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Endocrine regulation of puberty in cows and ewes

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Summary. Sexual maturation in cows and ewes is modulated through changes in hypothalamic inhibition. This inhibition results in little or no stimulation of the release of gonadotrophins from the anterior pituitary. The ovary has a primary role in inhibiting gonadotrophin secretion during the prepubertal period and the responsiveness to the negative feedback effects of oestrogen decreases during the peripubertal period. There is also an increased secretion of ovarian progesterone during the peripubertal period but its role in the process of sexual maturation is not clear. Photoperiodic cues and dietary intake act upon the hypothalamus to modulate gonadotrophin secretion during sexual maturation and, in turn, influence the time when puberty occurs.

Introduction

Puberty in this article is defined as the first behavioural oestrus accompanied by the development of a corpus luteum that is maintained for a period characteristic of a particular species. The maturation process that culminates in puberty occurs in a gradual fashion. It is initiated before birth and continues throughout the prepubertal and peripubertal periods of developing females. Some components of the endocrine system of prepubertal females are functional long before puberty occurs. For example, the gonadotrophs of the pituitary respond to hypothalamic secretagogues and the ovaries respond to exogenous gonadotrophins during the early phases of sexual maturation, well before first ovulation. Likewise the ovaries are able to respond to exogenous gonadotrophins administered before puberty. However, there appears to be at least one component of the endocrine system of the prepubertal ewe lamb and heifer that is incapable of functioning in an adult fashion until at or near the time of puberty. An alternative view is that all components of the endocrine system of prepubertal females are mature but one or more specific components are inhibited from functioning in an adult fashion.

Pituitary and ovarian function

Hypophysial stalk transection resulted in complete cessation of pulsatile release of luteinizing hormone (LH) at 5 months of age in heifers (Anderson *et al.*, 1981). Pituitary responsiveness to luteinizing hormone-releasing hormone (LHRH) was evaluated in prepubertal heifers by administration of LHRH at monthly intervals from 1 month of age to puberty (Schams *et al.*, 1981). Pituitary secretion of LH in response to LHRH was observed at all ages but the magnitude of this response increased with age. Increased follicle-stimulating hormone (FSH) secretion only occurred during the first 5 months of the 9-month prepubertal period. LHRH was administered at 4 and 10 months of age to prepubertal heifers (McLeod *et al.*, 1984). The amount of LH and FSH released in response to LHRH was greater in the older heifers. Pituitary concentrations of FSH and LH were higher in

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prepubertal than postpubertal heifers, indicating that the pituitary is able to synthesize and store LH and FSH before puberty (Desjardins & Hafs, 1968).

Exogenous administration of gonadotrophins has resulted in the production of fertile ova in heifers at 1 month of age (Seidel *et al.*, 1971). The number of induced ovulations resulting from exogenous gonadotrophins was greater in 5-month-old than in 1-month-old heifers. Therefore, both the pituitary and ovary respond to exogenously administered hormones early in life and maturation of these organs appears to occur before puberty.

Positive feedback of oestradiol in inducing the preovulatory surge of gonadotrophins

Increasing levels of oestradiol during the follicular phase of the oestrous cycle are thought to act at the hypothalamo–pituitary axis to induce the preovulatory surge of LH and FSH secretion in cattle (Echternkamp & Hansel, 1973). Induction of the preovulatory gonadotrophin surge by oestradiol is a component of the endocrine system that is essential for puberty to occur. Surges in LH secretion similar to those present before ovulation in mature cows have been induced in prepubertal heifers by administration of oestradiol (Schillo *et al.*, 1982). This component of the endocrine system appears to become functional between 3 and 5 months of age in heifers and the amount of LH released in response to oestradiol is actually greater in pre- than postpubertal heifers (Staigmiller *et al.*, 1979).

Squires *et al.* (1972) initially demonstrated that oestradiol would induce surges of LH in prepubertal ewe lambs that were similar to the preovulatory surge in sexually mature ewes. Foster & Karsch (1975) found that oestradiol failed to elicit a surge in secretion of LH at 3 weeks of age in ewe lambs, but surges progressively increased in magnitude as lambs were treated with oestradiol at 7, 12, 20 and 27 weeks of age. By 27 weeks of age, the surge in LH secretion was similar to the response of ewes given a similar dose of oestradiol during the anoestrous season. In that flock, puberty is normally attained in ewe lambs between 30 and 50 weeks after birth. Exogenous oestradiol does not appear to be effective in inducing ovulation and/or subsequent development of a corpus luteum that is maintained for a complete oestrous cycle (Foster & Karsch, 1975; Tran *et al.*, 1979).

Secretion of gonadotrophins during sexual maturation

Secretion of LH during the prepubertal period

Circulating concentrations of LH have been evaluated from birth to puberty in heifers (Schams *et al.*, 1981); values increased from birth to 3 months of age, declined from 3 to 6 months of age and then increased up to the time of puberty at 10 months of age. Frequency of pulses of LH increased and the amplitude of LH pulses decreased from 1 month of age until puberty. Swanson *et al.* (1972) reported that mean concentrations of LH increased during the 110-day period preceding puberty in heifers. An increase in mean concentration of serum LH and frequency of episodic LH pulses occurs during the 126 days preceding puberty (Day *et al.*, 1984; Table 1). This increase during the last 4 months before puberty coincides with the second increase in LH secretion reported by Schams *et al.* (1981).

Others have reported that serum concentrations of LH did not increase during the period of sexual maturation (Gonzalez-Padilla *et al.*, 1975b; McLeod *et al.*, 1984). The frequency and duration of blood collection is important for evaluating the dynamic changes that occur in gonadotrophin secretion before puberty. In a recent study we collected from heifers blood samples at 20-min intervals for 24 h every 2 weeks during the 140-day period before puberty. Our objective for this intensive evaluation of prepubertal secretion of LH was to develop a regression equation of LH

Table 1. Mean concentration of LH in serum and LH pulse frequency before puberty in intact heifers (N = 6) (from Day *et al.*, 1984)

Day*	Mean LH conc.† (ng/ml)	LH pulse frequency‡ (pulses/h)
−126	0·85 ± 0·26	0·04 ± 0·04
−112	1·22 ± 0·29	0·06 ± 0·06
− 98	0·93 ± 0·20	0·13 ± 0·06
− 84	1·11 ± 0·25	0·15 ± 0·05
− 70	1·33 ± 0·30	0·19 ± 0·07
− 56	1·16 ± 0·23	0·19 ± 0·08
− 42	1·48 ± 0·29	0·25 ± 0·08
− 28	1·48 ± 0·24	0·31 ± 0·05
− 14	2·30 ± 0·20	0·48 ± 0·10

*Days relative to puberty.
†$y = 0{\cdot}53 + 0{\cdot}15x$; $P < 0{\cdot}0001$, $R^2 = 0{\cdot}28$.
‡$y = 0{\cdot}36 + 0{\cdot}38x$; $P < 0{\cdot}0001$, $R^2 = 0{\cdot}36$.

