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Endocrine mechanisms governing transition into adulthood in female sheep

D. L. Foster and Kathleen D. Ryan*

Reproductive Endocrinology Program, Department of Obstetrics and Gynecology and Division of Biological Sciences, The University of Michigan, Ann Arbor, Michigan 48109, U.S.A.

Summary. It is proposed that the first follicular phase in the lamb is initiated when responsiveness to oestradiol inhibition of LH secretion decreases sufficiently to permit the expression of an inherent hourly LH pulse rhythm. The hourly LH pulse rhythm is believed to drive oestradiol production to levels that induce the first LH surge. This hypothesis is based upon several considerations. First, pulsatile LH secretion invariably occurs at low frequencies in immature lambs whereas hourly pulses are manifest in postpubertal lambs and mature ewes during the follicular phase of the oestrous cycle. Second, intravenous administration of LH at hourly intervals to immature lambs results in an increase in follicular size, induction of an LH surge, ovulation and corpus luteum formation. Third, hourly LH pulses will occur in the immature female if the ovaries are removed; the hourly frequency in the immature ovariectomized lamb can be reduced by exogenous oestradiol. Fourth, in the chronically oestradiol-treated ovariectomized lamb, responsiveness to oestradiol inhibition of LH secretion becomes markedly decreased during the pubertal period, and the hourly LH pulse rhythm is expressed. It is further proposed that a minimum body weight and short daylengths are necessary for the reduction in oestradiol negative feedback. With regard to photoperiod, the delay in onset of cyclicity in lambs born in the wrong season (autumn) may be due to retarded maturation or post-maturational seasonal anoestrus.

Introduction

The timing of the pubertal process in the female may be considered hypothetically from three different viewpoints. The first and simplest is that all of the components of the hypothalamo–hypophysial–ovarian axis which are necessary for ovulation would develop simultaneously and that, once sufficient maturation has been achieved, an ovulatory cycle would be generated. The second view is that the development of only one of the components would be rate limiting with respect to first ovulation, and therefore final maturation of a single neuroendocrine or ovarian element would lead to onset of puberty. The third, and perhaps most complex, view is that all of the components of the ovulatory mechanism would develop very early in life and that some inhibitory system would also develop to prevent their individual functions from becoming integrated prematurely. According to this scheme, first ovulation would be precipitated by removal of the inhibitory system. In considering the mechanism timing the initiation of ovulation in the sheep, a species which is relatively well developed at birth, this latter view is favoured.

* Present address: Department of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261, U.S.A.

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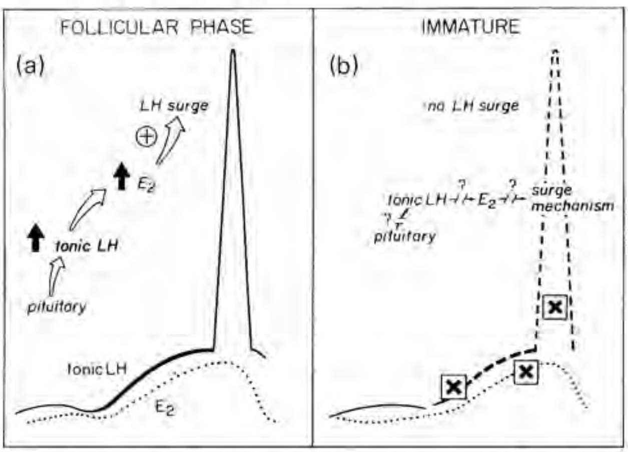
This paper is divided into two parts. The first is devoted to developing a working hypothesis for neuroendocrine disinhibition as the mechanism timing onset of ovulation in the lamb. The second part explores two factors which may influence the timing of this disinhibition, namely, body weight because of its postulated role in regulating puberty in other species and photoperiod because of its importance in governing reproductive endocrine function in the mature ewe. In an attempt to relate our most current thoughts on the final phases of sexual maturation, results of several studies in various stages of completion are summarized; preliminary data from ongoing investigations, as well as work previously published, are included. Studies reported in 1978 and thereafter use lambs of the Suffolk breed; prior to this time other breeds and their crosses were used. Unless denoted otherwise females were reared under natural environmental conditions (Ann Arbor, Michigan; 42° 18′ latitude).

Events leading to the first preovulatory surge of LH

Preovulatory events during the follicular phase of the adult oestrous cycle

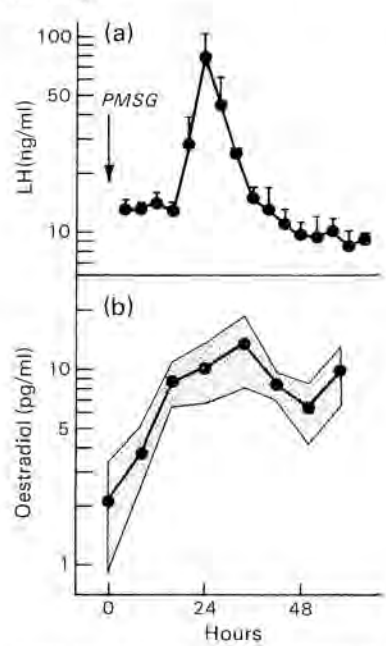
The unravelling of key events in the adult ewe during the follicular phase of the oestrous cycle has provided valuable insight into why the immature female does not exhibit spontaneous ovulatory cycles. In the mature female (Text-fig. 1a) it is proposed that the sustained increase in luteinizing hormone (LH) secretion during the 48-h follicular phase produces the requisite rise in circulating oestradiol to activate the preovulatory surge mechanism (Baird & Scaramuzzi, 1976; Karsch, Foster, Legan & Hauger, 1977). Bearing this sequence in mind, the failure of an LH surge to occur spontaneously in the immature female (Foster, Lemons, Jaffe & Niswender, 1975b) could be due to the inability of (a) the surge mechanism to respond to the positive (stimulatory) feedback action of oestradiol; (b) the ovary to produce large quantities of oestradiol; (c) the system governing tonic LH secretion to generate sufficient gonadotrophin for increased oestradiol production (Text-fig. 1b).



