

## Endocrine basis for puberty in heifers and ewes

J. E. Kinder, E. G. M. Bergfeld, M. E. Wehrman, K. E. Peters and  
F. N. Kojima

*Department of Animal Science, University of Nebraska, Lincoln, NE 68583-0908, USA*

Maturation processes that culminate in puberty and sexual maturity are initiated before birth, continue through prepuberty (> 50 days before puberty) and peripuberty (50 days before puberty) and are completed early after puberty. The hypothalamus is the primary site of change during transition to sexual maturity. Maturation of the hypothalamus results from decreased negative feedback of oestradiol that leads to increased frequency of release of LH pulses. Increased tonic release of LH pulses during sexual maturation is the primary endocrine factor that regulates the onset of puberty in ewe lambs and heifers. Increased frequency of release of LH pulses enhances development of ovarian follicles which produce enough oestradiol to induce behavioural oestrus and a preovulatory surge of gonadotrophins. In later stages of peripuberty, ovulation or luteinization of follicles results in transient increases in progesterone for shorter periods than is typical for luteal phases of the oestrous cycle of mature ewes and cows. Transient increases in progesterone are not generally preceded by behavioural oestrus. After the demise of the transient luteal structures, puberty is attained with occurrence of the first behavioural oestrus that is accompanied by ovulation and development of a corpus luteum with a typical lifespan. At puberty, all components of the hypothalamic–pituitary–ovarian axis are in place for oestrous cycles to be expressed. Factors that can influence the pubertal rise in release of LH pulses are genotype, gender, season of year when pubertal age is attained, growth or nutritional intake, social cues or treatment with exogenous progestins. Sexual maturation continues after puberty with an enhanced probability of pregnancy occurring from actions of ovarian steroids at the uterus.

### Prepubertal Reproductive System

The final component of the reproductive endocrine system to develop that regulates time of onset of puberty in heifers and ewes is the hypothalamus (Schams *et al.*, 1981; Foster *et al.*, 1986; Kinder *et al.*, 1987). Hypothalamic content of GnRH does not change during sexual maturation in heifers (Day, 1985). Treatment with *N*-methyl-D-aspartic acid (NMDA) elicits release of GnRH during prepuberty in primates (Plant *et al.*, 1989) and the numbers of pituitary receptors for GnRH do not change during sexual maturation (Day *et al.*, 1987). Release of GnRH from stores in the stalk median eminence, therefore, appears to be the final hypothalamic component to develop that results in onset of puberty. Maturation of the hypothalamus allows for function of the pituitary, ovaries and uterus in an adult fashion. Changes occur in the hypothalamus, therefore, that result in an increase in frequency of release of GnRH pulses into hypothalamic–portal vessels during peripuberty (Rodriguez and Wise, 1989). The pituitary can respond to GnRH early in life well before puberty (Schams *et al.*, 1981). Treatment of ewes or heifers with oestrogen will induce preovulatory surges of LH and FSH; thus mechanisms are in place before puberty to induce preovulatory surges of gonadotrophins. If appropriate GnRH stimulation occurs, the pituitary can function in an adult fashion before puberty.

Treatment of heifers with exogenous gonadotrophins can induce ovulation of fertile ova before puberty (Seidel *et al.*, 1971). Relatively few LH pulses are released from the anterior pituitary before puberty compared with after puberty in ewes (Foster and Ryan, 1979) and heifers (Day *et al.*, 1984; Day *et al.*, 1987; Wolfe *et al.*, 1989). There is a marked increase in frequency of release of LH pulses around the time of puberty which is the primary endocrine factor involved in onset of oestrous cycles at puberty. The ovary, therefore, has the capability of functioning in an adult fashion before puberty but gonadotrophic stimulation to allow ovulation to occur is absent.

Changes in uterine morphology that typically occur around the time of puberty would probably occur if heifers or ewes were treated with an appropriate milieu of oestradiol and progesterone before puberty. Lack of gonadotrophic stimulation of the ovaries before puberty limits production of the ovarian steroids, oestradiol and progesterone, and this in turn limits uterine development.

We conclude that administration of specific factors (i.e. NMDA) can induce the release of GnRH, that the pituitary can respond to GnRH by releasing LH and FSH and the ovary can respond to LH and FSH before puberty. The uterus could probably respond to gonadal steroids, oestradiol and progesterone before puberty. However, limited release of GnRH from the hypothalamus results in maintenance of the prepubertal state until physical maturation of females reaches a critical point.

### Peripubertal Maturation of the Hypothalamus

Neurones that contain GnRH are located in the anterior hypothalamus and medial basal hypothalamus. The putative generator of LH pulses that controls onset of puberty is thought to be in the arcuate nucleus in primates and deafferentation of the medial basal hypothalamus leads to decreased secretion of LH (Knobil *et al.*, 1980).