on days before puberty that could be used to predict the stage of sexual maturation in heifers that were killed before puberty. The heifers in this study reached puberty at a mean of 366 (s.e.m. = 4) days of age at a mean weight of 244 (s.e.m. = 3) kg. Mean concentrations of serum LH tended to increase in a gradual linear fashion during the 140-day period before puberty (Fig. 1). Amplitude of pulses of LH declined ($P < 0{\cdot}05$) in a cubic manner as puberty approached (Fig. 1). The regression line for frequency of LH pulses during the 130-day period preceding puberty was cubic ($P < 0{\cdot}01$; Fig. 2). Of the 3 measurements of LH secretion (mean LH concentration, frequency of LH pulses and amplitude of LH pulses), frequency of LH pulses was the best predictor of age at puberty. The correlation coefficient of the regression of frequency of LH pulses on days before puberty was 0·88. This compares to a correlation coefficient of 0·60 from an earlier study (Day *et al.*, 1984) in which blood was collected at 12-min intervals for 8 h. This may indicate that long sampling periods are necessary to obtain sufficient data to assess the characteristics of gonadotrophin secretion and to predict age at puberty based on these characteristics. From 130 to 46 days before puberty, frequency of LH pulses did not increase and there was considerable variation amongst animals. Prediction of the occurrence of puberty from LH data collected during this period was therefore difficult. However, during the last 46 days before puberty the prediction equation for age at puberty was more useful. Blood samples were taken over 24-h periods at 20-min intervals to determine the stage of sexual maturation at the time of slaughter in a group (N = 20) of contemporary heifers. Using the regression of pulse frequency on days before puberty developed with the 6 control heifers, it was estimated that the mean age at puberty in the 20 heifers that were slaughtered would have been 360 (s.e.m. = 7) days of age. In 10 heifers reared in conditions similar to those utilized to formulate the prediction equation and those that were slaughtered, the average age at puberty was 360 (s.e.m. = 6) days. The equation was surprisingly accurate in prediction of puberty in this group of animals. We believe the data from this study clarify some of the inconsistencies between studies regarding the change in secretion of LH in prepubertal heifers. A definite increase in frequency of LH pulses occurred during the 50 days before puberty but changes in frequency of LH pulses before the 50 days prior to puberty are less obvious in the heifer.

The onset of pulsatile secretion of LH begins at about 11 weeks after birth in the ewe lamb. At this time, concentrations of LH increase to levels similar to those detected at the time of puberty. Frequency of pulses in LH appear to vary dramatically as puberty (35 weeks of age) approaches in ewe lambs (Foster *et al.*, 1975). There is evidence of a marked increase in frequency but not amplitude of LH pulses shortly before first ovulation (Huffman & Goodman, 1985). Concentrations of

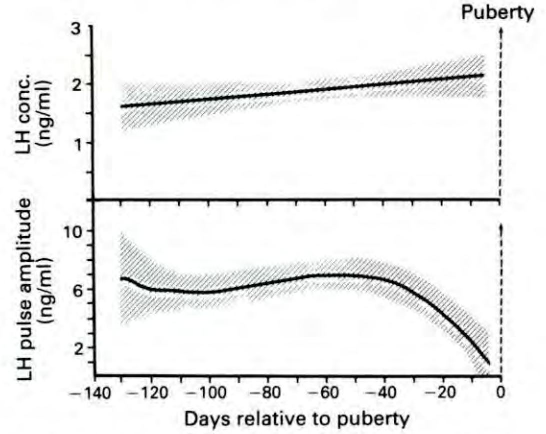

Fig. 1. Regression of mean concentration of LH and amplitude of LH pulses on days relative to puberty in heifers. Correlation coefficient for mean LH concentration; $r = 0{\cdot}2$. Correlation coefficient for LH pulse amplitude, $r = 0{\cdot}51$.

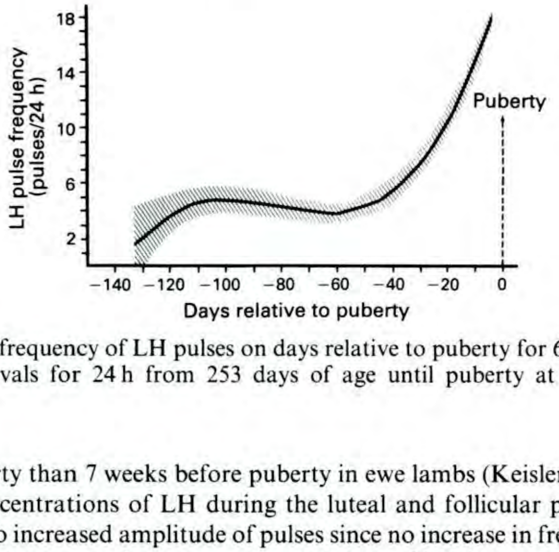

Fig. 2. Regression of frequency of LH pulses on days relative to puberty for 6 heifers that were bled at 20-min intervals for 24 h from 253 days of age until puberty at 366 days of age ($r = 0{\cdot}88$).

LH were higher at puberty than 7 weeks before puberty in ewe lambs (Keisler *et al.*, 1985). In that study, the increased concentrations of LH during the luteal and follicular phases preceding first oestrus were attributed to increased amplitude of pulses since no increase in frequency of LH pulses was detected. Therefore, it appears that mean concentrations of LH increase before puberty in the ewe lamb. However, it is not clear whether increased frequency or increased amplitude of LH pulses is responsible for the increase in mean LH concentrations.

Administration of purified LH at a level of 15·5 μg/h in a pulsatile fashion over a 48-h period induced an LH surge and ovulation in 2 of 3 lambs (Foster *et al.*, 1984). By contrast, administration of a lower dose or 15·5 μg LH at 3-h intervals did not induce any surge in LH or ovulation. In another experiment (Foster *et al.*, 1984), the same dose of 15·5 μg of purified LH was administered in hourly pulses. Of 7 lambs, 4 had a sustained increase in oestradiol, a preovulatory surge of LH and ovulation. However, the luteal phase was normal in only one of the lambs and a short luteal phase of 6–11 days occurred in the other 3 lambs that ovulated. Keisler *et al.* (1985) conducted a study in which ovine LH was pulsed at 7·5 μg/h, 15 μg/h, 30 μg/2 h, 45 μg/3 h or was infused continuously at 15 μg/h into ewe lambs for 48 h (Table 2). Pulses of about 24 ng LH/ml serum were produced by pulsatile administration of LH at 2- or 3-h intervals but this did not consistently induce preovulatory-like surges of gonadotrophin. In contrast, pulses of 6–12 ng LH/ml serum

Table 2. Peak and basal concentrations of LH in plasma after infusion or pulsed delivery of ovine LH into prepubertal lambs and occurrence of surges of LH in response to exogenous LH (from Keisler *et al.*, 1985)

Treatment (dose)	Conc. (ng/ml) of LH achieved: Peak	Conc. (ng/ml) of LH achieved: Basal	Lambs responding with a surge of LH
Control (saline)		1·6 ± 0·2	1/6
Infused (15 μg LH/h)	6·3 ± 0·3	6·3 ± 0·2	3/6
Pulsed (7·5 μg LH/h)	6·0 ± 0·4	2·0 ± 0·2	5/6
Pulsed (15 μg LH/h)	11·7 ± 0·6	3·9 ± 0·3	4/6
Pulsed (30 μg LH/2 h)	23·9 ± 1·1	2·6 ± 0·2	1/5
Pulsed (45 μg LH/3 h)	31·9 ± 2·4	1·7 ± 0·2	1/6

Values are mean ± s.e.m.

were observed after hourly administration of LH and were effective in inducing preovulatory LH surges. Thus, hourly administration of the purified LH resulted in a frequency and amplitude of LH pulses similar to that detected during the follicular phase associated with the first oestrus as puberty is attained in the ewe lamb.

When 2 μg injections of LHRH were administered at 2-h intervals for 72 h in 5-month-old prepubertal heifers, mean circulating concentrations of LH increased (McLeod *et al.*, 1985). Preovulatory-like surges of LH occurred in 8 of 12 heifers between 17 and 59 h after the start of the treatment. The frequency of LH pulses resulting from administration of LHRH corresponds to that seen about 20 days before puberty.