Text-fig. 1. Schematic diagram of (a) relationships between LH and oestradiol during the follicular phase of the oestrous cycle of the adult ewe and (b) 3 possible endocrine events that may fail in the immature female and could explain the absence of LH surges and ovulation before puberty (see text for explanation).

Competency of the hypothalamo–hypophysial–ovarian axis in the immature female

Preovulatory surge mechanism and ovary. The potential for ovulation is established long before the first oestrus, the outward sign that the pubertal process has been completed. For example, injection of exogenous gonadotrophin (PMSG) to immature females induces a rapid increase in circulating oestradiol (Text-fig. 2b) to levels comparable to those found during the follicular phase of the oestrous cycle (Hauger, Karsch & Foster, 1977). That the oestradiol rise was the stimulus for the LH surge which followed PMSG treatment (Text-fig. 2a) is supported by studies demonstrating that administration of this steroid alone induces a massive discharge of LH in the prepubertal female (Land, Thimonier & Pelletier, 1970; Squires, Scaramuzzi, Caldwell & Inskeep, 1972; Foster & Karsch, 1975). Finally, that LH is the normal stimulus for oestradiol secretion can be deduced initially from the observation that hCG, an LH-like gonadotrophin, is also able to initiate the sequence of events leading to an LH surge in the immature female (K. D. Ryan & D. L. Foster, unpublished). More direct evidence for LH stimulation of oestradiol secretion has been obtained from studies in the mature ewe (Legan & Karsch, 1979; Karsch, Goodman & Legan, 1980, for reviews).

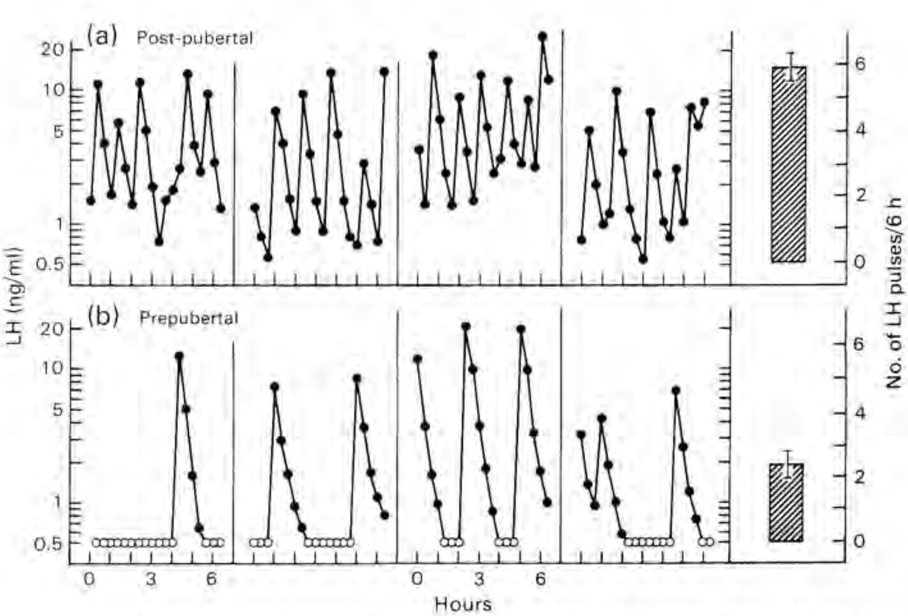


Text-fig. 2. (a) LH surge in immature female lambs in response to (b) endogenous oestradiol changes after injection of PMSG (750 i.u., i.m.) at 25 weeks of age. The high basal serum LH levels before the LH surge reflect cross-reaction of PMSG in the assay system used to measure LH. Note the logarithmic scales for hormone concentrations. (K. D. Ryan & D. L. Foster, unpublished.)

On the basis of the foregoing considerations, it could be proposed that tonic LH secretion is not sufficient to produce a major oestradiol rise, and, therefore, the LH surge mechanism remains dormant (functional, but inactive) in the immature female lamb. The failure of LH surges to occur spontaneously in the prepubertal female could also be explained by other possible mechanisms such as a low sensitivity to the positive feedback of oestradiol or a low sensitivity of the ovary to gonadotrophins. However, until evidence is produced for such developmental lesions the simpler hypothesis is favoured that the increase in circulating oestradiol does not occur because tonic LH secretion is inadequate. Two lines of evidence currently support this hypothesis, namely the differences in patterns and levels of circulating LH before and after puberty and the effects of exogenous LH on ovarian function in the immature lamb.

Tonic LH secretion. Tonic LH secretion in both the pre- and post-pubertal lamb (Bindon & Turner, 1974; Foster, Jaffe & Niswender, 1975a; Foster *et al.*, 1975b; Echternkamp & Laster,

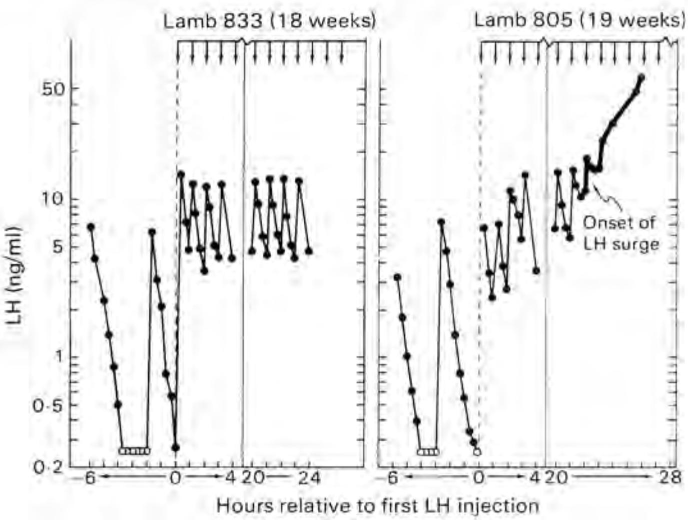
1976) is characterized by discrete LH pulses. A recent study in which serum concentrations of LH were monitored at 4-h intervals during the peripubertal period has yielded evidence that a subtle rise in the LH baseline begins several days before the first LH surge (Ryan & Foster, 1980). That this pubertal rise may be due to an increase in frequency of LH pulses is suggested by the different patterns of pulsatile LH release observed before and after first ovulation. In prepubertal females, as exemplified by the patterns at about 5 weeks before first ovulation (Text-fig. 3b), LH pulses occurred at intervals of 2–3 h or greater. This slow frequency of pulses in the sexually immature lamb, typical of the pattern of LH secretion from early postnatal life, permits circulating LH to fall to very low concentrations. By contrast, in postpubertal lambs during the 2–3-day follicular phase of either the first or second oestrous cycle, LH was discharged at approximately hourly intervals (Text-fig. 3a). Despite the obvious increase in pulse frequency, there was no evidence for an increase in pulse amplitude after puberty.