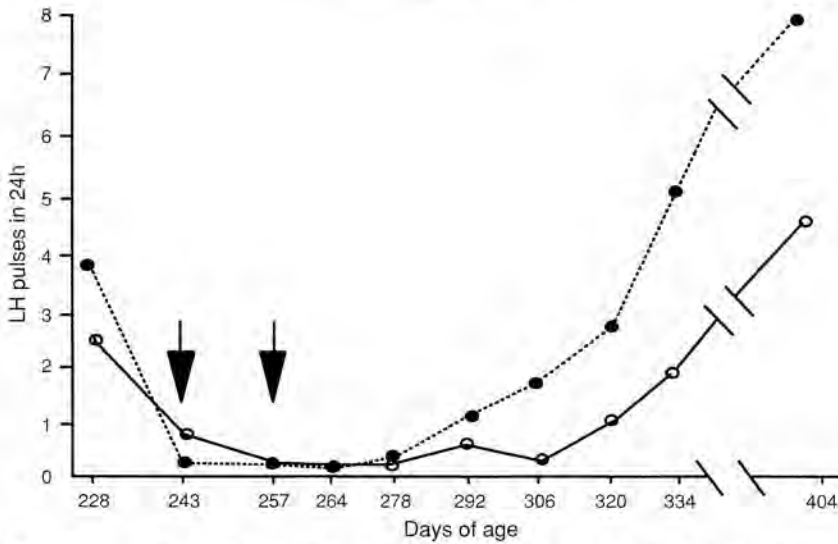
There is little information about changes in hypothalamic neurones during sexual maturation in ruminants. Changes have been noted in neurones that contain GnRH during sexual maturation in female rats (Wray and Hoffman, 1986). Morphology of neurones changes from cells with a smooth surface during early life to cells with irregular, spiny surfaces as puberty approaches. Spine-like processes are thought to form synaptic connections to neurones that contain GnRH. Most neurones of the anterior hypothalamus that contain GnRH in rats have terminals in the stalk median eminence (Merchenthaler *et al.*, 1984). Modifications in these neuronal processes during sexual maturation may result in increased frequency of release of tonic pulses of GnRH into the portal vasculature of the stalk median eminence.

Neurones that bind oestradiol contain various neurotransmitters including dopamine, gamma amino butyric acid, noradrenaline and  $\beta$ -endorphin. Neurones that bind oestradiol synapse with neurones that contain GnRH in the medial preoptic area (Lehman *et al.*, 1988).

The hypothalamus of prepubertal ewes and heifers is more responsive to inhibitory effects of oestradiol on release of GnRH before rather than after puberty (Foster *et al.*, 1986; Day *et al.*, 1986a) and this led to the development of the 'gonadostat hypothesis' (Ramirez and McCann, 1963). This change in responsiveness to oestradiol is a primary factor regulating time of onset of puberty in ewes and heifers (Foster *et al.*, 1986; Kinder *et al.*, 1987).

Low systemic concentrations of oestradiol before puberty reduce the frequency of GnRH pulses into the stalk median eminence. The effect of oestradiol is not thought to be directly on neurones that contain GnRH because these neurones do not concentrate radiolabelled oestradiol (Shivers *et al.*, 1983). Neurones that bind oestradiol are localized in the medial preoptic area, ventromedial hypothalamus and arcuate nucleus. These ventromedial neurones project into the medial preoptic area (Askesson *et al.*, 1988). This interneuronal network that binds oestradiol probably regulates the rate of release of GnRH pulses into the stalk median eminence. Modifications in binding of oestradiol by those neurones might result in reduction in the ability of oestradiol to inhibit release of GnRH. The number of receptors for the oestrogens in the hypothalamus declines during the peripubertal period in heifers (Day *et al.*, 1987) and this might reduce the response to oestradiol and increase the release of GnRH pulses.

Treatment of heifers before puberty with doses of oestradiol resulting in plasma concentrations greater than those for prepubertal heifers hastened the decline in oestradiol negative feedback on LH secretion during peripuberty (Dyer *et al.*, 1990; Fig. 1). This decline occurred in both intact and



**Fig. 1.** Frequency of LH pulses of heifers ovariectomized at 228 days of age and given a single implant containing oestradiol (OVXE). Heifers in the OVXE group (○) were treated with the single oestradiol implant throughout the treatment period and heifers in the OVXE + oestradiol group (●) were treated with the single oestradiol implant but received an additional oestradiol implant from day 16 to day 30 after initiation of the treatment (arrows indicate time of initiation and cessation of treatment with additional oestradiol). There was a treatment by time interaction ( $SEM = 1.3$ ). (Reproduced with permission from Dyer *et al.*, 1990.)

ovariectomized heifers treated with oestradiol. In rats, neurones responsive to oestradiol in the preoptic area of the hypothalamus sensitize neurones in the medial basal hypothalamus to negative feedback effects of oestradiol on release of GnRH. Transient exposure of preoptic neurones to high concentrations of oestradiol permanently alters neurones of the preoptic area so that reduction in oestradiol inhibition of GnRH release and enhanced release of LH pulses result, which are required for final maturation of preovulatory follicles.

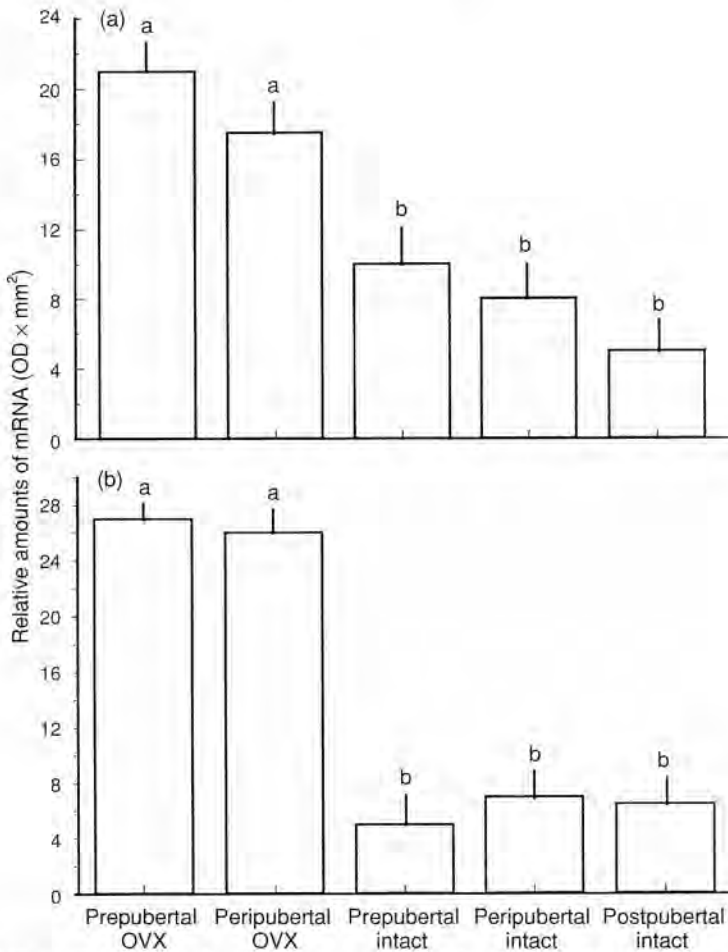
Similar transient exposure of heifers to supraphysiological concentrations of oestradiol might change the function of neurones of the hypothalamus so that release of GnRH pulses is enhanced. There are periods during sexual maturation when treatments with pharmacological agents such as high doses of oestradiol permanently alter release of LH pulses and in turn result in alterations in age at puberty.

