity and its effects are modulated by estradiol. Endocrinology 149:1979-1986.

Seminara S.B., S. Messager, EE. Chatzidaki, R.R. Thresher, J.S. Acierno, Jr, J.K. Shagoury, Y. Bo-Abbas, W. Kuohung, K.M. Schwinof, A.G. Hendrick, D. Zahn, J. Dixon, U.B. Kaiser, S.A. Slaugenhaupt, J.F. Gusella, S. O'Rahilly, M.B. Carlton, W.F. Crowley, Jr, S.A. Aparicio, and W.H. Colledge 2003. The GPR54 gene as a regulator of puberty. N. Engl. J. Med. 349:1614-1627.

Smith JT, Clay CM, Caraty A, Clarke IJ. 2007. KiSS-1 messenger ribonucleic acid expression in the hypothalamus of the ewe is regulated by sex steroids and season. Endocrinology 148:1150-1157

Smith JT, Rao A, Pereira A, Caraty A, Millar RP, Clarke IJ. 2008. Kisspeptin is present in ovine hypophysial portal blood but does not increase during the preovulatory luteinizing hormone surge: evidence that gonadotropes are not direct targets of kisspeptin in vivo. Endocrinology 149:1951-1959.

Taylor, St. C.S., and H.A. Fitzhugh, Jr. 1971. Genetic relationships between mature weight and time taken to mature within a breed. J. Anim. Sci. 33:726-731.

Wildt L, G. Marshall, and E. Knobil. 1980. Experimental induction of puberty in the infantile female rhesus monkey. Science 207:1373-1375.

Williams GL, Amstalden M, Garcia MR, Stanko RL, Nizielski SE, Morrison CD, Keisler DH. 2002. Leptin and its role in the central regulation of reproduction in cattle. Domest. Anim. Endocrinol. 23:339-349.

Wiltbank, J.N., K.E. Gregory, L. A. Swiger, J.E. Ingalls, J.A. Rothlisberger, and R.M. Koch. 1966. Effects of heterosis on age and weight at puberty in beef heifers. J. Anim. Sci. 25:744-751.