It appears that administration of LH or a secretagogue that increases LH secretion at 1- or 2-h intervals will induce a preovulatory LH surge and ovulation in a majority of the ewe lambs or heifers that are treated in such a fashion. Therefore, it is likely that the putative hypothalamic pulse generator that regulates the pulsatile secretion of gonadotrophins is suppressed in the prepubertal ewe lamb and heifer. Very few of the prepubertal animals treated with exogenous hormones had a normal luteal phase or continued to exhibit oestrous cycles after the treatment regimen.

Secretion of FSH, during the prepubertal period

Schams *et al.* (1981) reported that FSH was secreted in a pulsatile fashion at a frequency of 3–6 pulses/24 h in heifers at 1, 2, 5 and 10 months of age. There is no evidence that an increased frequency of pulses of FSH occurs during that period. Likewise, Foster *et al.* (1975) reported that FSH concentrations increased from 3 to 11 weeks of age in the ewe lamb, but the concentration detected at 11 weeks of age was maintained until the lambs reached puberty at 35 weeks of age.

Compensatory ovarian hypertrophy occurs in prepubertal heifers after unilateral ovariectomy. There is a transient rise in plasma concentrations of FSH that peaks at 24 h and a return to baseline concentrations of FSH occurs by 36 h after ovariectomy (Johnson *et al.*, 1985). The administration of bovine follicular fluid devoid of steroids (removed by charcoal adsorption) prevented the transient rise in FSH and the compensatory ovarian hypertrophy that occurred after unilateral ovariectomy. Secretion of LH did not change following unilateral ovariectomy. In a study with prepubertal ewe lambs, unilateral ovariectomy resulted in an increase in the number of large follicles/ovary by 12 h after ovariectomy. A transient rise in circulating concentrations of FSH but not LH occurred coincident with the increase in follicle numbers (Smith *et al.*, 1984). The possible role of FSH in the pubertal process is not clear, but the results from studies with unilaterally ovariectomized animals indicate that nonsteroidal ovarian factors inhibit secretion of FSH in the prepubertal ewe lamb and heifer.

The role of the ovary in regulating gonadotrophin secretion during sexual maturation

Circulating concentrations of LH increased more than 3-fold between 2 and 8 weeks after ovariectomy at 19 weeks of age (Foster & Ryan, 1979). Basal concentrations of LH increased between 168 and 192 h after bilateral ovariectomy of prepubertal heifers (Kiser *et al.*, 1981). Secretion of LH increased earlier (72–80 h) after ovariectomy in postpubertal heifers. Odell *et al.* (1970) detected an increase in secretion of LH following ovariectomy as early as 1 month of age. An increase in secretion of LH has also been detected after ovariectomy of heifers at 3, 6 or 9 months of age (Anderson *et al.*, 1985).

Intrauterine ovariectomy of the fetal ewe lamb resulted in an increase in concentrations of LH and FSH above those of intact lambs by 5 weeks of age. The levels of gonadotrophins were maintained above those of intact animals throughout the first year of life (Bremner *et al.*, 1981).

Ovariectomy resulted in a marked increase in secretion of LH by 8 days after ovariectomy in the prepubertal heifer and subtle increases in secretion of LH continued over the next 130 days (Day *et al.*, 1984). The average age of puberty in intact control heifers was at about 130 days after ovariectomy in the treated heifers. It has not been determined whether these subtle changes in secretion of LH that occur after the acute increase in LH after ovariectomy have any physiological significance.

The ovary becomes involved in inhibiting gonadotrophin secretion very early in life. The inhibitory activity of the ovary is apparently maintained throughout the prepubertal period. Ovarian removal results in increased secretion of LH to levels that are equal to or higher than those detected during the follicular phase of the oestrous cycle of the adult cow and ewe. The increase in LH appears to be the result of increased frequency of LH pulses, with pulses being generated at a rate of about 1/h after ovariectomy.

The role of the ovarian steroids in sexual maturation

Oestradiol. Pulses of LH are maintained at a low level during the prepubertal state. High-frequency pulses of LH are one of the primary components absent in the prepubertal ewe lamb and heifer. However, the ovaries of these young animals are capable of responding to high frequency pulses of LH by developing a preovulatory follicle when this gonadotrophin is administered exogenously and the neuroendocrine system is capable of generating high-frequency pulses of LH if the ovary is removed.

The 'gonadostat hypothesis' emerged about 50 years ago and explains how the pubertal increase in secretion of LH might occur. According to this hypothesis, low concentrations of LH are maintained because of the responsiveness of the hypothalamo–pituitary axis to the inhibitory feedback action of oestradiol. As sexual maturation occurs, the responsiveness to steroid negative feedback decreases and secretion of LH increases to the point that follicular growth is stimulated, oestrogen secretion is enhanced and in turn the preovulatory surge of gonadotrophins is induced by the increasing oestrogen. The process culminates in ovulation. This hypothesis can be applied to prepubertal lambs and heifers since concentrations of oestradiol-17β that inhibit secretion of LH in prepubertal animals are no longer effective in inhibiting secretion of LH after puberty. Physiological concentrations of oestradiol-17β inhibit the increase in secretion of LH after ovariectomy in the ewe lamb. The inhibitory action of oestradiol on secretion of LH decreases coincident with the onset of puberty in contemporary intact ewe lambs (Foster & Ryan, 1979; Fig. 3).

The duration of suppression of secretion of LH after acute administration of oestradiol was longer in heifers that were 4 months of age than in heifers that were 8 and 12 months of age at the time of oestradiol administration (Schillo *et al.*, 1982). Oestradiol inhibited the postovariectomy rise in secretion of LH in prepubertal heifers that were 60 or 200 days of age at the time of ovariectomy (Staigmiller *et al.*, 1979). There was no difference in the degree of inhibition by oestradiol between the heifers in the two age groups. The ability of oestradiol to suppress secretion of LH decreased in ovariectomized heifers implanted with oestradiol and the change was coincident with

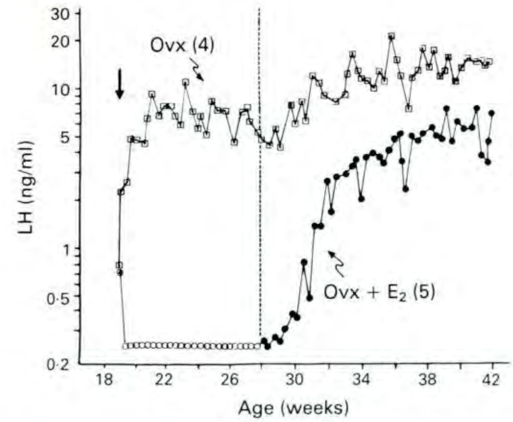

Fig. 3. Mean concentrations of circulating LH in ovariectomized (Ovx) lambs with or without chronic treatment with oestradiol (E_2). Arrow indicates the time of ovariectomy. Silastic capsules containing oestradiol-17β were inserted subcutaneously at the time of ovariectomy. Undetectable values for serum LH (0·25 ng/ml) are depicted by open symbols. (After Foster & Ryan, 1979.)