Text-fig. 3. Patterns of circulating LH at 20-min intervals over a 6-h period in individual lambs (a) after puberty during the follicular phase of first or second oestrous cycle (30–40 weeks of age) and (b) about 5 weeks before puberty (25 weeks of age). Hatched columns are mean ± s.e.m. values. LH values below assay sensitivity are indicated by open circles. Note the logarithmic scale for circulating LH concentrations. (Redrawn from Foster & Ryan, 1979b.)

It must be emphasized that the pattern of circulating LH has not been directly observed at extremely frequent intervals (e.g. 10 or 20 min) during the final days preceding the first preovulatory surge during puberty because it has not been possible to predict accurately when the first follicular phase will occur in any individual female. In the lamb, these events take place before any known overt sign is apparent to indicate that adulthood is impending. Therefore, the possibility cannot be excluded that a unique pattern of LH secretion occurs during the first follicular phase. For example, rather than the first follicular phase being driven by an increase in frequency of LH pulses, it could also be initiated by an increase in pulse amplitude or by an increase in basal concentrations of LH between pulses of similar amplitude and frequency as those occurring before puberty. Although this issue remains to be resolved fully, we have found that an hourly pulse frequency, when artificially produced in the immature female, can initiate the events leading to activation of the surge mechanism. The salient results of this study are presented below.

Purified ovine LH was injected (i.v.) every hour for 48 h at a dose which approximates the physiological LH pulse amplitude observed in the pre- or post-pubertal lamb. The patterns of circulating LH which resulted, as determined by frequent monitoring of this gonadotrophin during various phases of the experiment, are presented for two lambs in Text-fig. 4. As expected, before treatment the rate of endogenous LH release was low, one pulse every 3 h. Hourly injection of LH increased the 'LH pulse frequency' to which the ovary was exposed and, as early as 24 h, an LH surge was initiated in one of the lambs (Text-fig. 4b). This massive discharge, the early portion of which was detected fortuitously in one of the sampling periods, reflects the release of endogenous LH because the quantity of exogenous LH administered each hour was constant. Circulating oestradiol was not measured in this study, but it is suggested that the hourly injections of LH produced a sustained oestradiol rise, which, in turn, induced the preovulatory gonadotrophin surge. Luteal function was induced in 6 of 10 lambs treated at 18–19 weeks of age which is approximately 10 weeks before the normal age of first ovulation (~30 weeks of age). The remaining 4 lambs did not exhibit LH surges and examination of the ovaries revealed a mixture of results ranging from no obvious follicular stimulation to the presence of a large (13 mm) follicle. Perhaps the ovaries of some lambs require exposure of longer than 2 days to hourly LH pulses to stimulate development of a preovulatory follicle and to produce sufficient oestradiol to trigger the preovulatory gonadotrophin surge.

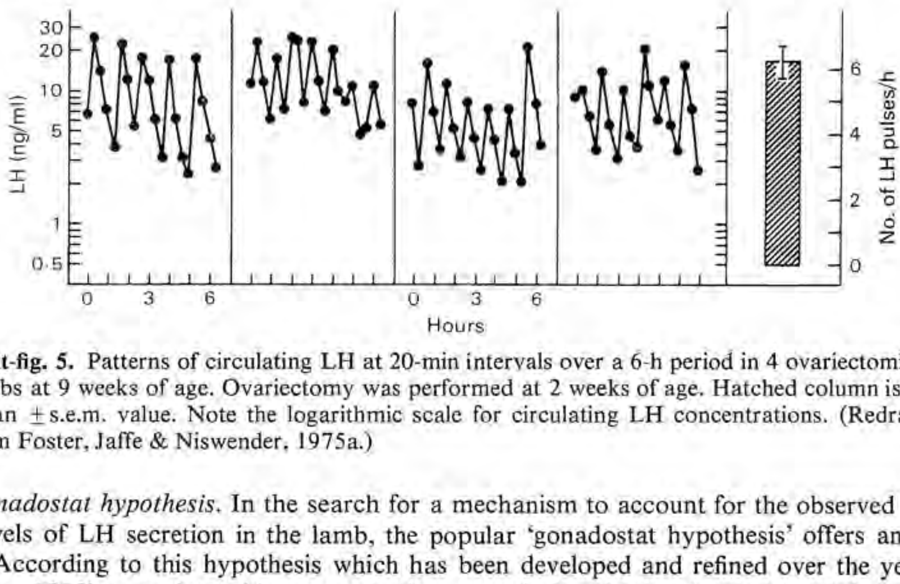


Text-fig. 4. Patterns of circulating LH at various times before and during hourly injections (arrows) of purified ovine LH (15·5 NIH-LH-S1 equivalents per injection) in 2 prepubertal female sheep. The preparation (G3-256D4) had an LH potency of approximately 2 × NIH-LH-S1 (FSH < 0·01 NIH-FSH-S1 equivalents). The high LH values after 24 h in Lamb 805 reflect the initial portion of a preovulatory LH surge. LH values below assay sensitivity are indicated by open circles. Note the logarithmic scale for circulating LH concentrations. (Redrawn from Ryan & Foster, 1980.)