In recent years there has been considerable study of endogenous opioids in modulation of release of LH pulses during sexual maturation. In prepubertal ewe lambs, blocking opioid receptors by treatment with naloxone resulted in an increase in frequency of LH pulses (Ebling *et al.*, 1989). Opioids interact with oestradiol to inhibit release of LH during prepuberty in heifers (Wolfe *et al.*, 1991), but the effects decline as heifers proceed from prepuberty to peripuberty (Wolfe *et al.*, 1991). There is no obvious change in inhibitory influence of opioids on secretion of LH during the period immediately before puberty in ewe lambs. Maturation of the neuronal system containing opioids may increase the frequency of LH pulses during sexual maturation but whether there is a reduced influence of opioids that corresponds to time of onset of puberty is equivocal.

## Synthesis and Release of Gonadotrophins during Sexual Maturation

### Synthesis of LH

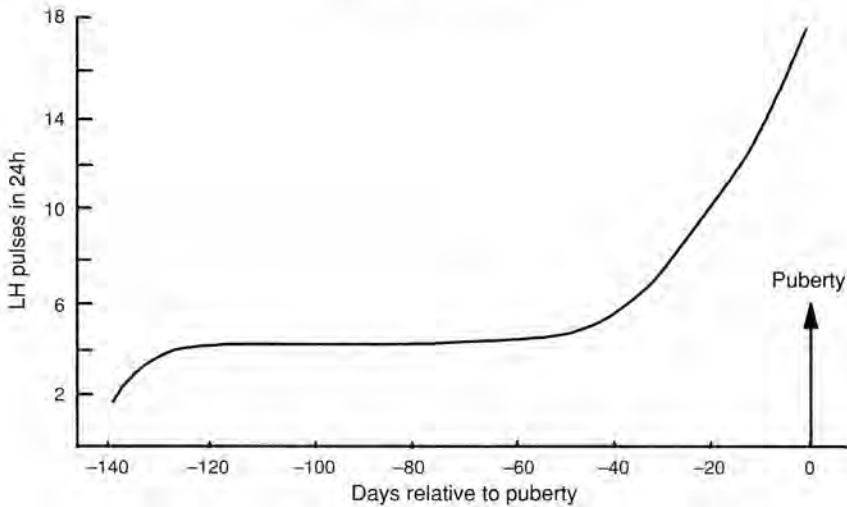
Concentrations of LH in the pituitary do not change from prepuberty to peripuberty in heifers (Stumpf *et al.*, 1992), but decline between peripuberty and postpuberty. Pituitary stores of LH are,



**Fig. 2.** Relative steady-state amounts of (a)  $\alpha$  subunit and (b) LH  $\beta$  subunit mRNAs corrected for tubulin. Heifers were ovariectomized (OVX) at approximately 210 days of age. Prepubertal: pituitaries were collected from heifers approximately 120 days before puberty; peripubertal: pituitaries were collected approximately 25 days before puberty; Intact: ovary-intact; postpubertal: pituitaries were collected 40 h after administration of PGF<sub>2 $\alpha$</sub> . No heifers were assigned to a postpubertal OVX group. Letters differing among treatments within mRNA type are significantly different. (Reproduced with permission from Roberson *et al.*, 1992.)

therefore, not limiting during sexual maturation in heifers. Amounts of mRNA encoding LH  $\alpha$  and  $\beta$  subunits do not differ among these heifers (Roberson *et al.*, 1992; Fig. 2). Therefore, mRNA encoding LH is not limited during sexual maturation in heifers. Ovariectomy of heifers results in greater amounts of mRNA encoding  $\alpha$  and  $\beta$  LH subunits both prepuberty and peripuberty than in intact heifers (Roberson *et al.*, 1992). The presence of ovaries before puberty, therefore, results in reduced amounts of mRNA encoding LH subunits which is probably the result of oestradiol suppression of GnRH release which maintains LH gene transcription (Hamernik *et al.*, 1986).

Ovariectomy resulted in a changed distribution of LH isoforms during prepuberty and peripuberty (Stumpf *et al.*, 1992). However, there was no detectable change in LH isoforms in the pituitary as sexual maturation progressed through prepuberty, peripuberty and postpuberty in intact heifers (Stumpf *et al.*,



**Fig. 3.** Change in frequency of LH pulses on days relative to puberty for heifers from which blood was collected at intervals of 20 min for 24 h every 2 weeks from 253 days of age until puberty occurred at an average of 366 days of age ( $r = 0.88$ ). (Reproduced with permission from Day *et al.*, 1987.)