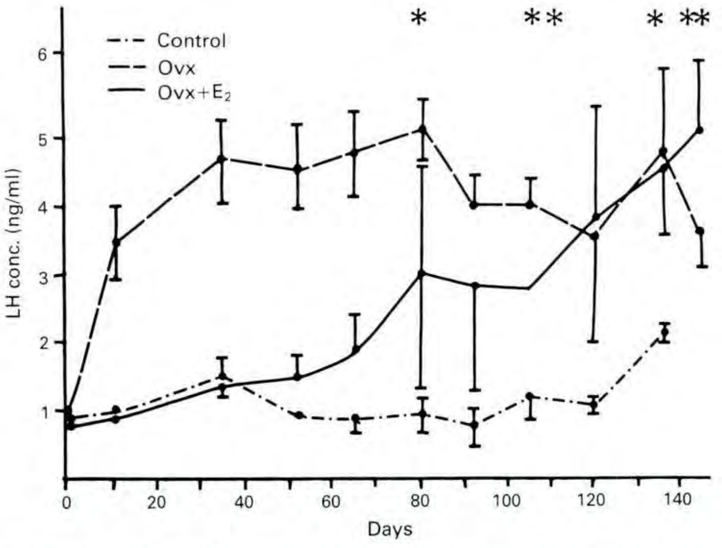

Fig. 4. Mean ± s.e.m. (indicated by bars) serum LH concentrations for intact (control, N = 6), ovariectomized (Ovx, N = 5) and ovariectomized, oestradiol-implanted (Ovx E_2, N = 5) heifers. * Indicates time of puberty for each control heifer. (After Day *et al.*, 1984.)

the time of puberty in contemporary intact heifers (Day *et al.*, 1984; Fig. 4). The increased mean concentrations of LH resulted from an increased frequency of LH pulses. Therefore, oestradiol can inhibit secretion of LH starting very early in life in heifers and ewe lambs and this inhibition is continued until the time when puberty occurs.

A decrease in concentration of cytosolic receptors for oestradiol in the anterior and medial basal hypothalamus and the anterior pituitary occurs during the period of sexual maturation in heifers (Fig. 5). This decline in receptors coincides with a decline in oestradiol negative feedback and an increased secretion of LH. The decline in receptor numbers may be responsible for the

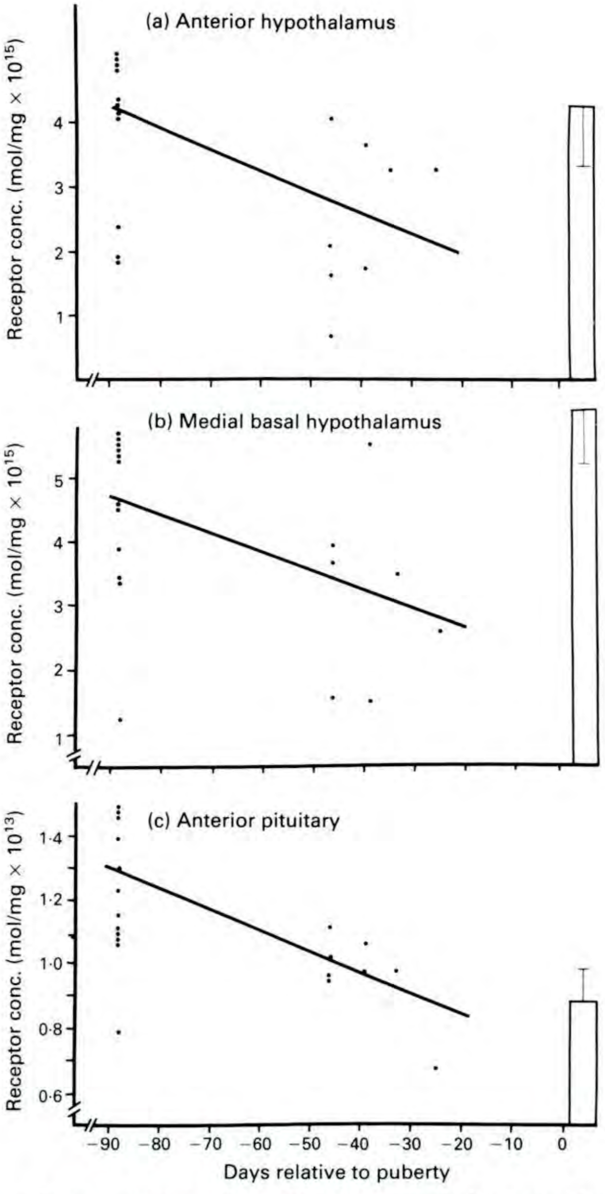

Fig. 5. Regression of concentration of receptors for oestradiol in cytosol of (a) the anterior hypothalamus, (b) the medial basal hypothalamus, and (c) the anterior pituitary of heifers on estimated days relative to puberty. Correlation coefficients for mean concentrations of receptors were $r = 0{\cdot}48$ in (a), $r = 0{\cdot}45$ in (b) and $r = 0{\cdot}53$ in (c). The histograms give the mean ± s.e.m. values for 5 post-pubertal heifers.

reduction in negative feedback of oestradiol on secretion of LH during this time. As the concentration of oestradiol receptors decreases during sexual maturation, the corresponding regulatory effect on secretion of gonadotrophins also decreases. Changes in the concentration of receptors for oestradiol in the preoptic area and stalk median eminence did not occur during the process of sexual maturation in heifers.

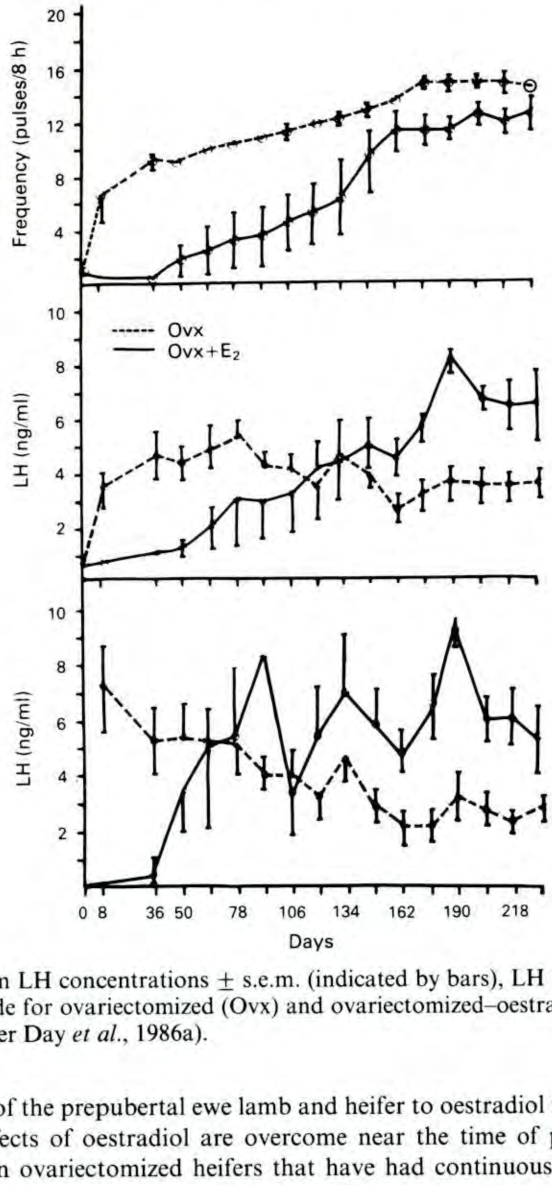

Fig. 6. Mean serum LH concentrations ± s.e.m. (indicated by bars), LH pulse frequency, and LH pulse amplitude for ovariectomized (Ovx) and ovariectomized–oestradiol implanted (Ovx $+ E_2$) heifers. (After Day *et al.*, 1986a).