The findings of this study are consistent with the postulate that the ovary of the immature sheep is inadequately supplied with LH and that the first follicular phase may be initiated by an increase in its secretion through hourly pulses. In addition, the study raises the interesting possibility that an increase in tonic FSH secretion may not be required for the onset of the pubertal process because highly purified LH alone was effective in initiating the events leading to the induction of the preovulatory gonadotrophin surge and ovulation. The lack of evidence for a major increase in tonic FSH secretion during the pubertal period (Foster *et al.*, 1975b; Fitzgerald, 1978) further supports this notion. Nevertheless, a more detailed characterization of circulating FSH during the pubertal period, as well as studies of the effects of physiological levels and patterns of FSH on the lamb ovary, will be required to substantiate this important, but tentative, conclusion.

Mechanism governing the pubertal process: de-repression of hypothalamic function

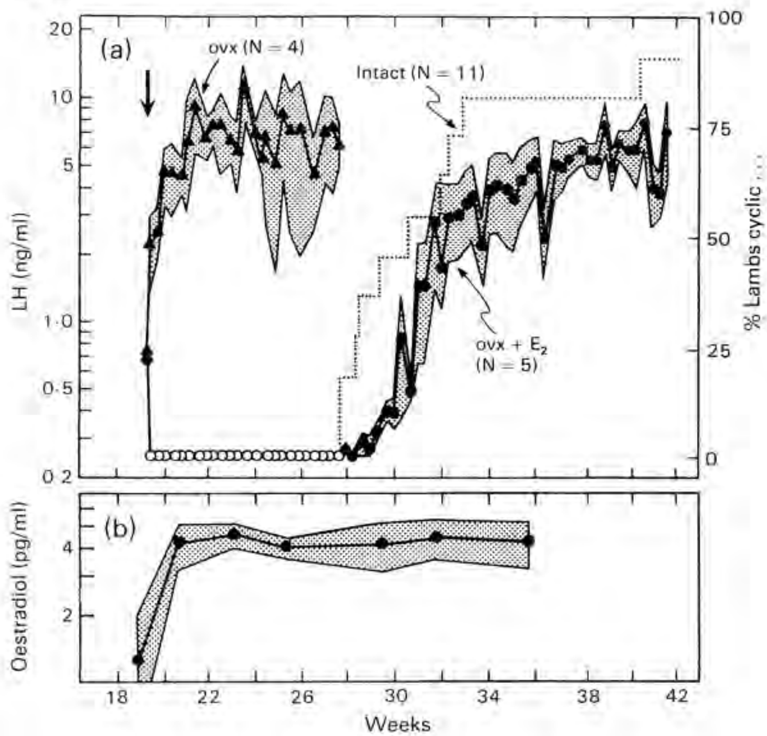
If the anovulatory condition of the immature lamb is due to the lack of hourly LH pulses, then it could be argued that the neuroendocrine system is not sufficiently developed to produce the high LH pulse frequency until the final stage of sexual maturity. This argument does not hold, however. Removal of the ovaries of the 2-week-old lamb increases the frequency of LH pulses such that by 9 weeks of age, hourly LH pulses occur (Text-fig. 5) and the pattern of LH secretion resembles that in postpubertal females during the follicular phase (Text-fig. 3a). This indicates that the potential for the production of hourly LH pulses is present from a very early age. It further suggests that, in the immature lamb, a mechanism involving the ovary represses high-frequency pulsatile LH secretion, and thereby prevents stimulation of the ovary early in life.



Text-fig. 5. Patterns of circulating LH at 20-min intervals over a 6-h period in 4 ovariectomized lambs at 9 weeks of age. Ovariectomy was performed at 2 weeks of age. Hatched column is the mean ± s.e.m. value. Note the logarithmic scale for circulating LH concentrations. (Redrawn from Foster, Jaffe & Niswender, 1975a.)

Gonadostat hypothesis. In the search for a mechanism to account for the observed patterns and levels of LH secretion in the lamb, the popular 'gonadostat hypothesis' offers an explanation. According to this hypothesis which has been developed and refined over the years (see Ramirez, 1973), gonadotrophin concentrations remain low in the circulation of the immature female because the hypothalamic mechanism governing tonic gonadotrophin secretion, the gonadostat, is highly sensitive to the negative (inhibitory) feedback of oestradiol. During puberty the sensitivity diminishes and gonadotrophins rise to levels sufficient to initiate a follicular phase.

Test of the gonadostat hypothesis in the developing sheep. The hypothesis of a changing response to steroid feedback was tested in the female lamb by examining the efficacy of a fixed concentration of circulating oestradiol to suppress chronically tonic LH secretion in the absence of the ovaries during maturation (Text-fig. 6). A constant level of serum oestradiol was produced by implanting Silastic capsules containing oestradiol. As expected, in untreated lambs a prompt increase in circulating level of LH occurred after ovariectomy at 19 weeks of age. In the group chronically treated with oestradiol, the ovariectomy-induced LH rise was prevented and circulating LH was suppressed to undetectable concentrations for several weeks. At about 28 weeks of age, coincident with the onset of ovarian cyclicity in intact lambs, a progressive rise in the serum concentration of LH was observed in oestradiol-treated lambs. Because the increase in circulating LH concentrations occurred in the presence of constant levels of exogenous oestradiol, it leads us to the conclusion that the pubertal process is associated with a marked reduction in responsiveness to oestradiol inhibition of tonic LH secretion.