1992). We do not consider therefore, that changes in the distribution of LH isoforms stored in the pituitary contribute to processes of sexual maturation in heifers.

#### Release of LH

Pulses of LH are released in ewes and cows. During prepuberty in heifers, pulses of LH are infrequent (1–4 pulses in 24 h) up until about 50 days before puberty (Day *et al.*, 1987). During the 50 days before puberty, frequency of LH pulses increases and approaches 24 pulses in 24 h during the few days before puberty (Day *et al.*, 1987, Fig. 3).

The onset of pulsatile release of LH begins about 11 weeks after birth in ewes and the frequency of LH pulses varies as puberty approaches at 35 weeks of age (Foster *et al.*, 1975). The increased frequency of release of LH pulses, therefore, is a prerequisite for the onset of puberty in heifers and ewe lambs.

When ewes or heifers are ovariectomized before puberty, frequency of LH pulses increases immediately and approaches 1 pulse per hour (Foster and Ryan, 1979; Day *et al.*, 1984; Wolfe *et al.*, 1989). When ovariectomized ewes or heifers are treated with oestradiol in amounts that result in physiological concentrations in the circulation, the frequency of LH pulses is reduced to values similar to those of age-matched intact control animals (Foster and Ryan, 1979; Day *et al.*, 1984; Wolfe *et al.*, 1989). The frequency of LH pulses in ovariectomized ewes and heifers treated with oestradiol increases markedly during the time that age-matched control ewes and heifers attain puberty (Foster and Ryan, 1979; Day *et al.*, 1984; Wolfe *et al.*, 1989). Thus, small amounts of oestradiol secreted by ovaries before puberty inhibit the release of LH pulses via inhibition by oestradiol of the GnRH pulse generator of the hypothalamus. In postpubertal cows, the same circulating concentrations of oestradiol that inhibit release of LH before puberty increase the amplitude of LH pulses and mean circulating concentrations of LH (Day *et al.*, 1986a; Stumpf *et al.*, 1988; Stumpf *et al.*, 1989). Increased amplitude of LH pulses as a result of treatment with low doses of oestradiol probably results from an increased response of the pituitary to each GnRH pulse that is released from the hypothalamus.

Of the three measurements of LH secretion (mean concentration of LH, frequency of release of LH pulses, and amplitude of LH pulses) in heifers, frequency of release of LH pulses is the best predictor of age at puberty (Day *et al.*, 1987). However, the frequency of release of LH pulses is not a reliable predictor of time of puberty until the last 50 days before puberty (Fig. 3).



Treatment of ewe lambs with repeated low doses of LH at frequent intervals induces a preovulatory surge of gonadotrophins followed by formation of corpora lutea (Foster *et al.*, 1984). Pulsatile administration of low doses of LH also induces preovulatory surges of gonadotrophins and ovulation in heifers (Tortonese *et al.*, 1990). Pulsatile administration of LH, therefore, stimulates ovarian follicular development to the point that sufficient amounts of oestradiol are secreted to induce ovulation in both ewes and heifers.

### *Synthesis of FSH*

Removal of ovaries either before or after puberty from heifers resulted in alterations in FSH isoforms stored in pituitaries compared with those of intact animals (Stumpf *et al.*, 1992). There were, however, no significant changes in the pattern of FSH isoforms in pituitaries of intact heifers as heifers progressed through prepuberty, peripuberty and postpuberty (Stumpf *et al.*, 1992).

### *Release of FSH*

In ewe lambs in which puberty is induced by pulsatile administration of GnRH, there are greater amounts of bioactive FSH in serum than in untreated control ewes (Padmanabhan *et al.*, 1992). Increased bioactivity of FSH is associated with a greater proportion of acidic FSH isoforms in serum of prepubertal compared with pubertal ewe lambs. There is no corresponding difference of acidic FSH isoforms in pituitaries of prepubertal compared with pubertal ewe lambs. There is, therefore, preferential secretion of acidic FSH isoforms during sexual maturation in ewes and changes in heterogeneity of FSH isoforms released into blood may have a role in the process of sexual maturation in ewes.

Concentrations of FSH in the circulation increase from 3 to 11 weeks of age in ewe lambs but no subsequent changes occur between 11 and 35 weeks of age when the onset of oestrous cycles occurs at puberty (Foster *et al.*, 1975). There is also no association between circulating concentrations of FSH and time of puberty in heifers (Schams *et al.*, 1981; Wolfe *et al.*, 1989). Changes in circulating concentrations of FSH, therefore, are not associated with onset of puberty in ewes or heifers.

## **Development of Ovarian Structures during Sexual Maturation**

### *Ovarian follicles*

Ovarian follicles in prepubertal heifers grow and regress in waves as occurs in postpubertal cows (Hopper *et al.*, 1993; Evans *et al.*, 1994). Increases in circulating FSH precede the emergence of waves of ovarian follicular growth in postpubertal cows (Adams *et al.*, 1992). Increases in serum concentrations of FSH occurred 1 to 2 days before emergence of a wave of growth of ovarian follicles. Concentrations of FSH in serum begin to decrease when follicles diverge into dominant and subordinate classes. Presumably similar changes in secretion of FSH regulate waves of follicular development in heifers before puberty. Adequate circulating FSH, therefore, probably stimulates development of waves of ovarian follicles in heifers during prepuberty and peripuberty.

We evaluated ovarian follicular development during the latter prepubertal period and peripubertal period of heifers (Bergfeld *et al.*, 1994). Compared with earlier prepuberty, dominant ovarian follicles were larger as puberty approached (Table 1). The greatest increase in size of the largest ovarian follicles occurred during the last 30 days before puberty corresponding to the time when there is a large increase in frequency of LH pulses. Increased frequency of LH pulses, therefore, may stimulate development of larger dominant ovarian follicles in the latter stages of peripuberty compared with earlier in life.