Chronic exposure of the prepubertal ewe lamb and heifer to oestradiol inhibits secretion of LH and the inhibitory effects of oestradiol are overcome near the time of puberty. An interesting phenomenon occurs in ovariectomized heifers that have had continuous exposure to oestradiol throughout the period of time that corresponds to the prepubertal, pubertal and postpubertal periods of contemporary intact heifers. The same dose of oestrogen that inhibited secretion of LH early in life enhances secretion of LH later in life (Day *et al.*, 1986b; Fig. 6). In our laboratory, we have seen this enhancement in secretion of LH in several studies in which cows that were ovariectomized after puberty were used. Heifers were ovariectomized after puberty and when oestradiol was administered at 3 different doses via implants, secretion of LH was increased above that detected in ovariectomized heifers without oestradiol (Kinder *et al.*, 1983; Table 3). Chronic implantation of ovariectomized cows that were sexually mature at the time of ovariectomy with oestradiol increases secretion of LH at the spring and autumn equinoxes and the summer and winter solstices above that of ovariectomized cows that did not receive oestradiol (unpublished data).

Although animals overcome the negative feedback effects of oestradiol at puberty, oestradiol can exert negative feedback on LH secretion in postpubertal animals in certain circumstances.

Table 3. Frequency and amplitude in pulses of LH and mean concentrations of oestradiol-17β and LH in serum of postpubertal heifers treated with different doses of oestradiol (taken from Kinder *et al.*, 1983)

Implant size (cm)	Oestradiol (pg/ml)	LH* (ng/ml)	Frequency of LH pulses (pulses/6 h)	Amplitude of LH pulses (ng)
0	2·1	2·35 ± 0·26	7·5 ± 0·5	0·98 ± 0·24
12·5	3·4	3·38 ± 0·17	6·0 ± 0·4	2·50 ± 0·41
27·0	4·7	3·60 ± 0·37	5·5 ± 0·7	3·24 ± 0·38
54·0	8·4	3·11 ± 0·42	4·0 ± 0·4	3·82 ± 0·58

Values are mean ± s.e.m. for 4 heifers/group.
*Concentrations of LH were evaluated at 30 days after ovariectomy and implantation of the oestradiol.

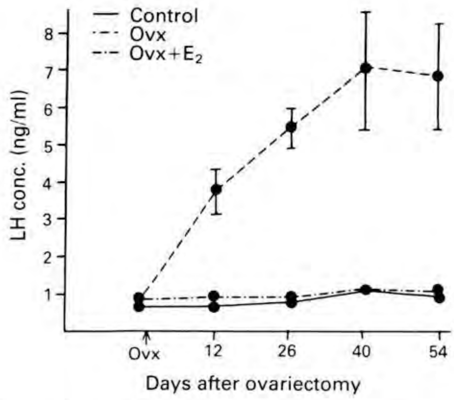

Fig. 7. Mean concentrations of serum LH ± s.e.m. (indicated by bars) in heifers. Heifers were fed diets restricted in energy until anoestrus resulted. At this time treatments were applied (Ovx, ovariectomized; Ovx + E_2, ovariectomized + oestradiol-17β) to the heifers and a diet adequate in energy was started. (After Imakawa *et al.*, 1986.)

Goodman & Karsch (1981) have shown that oestradiol suppresses secretion of LH in adult ovariectomized ewes during seasonal anoestrus. Oestradiol suppresses secretion of LH in cows during nutritionally-induced anoestrus (Imakawa *et al.*, 1986; Fig. 7). Oestradiol reduced frequency and amplitude of LH pulses during this physiological state. It would be useful to determine what happens to the concentration of oestradiol receptors at the hypothalamo–pituitary axis with the onset of anoestrus in animals of this type.

Oestrus without the initiation of oestrous cycles has been detected in prepubertal heifers and has been termed nonpubertal oestrus (Nelson *et al.*, 1985). The incidence of this phenomenon varied with breed of heifer during the 2-year study. This phenomenon could be related to photoperiod since, in the first year of the study, 64% of the heifers that exhibited behavioural oestrus before 1 January had a nonpubertal oestrus. In the 2nd year of the study, 80% of the heifers exhibiting oestrus before 1 January had a nonpubertal oestrus. The incidence of nonpubertal oestrus appears to decrease during the late winter, spring and summer months (Fig. 8). However, the possibility also exists that nonpubertal oestrus may be age related. In another study nonpubertal oestrus was exhibited in 62·8% of the heifers and there was a tendency for more of the heifers that were lighter in weight to exhibit nonpubertal oestrus than the heavier weight heifers (Rutter & Randel, 1986). More heifers that had a pubertal oestrus at the first observed behavioural oestrus also had a transient rise in serum concentrations of progesterone before their first oestrus

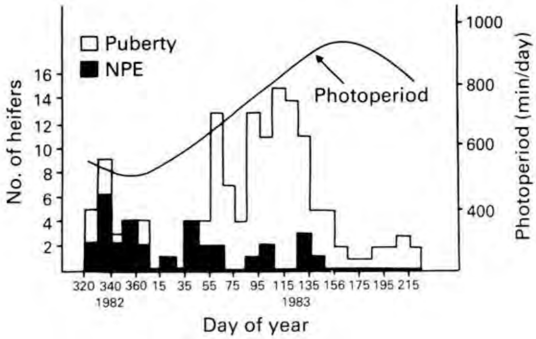

Fig. 8. Day of first behavioural oestrus for each heifer in Year 1. Puberty = oestrus accompanied by subsequent corpus luteum function; NPE = oestrus not accompanied by subsequent corpus luteum function. (After Nelson *et al.*, 1985.)

(64·3 *vs* 20%) and this transient elevation of serum progesterone was greater in heifers exhibiting the pubertal oestrus than in those showing the nonpubertal oestrus. On average, 63·5 days elapsed from the time that the nonpubertal oestrus was detected until the time of occurrence of pubertal oestrus. Therefore, situations appear to exist in which oestrogen secretion is occurring to the point that behavioural oestrus is induced but there is a failure of ovulation and/or subsequent luteal development to occur.

Progesterone. Two distinct elevations in progesterone concentration in blood serum were detected during the peripubertal period that immediately preceded puberty in the heifer (Gonzalez-Padilla *et al.*, 1975a). This increase in progesterone may have a role in the endocrine changes leading to the establishment of gonadotrophin and gonadal hormone secretions that are characteristic of the adult cow. The source of this progesterone is of ovarian origin in the heifer (Berardinelli *et al.*, 1979). When ovariectomy was performed 24–30 h after an increase in progesterone had been detected concentrations of progesterone returned to levels similar to those observed before the rise. Microscopic examinations of the ovaries revealed the presence of compact luteal tissue embedded within the ovary that could not be observed from the ovarian surface.

Fitzgerald & Butler (1982) have observed a transient increase in progesterone that lasts for 1 to 4 days before the time of the first ovulation in ewe lambs. Ovariectomy of ewe lambs within 8 h of the detected increases in progesterone concentration resulted in an immediate decline in serum progesterone concentration (Berardinelli *et al.*, 1980). It was determined that progesterone concentrations were significantly higher in the venous blood from the ovary containing luteal tissue. As in heifers, there were no ovulation papillae observed on the luteal structures of the ovaries that were removed at the time of the first rise in progesterone. Therefore, the source of the progesterone secreted in the short luteal phases preceding puberty in the ewe lamb and heifer is in the ovary but ovulation is not necessary for the formation of these structures.