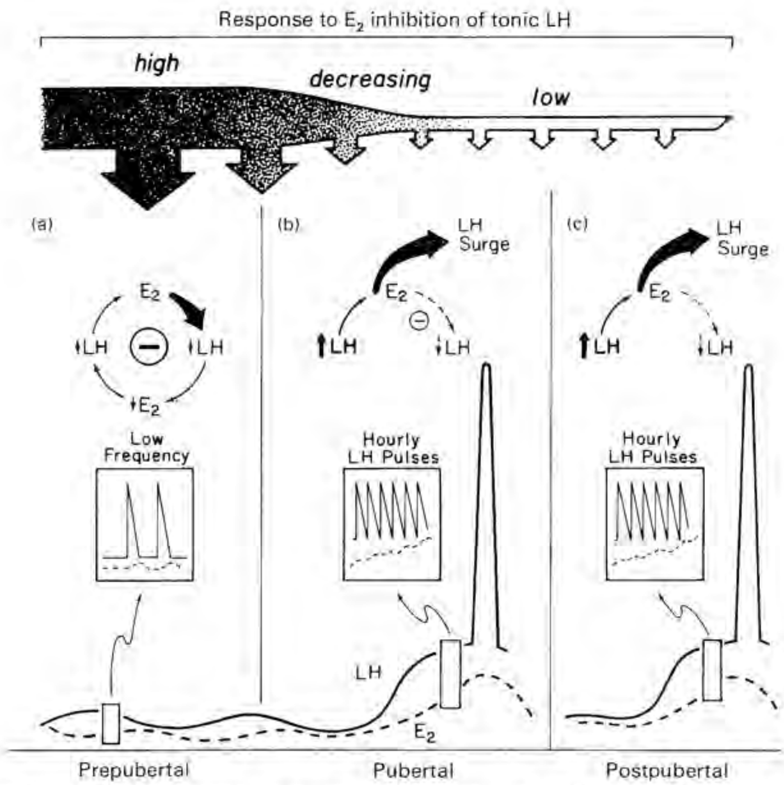
Text-fig. 6. Decrease in response to oestradiol inhibition of tonic LH secretion in lambs. In (a) the mean ± s.e.m. LH levels are presented for lambs following ovariectomy (ovx, arrow) with (+ E_2, 5 lambs) or without (4 lambs) oestradiol replacement. In (b) mean ± s.e.m. serum oestradiol concentrations are for the ovx + E_2 lambs. The histogram (dotted line) indicates onset of ovulations in 10 of 11 intact lambs born at the same time (March) as the experimental lambs; first ovulation was based upon the presence of luteal-phase levels of circulating progesterone. LH values below assay sensitivity are indicated by open circles. Note logarithmic scales for circulating hormone concentrations. (Redrawn from Foster & Ryan, 1979a.)

This concept can be extended and related to our hypothesis concerning LH pulse frequency. Measurement of circulating LH at frequent intervals (12 min for 6 h) at 24 and 38 weeks of age in another group of 5 oestradiol-treated ovariectomized lambs revealed that at 24 weeks the consistently undetectable concentrations of serum LH were due to the absence of LH pulses, and at 38 weeks the high levels of circulating LH were due to hourly LH pulses (D. L. Foster, unpublished). This provides evidence that, in the oestradiol-treated ovariectomized lamb, oestradiol modulates the frequency of LH pulses and that the inherent hourly LH pulse rate is expressed after the pubertal reduction in oestradiol inhibition. As mentioned above, however, we now face the challenge of identifying the first follicular phase to determine if a similar frequency exists in the normal lamb during the transition into adulthood.

Summary of hypothesis for events leading to the first LH surge in the lamb

The foregoing observations and considerations may be summarized by means of an hypothesis for the pubertal process in the lamb (Text-fig. 7). Beginning early in life a neuroendocrine rhythm becomes established in the female to produce hypothalamic gonadotrophin-releasing hormone (GnRH) discharges at a frequency of approximately 1 per hour. Although the resultant hourly LH pulses can be elicited within a few weeks after birth, they are not present in the immature female because the response to the inhibitory feedback action of oestradiol is high (Text-fig. 7a). LH pulses occur at a reduced frequency and concentrations of circulating LH between each pulse return to low levels. It is postulated that these pulses, acting on the ovary, produce only transient rises in circulating oestradiol, rises that are insufficient to trigger an LH surge. During puberty, a pronounced reduction in responsiveness to oestradiol feedback occurs and the mechanism modulating tonic LH secretion becomes de-repressed (Text-fig. 7b). The inherent neuroendocrine rhythm driving GnRH release is allowed to run free and the hourly pulse rhythm is first expressed. In response to this higher frequency of LH stimulation, one or more ovarian follicles increase their oestradiol production and circulating oestradiol rises progressively to levels which exert a positive feedback action on the system regulating the surge mode of LH secretion. As a consequence, the dormant surge mechanism is activated and a massive preovulatory discharge of LH is produced. The next sequence of events during the pubertal transition, which include a short luteal phase and first expression of oestrous behaviour, is presented and discussed elsewhere (Foster & Ryan, 1979b; Berardinelli, Daily, Butcher & Inskeep, 1980).

The foregoing scheme should be considered as a working hypothesis based upon the



Text-fig. 7. Hypothetical scheme for events leading to the first LH surge in the lamb in which (a) before puberty, high responsiveness to oestradiol inhibitory feedback permits only a low frequency of LH pulses, and (b) during puberty, responsiveness to inhibitory feedback decreases to allow high frequency LH pulses. This scheme is based upon the assumption that the first follicular phase (b, puberty) is similar to subsequent follicular phases (c, postpubertal).

available descriptive information and limited experimental manipulation of hypothalamo–hypophysial–ovarian function in the developing lamb. As with any working hypothesis, its validity will be tested by its predictive ability and the design of many future studies must be dictated by the hypothesis itself. A number of important gaps in our knowldege must be filled, concerning, for example, LH pulse frequency during the first follicular phase, peripubertal oestradiol secretion, prepubertal sensitivity of the ovary to LH, prepubertal sensitivity of the gonadotrophin surge mechanism to oestradiol positive feedback and the role of FSH during the final period of sexual maturation.

Our understanding of ovarian-independent regulation of LH secretion during maturation must be expanded in view of other hypotheses for puberty. For example, in man it is postulated that the central nervous system imposes a severe restraint on gonadotrophin secretion during most of childhood by a mechanism independent of gonadal steroids and that, during puberty, this restraint is removed (Winter & Faiman, 1972; Grumbach, 1980). Similar evidence is available in the prepubertal lamb although the degree of steroid-independent inhibition of gonadotrophin secretion is much less than in children. In the developing sheep, the high concentrations of circulating LH in the untreated castrated female increase even further at puberty (Foster & Ryan, 1979a). Since this secondary rise in LH secretion in the absence of the ovaries and the postulated decrease in feedback responsiveness occur concomitantly, it leads to the question of whether the two phenomena are functionally related. This issue must be resolved to determine if the increased tonic LH secretion at puberty in the intact female is solely due to a reduction in negative feedback or if steroid-independent mechanisms also play a role. Our current hypothesis emphasizes the steroid-mediated control system because the potential to secrete LH at a rate adequate to initiate a follicular phase (hourly LH pulses) is inherent from very early postnatal life and can be readily expressed if inhibitory steroid feedback is reduced sufficiently (ovariectomy) (compare Text-figs 3a, 4 and 5).