### *Luteal structures*

There are transient increases in circulating concentrations of progesterone of ovarian origin during the peripubertal period (Berardinelli *et al.*, 1979). Ovulation can occur before development of these

**Table 1.** Maximum size (mm) of the dominant ovarian follicles of prepubertal heifers fed either a high ( $n = 5$ ) or low ( $n = 5$ ) energy diet

Energy diet	Month of treatment						
	1	2	3	4	5	6	7
High	11.05	11.96	12.14	12.02			
Low	9.16	10.54	10.45	10.85	11.86	12.60	12.02

Data from Bergfeld *et al.* (1994).

transient increases in progesterone (Quirke *et al.*, 1985). In other studies no ovulation papillae were observed on luteal structures that were removed when progesterone concentration first increased (Berardinelli *et al.*, 1979). Apparently there was enough LH support to luteinize dominant ovarian follicles in the absence of ovulation during peripuberty when luteal structures developed in the absence of ovulation. Ovulation is, therefore, not necessary for the rise in progesterone during peripuberty.

Behavioural oestrus does not generally precede transient luteal phases before puberty possibly because of the lower concentrations of oestradiol in circulation before development of transient luteal structures compared with concentrations in the circulation before pubertal oestrus (Glencross *et al.*, 1984). The time of puberty is not affected by removal of luteal tissue immediately after the rise in progesterone is detected (Keisler *et al.*, 1983).

### Development of the Uterus during Sexual Maturation

In heifers, the uterus grows at a similar rate to the rest of the body until 6 months of age but then grows more rapidly until puberty (Desjardins and Hafs, 1968). Concentrations of oestradiol in circulation increase as puberty approaches in heifers (Bergfeld *et al.*, 1994) and might contribute to increased rate of uterine growth particularly during the last stages of peripuberty.

Luteal function before the pubertal oestrus might serve to provide a more desirable uterine environment for the embryo should conception occur as a result of mating at the pubertal oestrus. Whatever the case, there is a marked change in the size of the uterus during peripuberty as evidenced by large increases in uterine mass subsequent to the first rise in circulating progesterone in heifers (Day, 1985). Development of luteal function in heifers is, therefore, associated with marked changes in uterine growth.

### Factors Influencing Time of the Peripubertal Increase in Frequency of LH Pulses

#### Genotype

There is a negative within-breed correlation between age at puberty in heifers and subsequent milk production of females as adults (Laster *et al.*, 1979). When all data are taken together, heifers of dairy or beef breeds with greater genetic ability for milk production reach puberty earlier than do heifers of breeds with lower milk production. Heritability estimates for age at puberty in beef heifers are about 0.50. There is a genetic correlation between reproductive characteristics of males and females based on common hormonal relationships in both sexes. There is a high negative correlation between scrotal circumference (testis size) in developing bull calves and age at puberty in genetically related heifer calves. Selection for increased scrotal circumference during sexual maturation results in an earlier puberty in daughters of these bulls (Morris *et al.*, 1992). Because of high heritability for age at puberty, genetic selection can result in shifting of the sequence of endocrine events that modulate the onset of puberty.

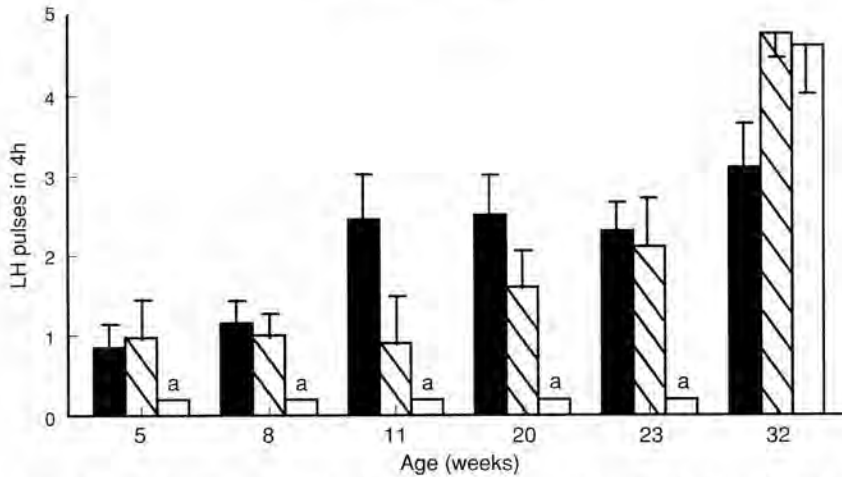


Fig. 4. Mean frequency of LH pulses in gonadectomized, oestradiol-treated males (control males; ■), females (control females; □) and androgenized female lambs (▨). Androgenized ewe lambs were produced by treating pregnant ewes with 100 mg testosterone cypionate i.m. each week from day 30 to day 90 of gestation and then using the ewe lambs that resulted from these pregnancies in the experiment. (Reproduced with permission from Wood *et al.*, 1991; ©The Endocrine Society.)

As a result of the relationship between testis development in males and age at puberty in related females, age at puberty can be changed in herds and flocks by applying these practices for genetic selection.

### Gender

The time when there is an increase in frequency of release of pulses of LH that stimulate gonadal development varies significantly between male and female lambs. Production of androgens during the prenatal period by male lambs alters the time of neuroendocrine maturation of the LH pulses (Wood *et al.*, 1991; Fig. 4). Treating pregnant ewes carrying female fetuses at 30–90 days of gestation with testosterone results in an earlier increase in release of tonic LH pulses in ewe lambs born from ewes treated with testosterone compared with ewe lambs from untreated control ewes (Wood *et al.*, 1991). Androgen production prenatally, therefore, causes increases in tonic release of LH pulses earlier in male than in female lambs.