The effect of progesterone during the short luteal phases upon subsequent endocrine and ovarian function in the prepubertal heifer and ewe lamb is unclear. Keisler *et al.* (1983) removed the ovary containing luteal tissue from 1 group of ewe lambs and removed the ovary without luteal tissue in another group of lambs within 24 h of the first increase of plasma progesterone concentrations. There was no difference in the time from the transient rise in progesterone concentrations to the initiation of the first luteal phase of normal duration in the ewe lambs of the two groups. Also, no difference in the duration of the normal luteal phase was detected (Table 4). The transient luteal structure that develops in the ewe lamb before development of the first normal corpus luteum therefore does not appear to be required for sexual maturation.

Quirke *et al.* (1985) found that the mean number of ovulations before the first behavioural oestrus averaged 1·6 in ewe lambs of several different breeds. It is therefore not uncommon for ewe lambs to ovulate more than once before the time when first behavioural oestrus is exhibited.

Table 4. Characteristics of profiles of progesterone exhibited before the first behavioural oestrus in ewe lambs (taken from Keisler *et al.*, 1983)

Treatment*	No. of lambs	Average maximum magnitude (ng/ml)†	Average duration (days)†	Days from transient rise to luteal phase preceding first oestrus†	Luteal phase preceding first oestrus	
					Average conc. of progesterone (ng/ml)†	Average duration (days)†
Sham-operation	5	0·50 ± 0·13	3 ± 1	4 ± 1	1·30 ± 0·25	12 ± 1
Nonluteal ovary removal	5	0·30 ± 0·02	3 ± 1	4 ± 1	1·20 ± 0·10	11 ± 1
Luteal ovary removal	6	0·32 ± 0·04	1	5 ± 1	1·23 ± 0·10	11 ± 1

*At the first rise in progesterone in the plasma.
†Determined from consecutive concentrations of progesterone that equalled or exceeded average baseline plus 3 s.e.m. concentrations.

The use of progestagen implants for induction of puberty in prepubertal heifers has been studied extensively (Gonzalez-Padilla *et al.*, 1975b; Short *et al.*, 1976; Rajamahendran *et al.*, 1981, 1982; Sheffield & Ellicott, 1982). Removal of the progestagen implant can induce puberty in a high percentage of heifers but the fertility at first oestrus increases with age of the heifer (Short *et al.*, 1976). The role that progesterone has in the process of sexual maturation is not well understood at the present time.

The influence of photoperiod on sexual maturation

The sheep is a seasonal breeder, and so it is not surprising that seasonal factors modify the time of puberty in the ewe lamb. The primary seasonal factor thought to be responsible for modification in age at puberty in the ewe lamb is daylength. Ewe lambs can attain puberty as early as 150 days of age, but the season dictates age at first ovulation. A delay in the onset of puberty occurs in ewe lambs born in the spring or autumn if they reach pubertal age during the nonbreeding season. Fitzgerald & Butler (1982) demonstrated that ewe lambs born in March were older at puberty than lambs born in July or August. Spring-born lambs were shown to begin reproductive cycles at 25–35 weeks of age (Foster *et al.*, 1986; Fig. 9). However, lambs born in the autumn attained the age at which puberty would have normally occurred (25–35 weeks) during the anoestrous season and thus remained anovulatory until the autumn when they were 48 to 50 weeks of age. Foster *et al.* (1986) have shown that a sequence of long days, followed by short days, was required to initiate and sustain oestrous cycles in ewe lambs. In addition, the age when long days were experienced was important, with later exposure to long days being more effective in producing oestrous cycles of normal duration than exposure to long days at an earlier age. There may therefore be a critical period during sexual maturation during which ewe lambs must be exposed to long days in order for normal sexual maturation to occur.

The re-establishment of oestradiol negative feedback on secretion of LH which accompanies seasonal anoestrus occurs about 3 weeks earlier in ewe lambs than in adult ewes. The timing of the increased negative feedback of oestradiol coincides with cessation of oestrous cycles in lambs and adult ewes (Foster & Ryan, 1981; Fig. 10). Ewe lambs may therefore maintain an enhanced response to oestradiol negative feedback throughout the first breeding season.

Even though reproductive activity in the cow is not limited to one season of the year, season appears to be able to modulate reproductive function. This action probably influences the hormonal secretions of the hypothalamo–pituitary–gonadal axis. Through this route, age at puberty in the

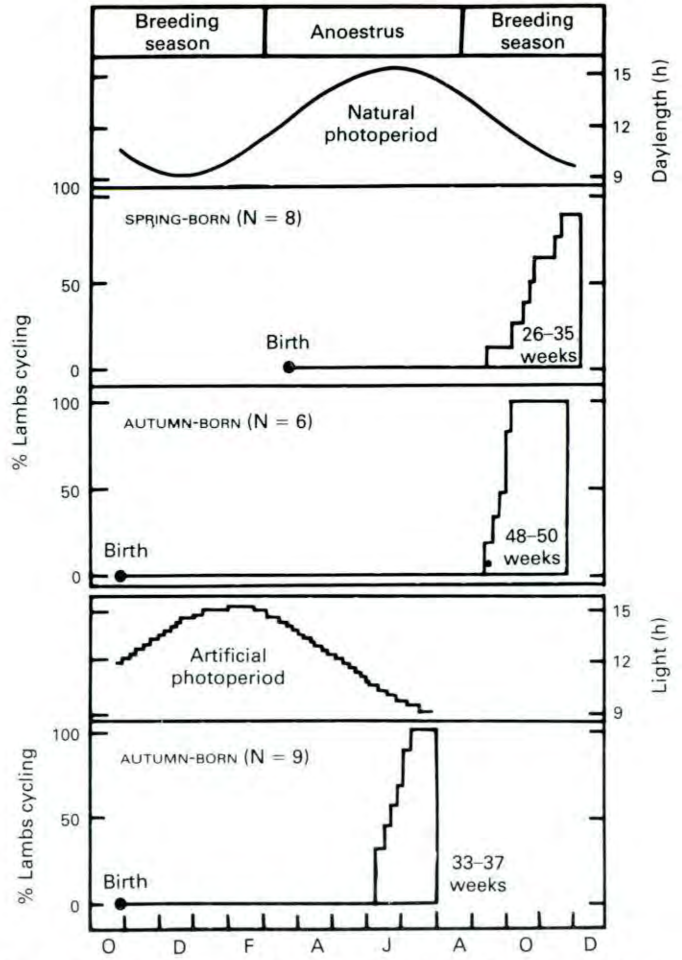

Fig. 9. Time of puberty in the spring- and autumn-born lamb. Growth and development occurs during the long days of the spring and summer anoestrous season, and reproductive cycles and matings begin during the decreasing daylengths of the autumn breeding season. (After Foster *et al.*, 1986.)

heifer could be influenced by photoperiod much as it is in the ewe lamb. Several studies have indicated that seasonal factors influence age at puberty in heifers. However, it is not possible to determine the time at which season influences sexual maturation because season of birth is confounded with the season when sexual maturation occurs (Petitclerc *et al.*, 1983; Hansen *et al.*, 1983). An intricate experiment was performed in a group of Angus–Holstein crossbred heifers that were born on 21 March 1978 or 23 September 1978 (Schillo *et al.*, 1983). All heifers were exposed to their natural environment during the first 6 months of life. However, during the second 6 months of life, heifers were exposed to a controlled environment in which temperature and photoperiod were both manipulated to give either a spring to autumn sequence of environmental events or an autumn to spring sequence of environmental events. Age at puberty was influenced by date of birth, indicating that the natural environmental conditions to which the heifers were exposed during the first 6 months of life influenced age at puberty. Heifers born in the autumn tended to reach puberty earlier in life than heifers born in the spring. March- and September-born heifers exposed to the spring to autumn climatic changes (controlled environment) during the second 6 months of life reached puberty earlier than the heifers

Table 5. Effects of month of birth and photoperiod chamber on age and body weight of heifers at puberty (from Schillo *et al.*, 1983)

Date of birth	Chamber* (after 6 months)	Age at puberty† (days)	Weight at puberty‡ (kg)
21 March	Sp–F	321 ± 29·5	281 ± 39·0
21 March	F–Sp	346 ± 50·1	318 ± 43·4
23 September	Sp–F	295 ± 12·6	268 ± 9·9
23 September	F–Sp	319 ± 32·5	306 ± 25·5
Date of birth means			
21 March		334	300
23 September		307	287
Chamber means			
Sp–F		308	274
F–Sp		333	312

Values are mean ± s.d. for 14 heifers born in March and 14 heifers born in September.