Finally, the 'pulse generator', the putative neural oscillator from which the periodicity arises for the ultimate timing of LH pulses (Goodman & Karsch, 1981), must be defined both anatomically and functionally. Identification of the neural organization of this mechanism should then provide a means to understand the neuroendocrine basis for maturational alterations in 'sensitivity' to inhibitory steroid feedback and for developmental changes in 'steroid-independent' regulation of tonic LH secretion. Presently, such control systems can only be considered as descriptive concepts rather than as definable neurocellular mechanisms.

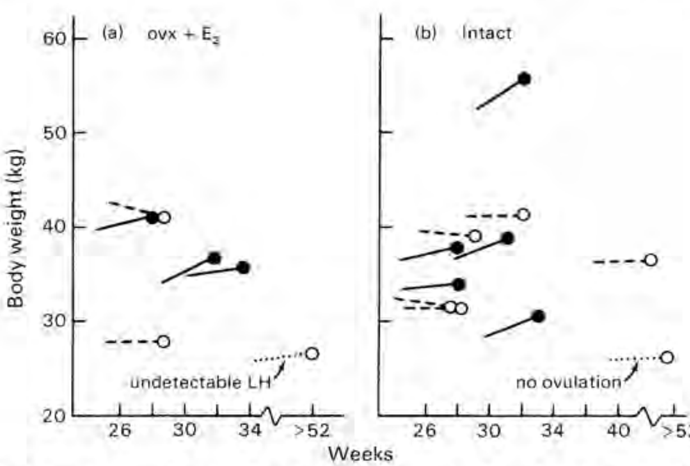
Factors governing the time of puberty

It has long been recognized that there is variation in the time of puberty in the lamb. It is less clear, however, to what extent the tempo of maturation is being driven by inherent processes (genetically determined) and to what extent it is influenced by external factors (season, nutrition, social interactions, etc.). Genetic variability as it relates to breed differences in the onset of fertility in sheep has been considered by other workers (Dyrmundsson, 1973; Land, 1978; Land & Carr, 1979) and will not be dealt with here.

The early work of numerous investigators concerning the influence of internal and external factors, primarily body weight and season on sexual development has been summarized by Dyrmundsson (1973); the work of Keane (1974), Christenson, Laster & Gimp (1976), Land (1978) and Quirke (1979) has provided further insight. The following generalizations can be obtained from the results of these studies. Normally growing spring-born lambs exhibit first oestrus during the first breeding season (autumn and winter), but over a wide range of body weights; very slow growing lambs do not display mating behaviour until the next breeding season when they are well over 1 year of age. On the other hand, lambs born early in the year (winter) usually attain an appropriate body size sometime during the 5–6-month non-breeding season (anoestrus, spring and summer), but the onset of first oestrus is delayed until the beginning of the breeding season. Finally, lambs born late in the year (e.g. autumn) may not attain the minimum size for adulthood during the confines of the first breeding season, and, therefore, the first oestrus is normally delayed until the next year when they are much older and larger. This leads to two conclusions. The first is that a minimal body weight, used as an index of body size, is necessary for onset of mating behaviour (first oestrus). The second is that this size must be attained during the 6-month breeding season of the sheep (fall and winter). The requirement of a minimum body size has been suggested to provide an adaptative mechanism for prevention of pregnancy during a formative growth stage when there could be disastrous effects of competition between tissues of the fetus and the physiologically underdeveloped mother (Allen & Lamming, 1961). The requirement for a particular season for puberty is probably related to synchronization of the time of conception in young females with that of fully mature females, which, in turn, has evolved so that births will occur at a time of the year when environment most favours survival of the offspring. In view of these observations and considerations, this section attempts to related the influence of body size and environment to the hypothesis developed in the preceding section wherein a reduction in responsiveness to oestradiol negative feedback leads to initiation of ovarian cyclicity at puberty (see Text-fig. 7 for summary).

Body size as determinant for puberty

An attractive hypothesis for the role of size in timing the onset of adulthood is that a 'critical body weight or composition' exists for puberty and that this reflects attainment of a 'critical metabolic rate' which causes a 'resetting of hypothalamic sensitivity to oestrogen negative feedback responsiveness' (rat: Kennedy & Mitra, 1963). Such an hypothesis provides the setting for the results in Text-fig. 8 which illustrates the body weight in individual females at the beginning of the pubertal decrease in response to oestradiol inhibition of tonic LH secretion (oestradiol-treated ovariectomized lambs) and first ovulation (intact lambs). The reduction in feedback response was initiated over a wide range of weights (28–42 kg) in 5 of 6 females; in 3



Text-fig. 8. Body weight and age of individual lambs in Text-fig. 6 at (a) the beginning of decrease in oestradiol inhibition of LH secretion [first detectable value for circulating LH in ovariectomized oestradiol-treated females (ovx + E_2)] and (b) at first ovulation in untreated (intact) females. The lines indicate the general slope of the growth curve during the 4 weeks before either event; in some lambs, body weight was increasing (——●) while in others it was not (– – –○). (Redrawn from Foster & Ryan, 1979a.)