### Season of year

Ewes are seasonal breeders and photoperiodic cues therefore modify time of onset of puberty in ewe lambs (Foster *et al.*, 1986). A delay in the onset of puberty occurs in ewe lambs that attain pubertal age during nonbreeding seasons (Foster *et al.*, 1986). A decrease in photoperiod is necessary to signal timing of puberty of ewe lambs but seasonally anoestrous ewes can enter the breeding season in the absence of decreased photoperiod (Ebling and Foster, 1988). Photoperiodic signals that affect age at puberty are mediated by melatonin from the pineal gland as a result of perception of daylength and transmission of photoperiodic signals to the hypothalamus.

There are also seasonal influences on age at puberty in heifers, even though cattle are not seasonal breeders (Schillo *et al.*, 1992). The influence of photoperiod on age at puberty in heifers was reviewed by Schillo *et al.* (1992). Brahman heifers exhibit a definite seasonal variation in the presence of corpora lutea (Plasse *et al.*, 1968). Incidences of corpora lutea increase during spring, are greatest in summer, decline during winter and increase during the second spring. Season, therefore, affects age at puberty in heifers even though cattle are not seasonal breeders.



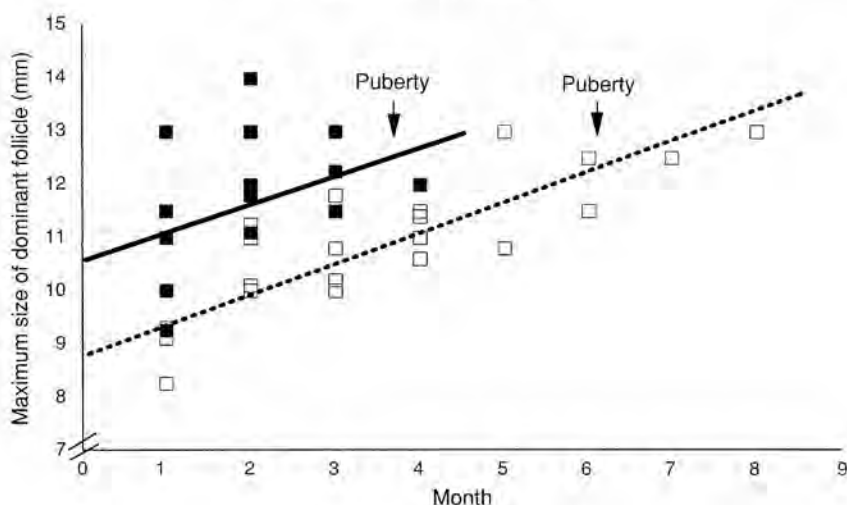


Fig. 5. Regression of dominant follicle across time for heifers fed a diet of either high (solid line; ■;  $r = 0.76$ ) or low (dashed line; □;  $r = 0.43$ ) energy content. Heifers averaged 240 days of age at initiation of the experiment (month 0 on graph). Time of puberty during the treatment period is indicated by the arrows that depict when heifers of each group attained puberty. (Reproduced with permission from Bergfeld *et al.*, 1994.)

Season of year has marked effects on secretion of LH in adult cows (Stumpf *et al.*, 1988). Treatment of heifers during prepuberty with melatonin hastens the onset of puberty (Tortonese and Inskeep, 1992). Melatonin, therefore, modulates the onset of puberty in heifers in a similar way to that in ewe lambs except long days are perceived as stimulatory in heifers, whereas short days are stimulatory in ewe lambs. In summary, during prepuberty, heifers can respond to seasonal cues by modulating release of LH from the anterior pituitary.

#### Growth-nutritional intake

Nutritional intake and growth rate influence age at puberty (Foster *et al.*, 1986). We do not understand how nutritional cues affect the central nervous system to modulate frequency of release of LH pulses from the anterior pituitary (Schillo *et al.*, 1992). Fluctuations in intermediary metabolism associated with changes in body mass or body fatness somehow modulate release of LH pulses. However, fatty acids or growth hormone do not affect frequency of release of LH pulses in sheep or cattle (Schillo *et al.*, 1992).

Heifers were divided into two groups and fed two different amounts of dietary energy (Bergfeld *et al.*, 1994). Heifers fed greater amounts of energy were expected to attain puberty earlier than those fed diets with lower energy (372 versus 435 days of age at puberty). Larger dominant ovarian follicles were detected in heifers fed the diet with greater energy at a younger age than in heifers fed diets with lower energy content (Fig. 5). Release of LH pulses is suppressed in heifers fed limited amounts of dietary energy before puberty compared with heifers fed diets with greater energy content (Day *et al.*, 1986b). Increases in frequency of LH pulses occurred earlier in heifers fed diets with greater energy content (Day *et al.*, 1986b), which might lead to development of larger ovarian follicles earlier in life (Bergfeld *et al.*, 1994). The size of ovulatory follicles at puberty did not differ among heifers fed diets with low or high energy content. Ovulation occurred when dominant follicles reached approximately 13 mm in diameter (Fig. 5). Dominant ovarian follicles of 13 mm in diameter were attained on average 63 days earlier in heifers fed diets with greater energy content than in heifers fed the diet with lower energy content.