*Sp–F = spring to fall climatic changes; F–Sp = fall to spring climatic changes during second 6 months of life.

†There were effects of date of birth ($P < 0·06$) and chamber ($P < 0·08$).

‡There was an effect of chamber ($P < 0·01$).

exposed to the autumn to spring climatic changes. Therefore, exposure during the second 6 months of life to the short photoperiods and temperatures that would exist during the first 6 months of life in autumn-born heifers was associated with an earlier age at puberty (Table 5). The younger age at puberty in September-born heifers and in heifers exposed to the controlled environment that represented spring to autumn climatic changes suggests a mechanism by which heifers would tend to give birth to offspring in the spring and summer regardless of their own birth date. Heifers born in the autumn would tend to reach puberty at less than 1 year of age in the summer or early autumn after their birth. Spring-born heifers would tend to reach puberty later in life and give birth to offspring in the spring or early summer of the next year.

Season has been shown to influence secretion of LH in cows. Increased secretions of LH occur during the spring and suppression of secretion occurs during the autumn months in mature ovariectomized cows (Fig. 11). The fluctuation in concentration of LH with season results from changes in the amplitude of pulses (Day *et al.*, 1986a). The cow therefore has the ability to receive and integrate seasonal cues that modulate gonadotrophin secretion.

The influence of growth and nutrition on sexual maturation

It appears that growth-related cues influence the rate of sexual maturation and the onset of puberty in the ewe lamb and heifer. Low nutritional intake will delay the onset of puberty in the ewe lamb and heifer. Lambs remained anovulatory during the first breeding season when growth was retarded (Foster *et al.*, 1986). The frequency of pulses of LH was reduced and oestradiol continued to exert the negative feedback action on secretion of LH when growth rate was retarded in the ewe lamb. Therefore, the rate at which pulses were generated was much slower in ewe lambs that were given restricted amounts of food. When ewe lambs that had been fed diets restricted in quantity were switched to diets that were fed *ad libitum*, the sensitivity to oestradiol negative feedback on LH secretion was reduced and an increase in secretion of LH occurred (Foster & Olster, 1985; Fig. 12). Restricted food intake also resulted in a suppression of the preovulatory-like surge of LH secretion that resulted after oestradiol administration (Foster & Olster, 1985). Therefore, the

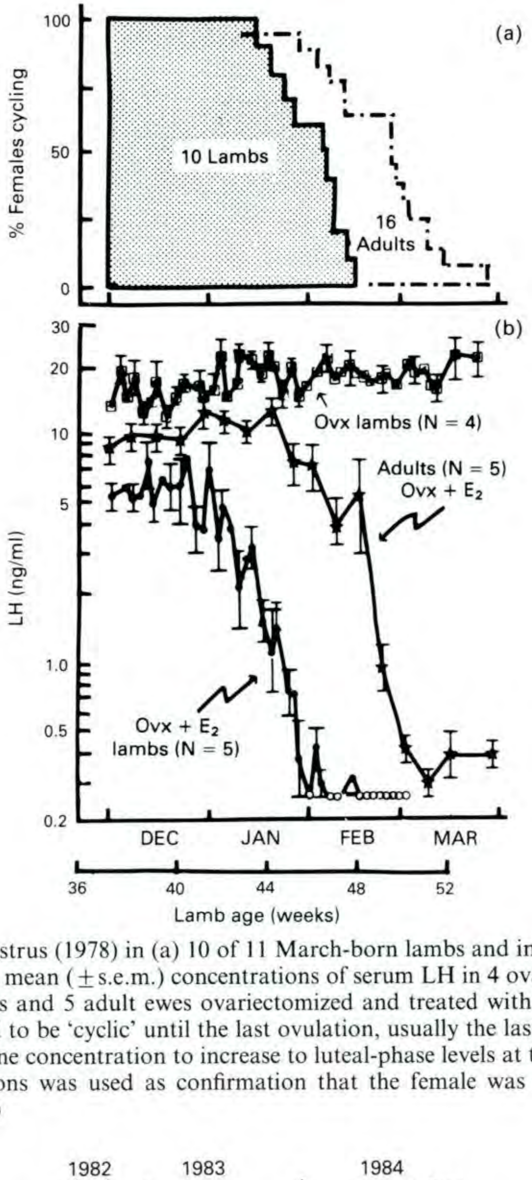

Fig. 10. Onset of anoestrus (1978) in (a) 10 of 11 March-born lambs and in 16 adult ewes and (b) seasonal change in mean ($\pm$s.e.m.) concentrations of serum LH in 4 ovariectomized (Ovx) lambs, in 5 of 6 lambs and 5 adult ewes ovariectomized and treated with oestradiol (E_2). A female was considered to be 'cyclic' until the last ovulation, usually the last oestrus; failure of circulating progesterone concentration to increase to luteal-phase levels at the time of the next two expected ovulations was used as confirmation that the female was anoestrous. (After Foster & Ryan, 1981.)

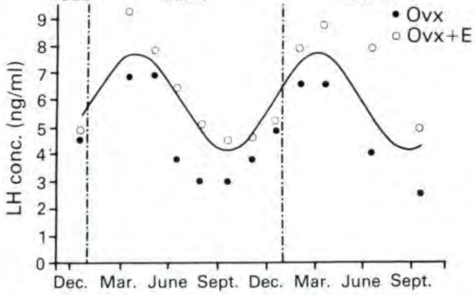

Fig. 11. Regression lines for mean concentration of LH on day of bleeding of cows ovariectomized (Ovx) and ovariectomized and treated with oestradiol-17β (Ovx + E). (After Day *et al.*, 1986a.)

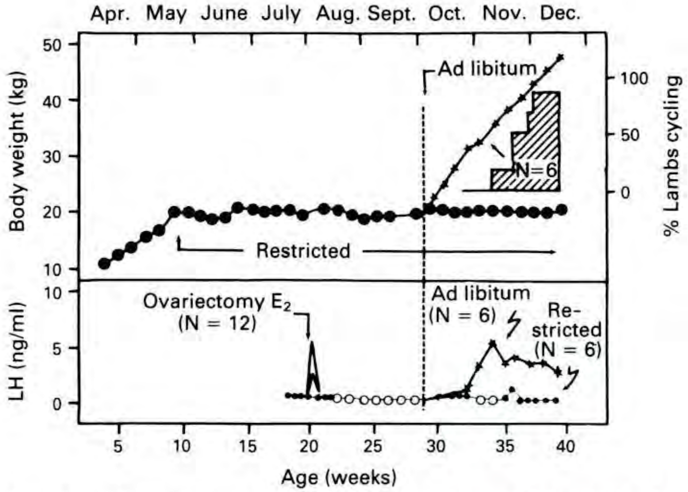

Fig. 12. Mean body weights and mean (±s.e.m.) concentrations of circulating LH in ovariectomized lambs (bottom panel) chronic treatment with low levels of oestradiol (Silastic implant). Results obtained during restricted feeding are presented as solid circles (●) while those obtained during ad-libitum feeding are presented as solid stars (★). Open circles (○) designate undetectable (<0·2 ng/ml) LH values. Histogram in top panel shows onset of reproductive cycles in lambs during ad-libitum feeding. (After Foster & Olster, 1985.)

pituitary reserves of LH to be released at the oestradiol-induced preovulatory LH surge were less, the amount of LHRH released was less or the response to LHRH was reduced in ewe lambs that received oestradiol and were fed restricted quantities of food.