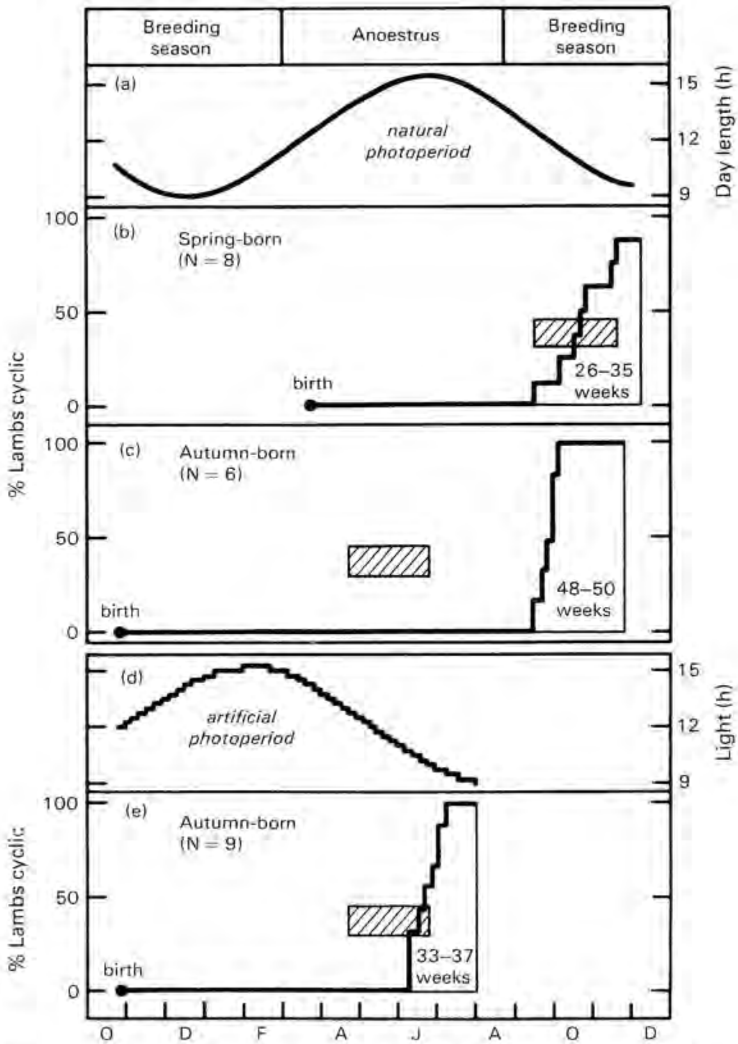
lambs, weight was increasing while in the other 2 body weight had attained a plateau or was slightly decreasing during the preceding 4 weeks. Similarly, first ovulation occurred over a wide weight range in intact lambs; again, half of the lambs were increasing in weight at first ovulation while the other half were not as evidenced by the plateau in their growth curves during the preceding 4–6 weeks. Finally, in the slowest growing oestradiol-treated ovariectomized lamb, the reduction in oestradiol feedback responsiveness did not occur, and circulating LH was still suppressed by 1 year of age when the study was terminated. The smallest intact lamb did not ovulate during the study.

These observations extend those of others in the sheep by demonstrating that the reduction in negative feedback responsiveness and first ovulation do not occur at a constant weight nor are they necessarily precipitated by an immediate and sustained increase in weight. The failure of the 2 smallest lambs (one oestradiol-treated ovariectomized lamb and one intact lamb) to exhibit pubertal changes suggests that they had not achieved the minimal size necessary for the decrease in response to oestradiol inhibitory feedback and, therefore, the onset of adulthood. In this experiment, the minimum weight was between 28 and 32 kg, a size similar to that reported by others for the weight below which onset of mating activity does not occur (Hafez, 1952; Dyrmundsson & Lees, 1972; Keane, 1974). Viewed in the context of the hypothesis for metabolic regulation of hypothalamic sensitivity to the negative feedback of oestradiol (Kennedy & Mitra, 1963), it could be postulated that the hypothetical 'critical metabolic rate' varies genetically among lambs and accounts for puberty occurring at various body sizes. Further, some lambs, because of slow genetic growth potential or under-nutrition, may not achieve the requisite metabolic rate to alter feedback sensitivity during the first year after birth. While this hypothesis remains to be tested, it is suggested that experimentally growth-retarded lambs (oestradiol-treated ovariectomized animals) may offer a novel model system for future studies to examine the role of metabolism as a factor timing the reduction in oestradiol negative feedback responsiveness.

Photoperiod as a determinant for puberty

In the fully mature female of several breeds of sheep, daylength is the most important seasonal variable governing sexual activity, with long photoperiods of the spring and summer inhibiting, and short photoperiods of the autumn and winter favouring reproductive endocrine function (Yeates, 1949; Hafez, 1952; Legan & Karsch, 1979). Because of this, several investigators have addressed the question of whether photoperiod influences the time of puberty by rearing lambs under a variety of experimental light regimens (Radford, 1961; Smith, 1967; Ducker, Bowman & Temple, 1973). Despite some differences for the age at puberty (first ovulation or oestrus) among lambs in the various light treatments, onset of cyclicity occurred within the normal age range. The failure to demonstrate any pronounced effect of artificial photoperiod on the age at puberty has led to the conclusion that inherent factors time the onset of adulthood in female sheep.

The foregoing conclusion, which implies that the lamb is not strongly photoperiodic, becomes intriguing in view of numerous reports that seasonal factors do influence the time of first ovulation in the lamb (see Dyrmundsson, 1973, for review). This seasonal influence is exemplified in Text-fig. 9 (b and c) which presents the ages at first ovulation in lambs born in the two different seasons, spring and autumn, and reared in natural environment. The majority (80%) of lambs born in spring (March), the natural season of birth, initiated ovarian cyclicity around 30 weeks of age whereas lambs born out of season, in the autumn (October), did not. Although by 30 weeks the weights of autumn-born females were well within the pubertal range, first ovulation was delayed for nearly another 20 weeks, and it finally occurred when they were nearly 1 year old. Other studies indicate that the failure of autumn-born females to ovulate at 30 weeks, which occurs in the anoestrous season rather than in the breeding season as is the case for spring-born females, is due to the failure of a reduction in the feedback response to oestradiol to occur at the normal age (data not shown, Foster, 1981). Serum LH remained suppressed in autumn-born oestradiol-treated ovariectomized lambs between 25 and 35 weeks of age when the decrease in response should normally occur based on the age and body weight; at 45–50 weeks of age, coincident with the onset of ovulation in intact autumn-born lambs during the breeding season (Text-fig. 9c), circulating LH then increased to high levels. These results suggest that the pronounced shift in age at first ovulation in the autumn-born lamb is due to some inhibitory environmental factor(s) altering the time of the decrease in oestradiol negative feedback to accord with the appropriate season for mating.