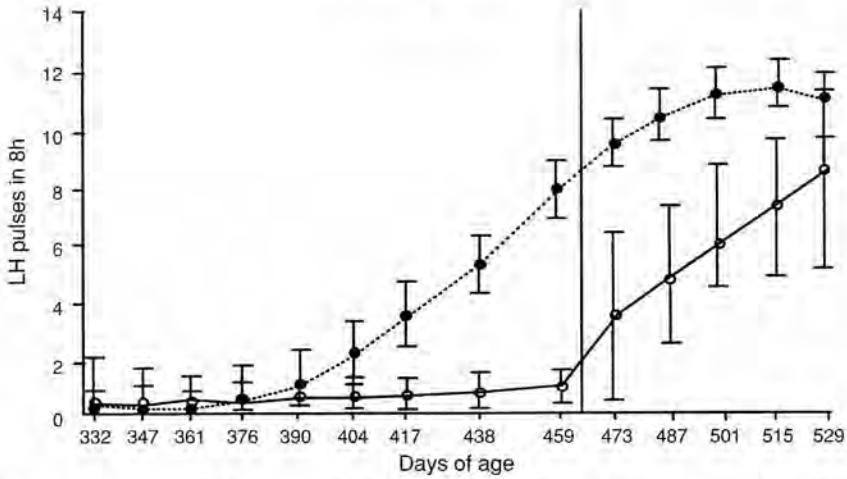


Fig. 6. Regression of frequency of LH pulses on day of age for ovariectomized heifers treated with oestradiol (OVXE) fed either a two-phase, low to high energy diet (○; solid line) or a control (●; broken line) diet during the experimental period. The vertical line separates the period of feeding of the low energy diet (0–127 days of experimental period) from the high energy diet (128–197 days of experimental period) to heifers in the ovariectomized low to high energy group. Error bars represent 95% confidence limits for each mean. (Reproduced with permission from Kurz *et al.*, 1990.)

There is a prolonged response to inhibition by oestradiol on release of LH pulses in heifers (Kurz *et al.*, 1990; Fig. 6) and ewe lambs (Foster *et al.*, 1986), if the amount of dietary energy is limiting during prepuberty. Increases in frequency of release of LH pulses that occur at puberty are delayed until later (Day *et al.*, 1986b) and this is the underlying cause of delayed puberty if nutritional content of diets is limited.

Immunization against growth hormone releasing hormone delays the onset of puberty in heifers, resulting in a decrease in concentrations of insulin-like growth factor I in serum and ovarian follicular fluid and suppression of ovarian follicular growth without affecting concentrations of LH in serum (Simpson *et al.*, 1991). Chronic treatment with bovine somatotrophin did not alter age at puberty in heifers (Hall *et al.*, 1994). Enhanced amounts of circulating growth hormone, therefore, do not result in delayed or earlier induction of endocrine processes involved in modulation of age at puberty in heifers, but when low concentrations of growth hormone are present before puberty, the onset of puberty is delayed.

### Social cues

Administration of bull urine via the nasal cavity to prepubertal heifers resulted in an increased proportion of heifers attaining puberty during an 8 week treatment (Izard and Vandenberg, 1982). Early studies indicated that there was no influence of presence of bulls on age at puberty in heifers (Roberson *et al.*, 1987), but a later study showed that the presence of bulls with heifers did influence the age at puberty in heifers (Roberson *et al.*, 1991). There was an interaction between growth rate of heifers before puberty and presence of bulls on age at puberty of heifers (Roberson *et al.*, 1991; Table 2). We hypothesized that the presence of bulls with heifers before puberty would influence age at puberty in heifers gaining weight at moderate rates but not at high rates of weight gain; however, there was an influence of the presence of bulls on age at puberty in heifers gaining weights at either moderate or high rates during periods of sexual maturation.

In a recent study, the presence of bulls resulted in enhanced tonic release of LH pulses in postpartum anoestrous cows (Berardinelli *et al.*, 1994). On the basis of existing data, we assume that the presence of

**Table 2.** Growth rates, age at puberty and pregnancy rates in heifers that were artificially inseminated for 21 days

Treatment <sup>b</sup>	Growth rate (kg day <sup>-1</sup> )	Age at puberty (days) <sup>a</sup>		AI pregnancy <sup>c</sup> (pregnancies per group)
		Year 1	Year 2	
BE × HIGHGR	0.82	358	391	20/40
BE × MODGR	0.63	417	427	20/39
NE × HIGHGR	0.77	427	428	10/40
NE × MODGR	0.68	456	441	3/40
Pooled SEM	0.02	8.5	8.7	

<sup>a</sup>Bull exposure × growth rate interaction.

<sup>b</sup>BE: exposed to bulls; NE: not exposed to bulls; HIGHGR: high growth rate; MODGR: reduced growth rate; *n* = 19 or 20 per group; treatments were initiated when heifers were 268 ± 6 days of age and 248 ± 7 kg per body mass.

<sup>c</sup>Percentage of all heifers in treatment group that conceived during 21 days of artificial insemination which was initiated when heifers were at an average of 420 days of age.

Data from Roberson *et al.* (1991).

bulls is perceived by heifers via pheromonal cues and transmitted via neurones to the hypothalamic pulse generator that controls release of GnRH. Enhanced secretion of LH pulses and onset of oestrous cycles at puberty would be the end result.

### Exogenous progestin

Short-term treatment with progestins during peripuberty induced puberty in heifers (Short *et al.*, 1976). Induction of puberty in lightweight heifers occurred, but conception at the induced oestrus was low (Short *et al.*, 1976). Prepubertal heifers treated with progestins to induce puberty exhibited behavioural oestrus but failed to ovulate after removal of progestin implants (Escobedo *et al.*, 1989).