Mean concentrations of LH were suppressed in prepubertal heifers fed restricted levels of dietary energy as compared to heifers fed higher energy diets during the 120 days preceding puberty (Day *et al.*, 1986c; Fig. 13). Both frequency and amplitude of pulses of LH were suppressed in the heifers fed restricted levels of dietary energy as compared to heifers that were fed a diet adequate in energy. Thus, as in the ewe lamb, the rate at which pulses of LH are generated was slower in heifers fed restricted levels of dietary energy during the period that sexual maturation would normally have occurred. The magnitude of the peak response of LH after administration of LHRH was less in the heifers fed the diet restricted in energy during the first 84 days following initiation of feed restriction as compared to heifers fed diets adequate in energy (Fig. 14). Therefore, the results of the studies in ewe lambs and heifers both indicate that pituitary responsiveness to exogenous secretagogues is decreased when quantities of food intake are restricted.

In a series of experiments in prepubertal heifers, increased concentrations of the volatile fatty acid propionate in the rumen resulted in an increased release of LH from the pituitary after administration of LHRH (Randel & Rhodes, 1980; Rutter *et al.*, 1983). In addition, an increase in propionate resulted in an increase in the amount of LH released during the preovulatory-like surge of gonadotrophin secretion after oestradiol administration in the prepubertal heifer (Randel *et al.*, 1982). It has also been reported that prepubertal heifers fed an additive that increases the propionate to acetate ratio in the rumen had an enhanced ovarian response with an increased ovarian weight, more corpora lutea and more follicular growth after administration of FSH and human chorionic gonadotrophin (Bushmich *et al.*, 1980). Therefore, increased concentrations of propionate result in an increased responsiveness of the pituitary to secretagogues, and thus an enhanced secretion of LH. In addition, ovarian responsiveness to the gonadotrophins is increased in heifers with a higher propionate to acetate ratio. The volatile fatty acid, propionate, might therefore be involved in modulating endocrine secretions in prepubertal heifers.

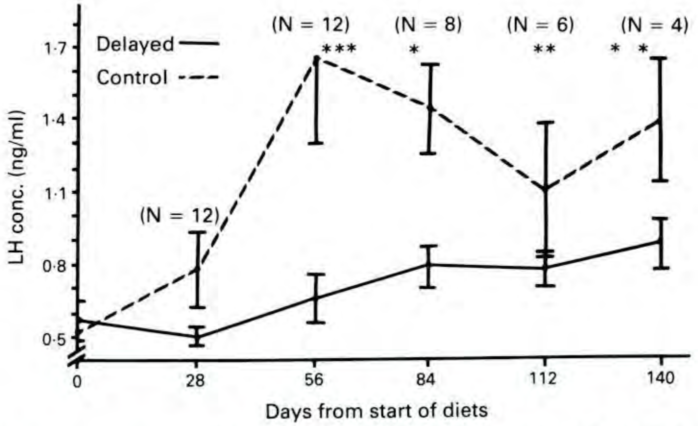

Fig. 13. Mean serum concentration of LH in heifers after start of feeding a growing diet (average daily gain = 0·79 kg/heifer/day; control) or energy-restricted diet (average daily gain = 0·21 kg/heifer/day; delayed). Day of puberty in individual control heifers is indicated by *; vertical bars indicate s.e. of each mean. Numbers in parentheses indicate the number of control heifers that were prepubertal at this period of blood collection. (After Day *et al.*, 1986c.)

Fig. 14. Mean magnitude of the peak response of LH in heifers to 0·5 μg LHRH after start of feeding a growing diet (average daily gain = 0·79 kg/heifer/day; control) or energy-restricted diet (average daily gain = 0·21 kg/heifer/day; delayed). Vertical lines indicate s.e.m. (After Day *et al.*, 1986c.)

Puberty: a conceptual view

The low frequency endogenous pulses of LH generated in prepubertal sheep and cattle are not capable of driving follicle growth to the preovulatory stage. Ovarian oestradiol acts upon the hypothalamus to suppress the generation of pulses of LHRH which maintain the secretion of LH at low levels and in turn prevents the growth of ovarian follicles to the point that ovulation can occur.

Involvement of the opiates in modulating the responsiveness of the hypothalamus to oestradiol in female rats has been described (Bhanot & Wilkinson, 1983). The endogenous opiates sensitize the hypothalamus to oestradiol and thus inhibit generation of pulses of LH. The endogenous opiates may carry out this role by modulating the level of oestradiol receptors in the medial basal hypothalamus on which oestradiol can act in the inhibition of secretion of LH. Docke *et al.* (1984) suggested that the sensitivity of the medial basal hypothalamus to negative feedback of oestradiol

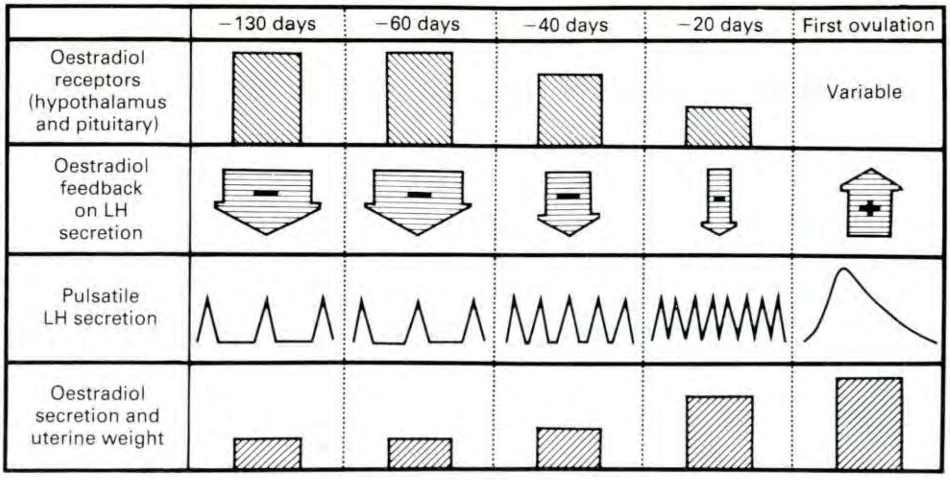

Fig. 15. Model for endocrine control of puberty in heifers.

decreased as puberty approached in the female rat. These data from the rat would be consistent with the data from heifers in which the population of oestradiol receptors in the medial basal hypothalamus declines as puberty approaches. As the concentration of oestradiol receptors in the medial basal hypothalamus declines, the frequency with which pulses of LH are generated increases. Follicles can develop to more advanced stages as frequency of pulses of LH increase and produce concentrations of oestrogen that stimulate uterine growth and development. The frequency at which pulses are generated reaches one pulse of LH per hour during the final stages of sexual maturation. At this point concentrations of LH are attained that drive ovarian follicle growth to the preovulatory stage, oestradiol induces a preovulatory surge of gonadotrophins and ovulation occurs as a result of gonadotrophins acting on the mature ovarian follicle (Fig. 15).

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