The reason why short-term treatment with progestins induces puberty has not been elucidated. It would be anticipated that treatment with progestins, such as norgestomet, during prepuberty when the pulse generator for GnRH is being suppressed by oestradiol would result in further reduction in frequency of LH pulses. Norgestomet has been used most frequently to induce onset of puberty in previous experiments. Inhibitory feedback of norgestomet on LH pulses occurs in adult bovine females (Sanchez *et al.*, 1995). However, treatment of peripubertal heifers with norgestomet resulted in enhanced release of LH pulses during periods of norgestomet treatment (Anderson and Day, 1994). On the basis of these data, we speculate that enhanced release of LH pulses during periods of norgestomet treatment stimulate development of dominant ovarian follicles to a greater degree than would occur in untreated control heifers resulting in enhanced production of oestradiol subsequent to progestin withdrawal. Enhanced oestradiol would induce behavioural oestrus and preovulatory surges of gonadotrophins and subsequent pubertal ovulation.

### Sexual Maturation after Puberty

Sexual maturation continues after puberty in heifers. Fertility was greater at the third oestrus after puberty than at the pubertal oestrus in heifers (Byerley *et al.*, 1987). When embryos from mature cows were transferred to heifers at their pubertal oestrus and third oestrus after puberty, conception was lower at the pubertal oestrus (Staigmiller *et al.*, 1993). Uterine environment subsequent to the pubertal oestrus, therefore, is not adequate for maintenance of pregnancy in some heifers, and maturation of the uterus continues after puberty.

### Variation in Age at Puberty of Heifers

There is tremendous variation in age at puberty among females within a species. Because of all the factors that can affect age at puberty, a large variation in age at puberty is expected. Age at puberty in heifers ranges from 6 months to more than 24 months of age. Oestrus without initiation of ovarian cycles occurs in some heifers during prepuberty and this has been called nonpubertal oestrus (Nelson *et al.*, 1985). Nonpubertal oestrus varies with breed of heifer and incidence of nonpubertal oestrus may be related to season (Nelson *et al.*, 1985). Nonpubertal oestrus might result when oestrogen production is sufficient to induce behavioural oestrus but not ovulation.

On the basis of two observations in our research herd at the University of Nebraska we hypothesized that age at puberty was a variable that was not normally distributed. We determined that age at puberty ranged from 427 to 456 days of age for four groups of heifers over a two-year study if heifers were not in the presence of bulls during the period of sexual maturation (Roberson *et al.*, 1991). However, on the basis of calving records, there were some years in which as many as 5% of the heifers of this herd calved at less than 15 months of age. These two observations are not consistent if age at puberty is a normally distributed variable.

We recently performed a study to determine whether age at puberty of heifers in our research herd is normally distributed and to determine whether precocious puberty occurred in some heifers (Wehrman *et al.*, 1994). Precocious puberty was defined by transient cyclic increases of progesterone in the circulation. If these transient increases in progesterone were detected before 300 days of age precocious puberty was considered to have occurred. The study was replicated over two years and half of the heifers each year were placed in paddocks with sterile bulls whereas the other half of the heifers were isolated from bulls. There was a year effect on incidence of precocious puberty with incidence of precocious puberty being 25% and 8% for the two years. There was no influence of presence of bulls on incidence of precocious puberty. Average age at which precocious puberty occurred was  $194 \pm 12$  days. Cyclic luteal function continued for 66 days with resumption of anoestrus occurring at  $260 \pm 15$  days of age. We believe cyclic luteal function was indicative of a true precocious puberty because some heifers in previous years became pregnant at ages similar to those when heifers exhibited cyclic profiles of luteal function in the present study. The endocrine basis for precocious puberty in heifers is not understood.

### Conclusion

Maturation of the hypothalamic–pituitary–ovarian–uterine axis must occur during sexual maturation if heifers and ewe lambs are to become capable of reproducing. Before puberty in heifers and ewes, neurones that contain GnRH are inhibited from releasing GnRH at a rate that would result in pubertal onset. Inhibition of tonic GnRH release results from negative feedback effects of oestradiol on the GnRH pulse generator. There are many factors (genotype and gender of animals, season of year when pubertal age is attained, growth rate or level of nutritional intake during period of sexual maturation and presence of males) that determine time when reduction in negative feedback actions of oestradiol occurs to the point that frequency of release of GnRH pulses is adequate to allow the onset of puberty. All of these factors probably affect the age at puberty by modulating the frequency of release of GnRH pulses during sexual maturation. As sensitivity to oestradiol inhibition on the GnRH pulse generator declines during the peripubertal period preceding puberty, there is an increased frequency of release of GnRH and increased frequency of release of LH from the anterior pituitary. Increased frequency of LH pulses stimulates development of dominant ovarian follicles and these follicles grow larger before undergoing atresia as peripuberty progresses. Eventually there is enough LH support to induce luteinization of granulosa and thecal cells (either with or without ovulation) of large ovarian follicles resulting in transient increases in circulating concentrations of progesterone of shorter duration than typically present during luteal phases of adult females. Greater amounts of oestradiol are secreted as larger dominant ovarian follicles develop during the peripubertal period preceding puberty. Increased oestradiol combined with transient increases in progesterone probably induce significant changes in uterine morphology during the final stages of sexual maturation. Dominant follicles finally develop to the point at which they produce enough oestradiol to induce behavioural oestrus and preovulatory

surges of gonadotrophins. Pubertal oestrous cycles ensue with development of corpora lutea that are maintained for periods that are typical for heifers or ewes. Sexual maturation continues after puberty because conception rate is lower if mating occurs at the pubertal oestrus than if mating occurs during oestrus periods that are further removed from time of puberty. Sexual maturation subsequent to puberty probably results in uterine development. When postpubertal sexual maturation is completed, all functional components of the hypothalamic-pituitary-ovarian-uterine axis are in place for rates of reproduction to occur that are typical for ovine and bovine females.

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