Text-fig. 9. Month and age at first ovulation in (b) spring-born (March) and (c) autumn-born (October) lambs in (a) natural photoperiod, and in (e) autumn-born lambs reared in (d) artificial photoperiod from birth. The general times of the adult breeding and anoestrous seasons are indicated. First ovulation was based upon the presence of luteal-phase levels of circulating progesterone. In (c) and (e) the hatched bar provides a reference for the age range (26–35 weeks) over which ovulation was initiated in control spring-born lambs. (Redrawn from Foster, 1981.)

Evidence that photoperiod is the environmental factor responsible for the shift in first ovulation to a greater age in autumn-born lambs has recently been obtained (text-fig. 9d and e). When autumn-born lambs were reared from birth under a gradually changing photoperiod, one simulating that had they been born in the spring, the age at first ovulation was virtually restored to that of spring-born lambs. Furthermore, in oestradiol-treated autumn-born ovariectomized lambs reared in this 'reversed photoperiod', the decrease in negative feedback was also restored to the normal age (Foster, 1981). Such findings indicate that the lamb is indeed photoperiodic and that short photoperiods are necessary for the reduction in oestradiol negative feedback responsiveness and first ovulation. The discrepancy between the conclusions derived from this study and those of others (Radford, 1961; Smith, 1967; Ducker *et al.*, 1973) in which long artificial photoperiods were unable to delay the onset of cyclicity remains to be resolved.

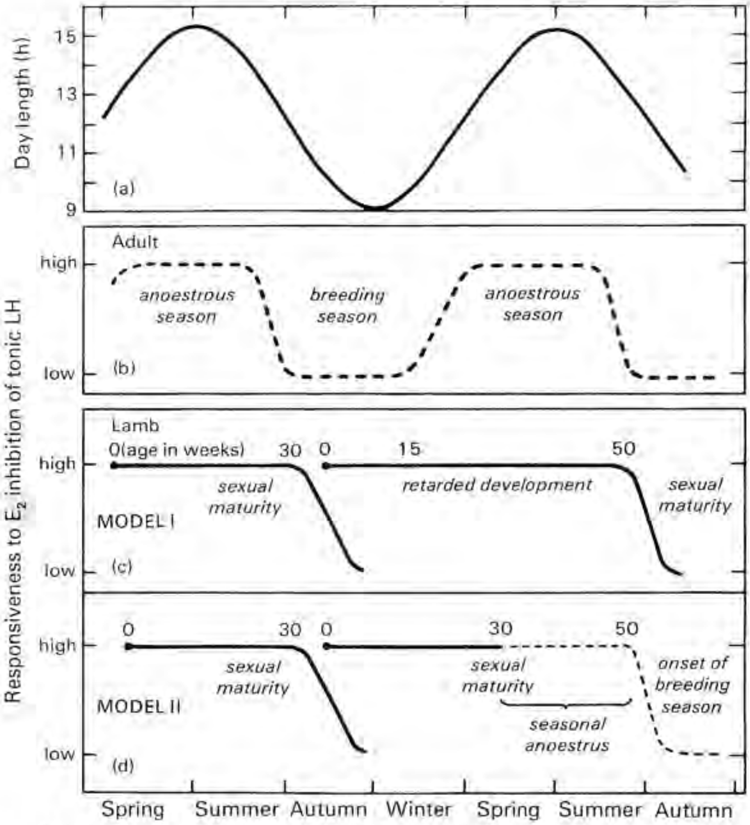
Models for influence of photoperiod on the initiation of cyclicity in the lamb

In considering puberty in a seasonally breeding species such as the sheep the fundamental question is, when does sexual development end and environmental regulation of ovarian cyclicity begin? The finding that the anovulatory condition of the mature female during anoestrus is also mediated by hyper-responsiveness to oestradiol inhibition of tonic LH secretion (Legan, Karsch & Foster, 1977) has led to the postulate that the immature lamb and seasonally anoestrous adult use a common final mechanism to prevent ovulation (Karsch & Foster, 1980). In the mature ewe, daylength is responsible for modulating changes in negative feedback because long artificial photoperiods induce hyper-responsiveness and short photoperiods induce hypo-responsiveness (Legan & Karsch, 1979). Although the developmental stimuli which drive oestradiol feedback responsiveness to low levels at puberty remain to be identified, it has become apparent that long daylengths can extend the anovulatory period by prolonging the time of oestradiol hyper-responsiveness (e.g. autumn-born lamb, Text-fig. 9). Bearing in mind these considerations for the lamb and adult, the above question relating to how much of the apparent 'prepubertal' period of the female is due to true sexual immaturity and how much is due to seasonal photoperiodic inhibition of ovulation can be rephrased more explicitly as, when does developmental hyper-responsiveness to oestradiol inhibition end and seasonal photoperiod-induced hyper-responsiveness to oestradiol inhibition begin?

In view of the foregoing questions two general models are presented, the important difference between the models being whether photoperiod influences maturational (Model I) or post-maturational (Model II) neuroendocrine events (Text-fig. 10). The assumption underlying these models is that approximately 30 weeks (25–35 weeks) is the minimum developmental period in the female sheep. The inability of lambs to initiate cycles far earlier (e.g. at 10–15 weeks of age) when reared in short photoperiods from birth (Ducker *et al.*, 1973) provides some support for this assumption.

Model I proposes that photoperiod modulates neuroendocrine maturation with development proceeding more rapidly under short photoperiods than under long photoperiods. The last half of the 30-week developmental period of the spring-born lamb occurs in short daylengths, and through some yet unknown maturational process it culminates in the decrease in negative feedback responsiveness. In the autumn-born lamb, although development is rapid to 15 weeks of age under short days, it is insufficient to effect a reduction in oestradiol inhibitory feedback responsiveness by this age. First ovulation cannot therefore occur during the first breeding season in such females. According to this model, after 15 weeks neuroendocrine development is retarded in the face of long daylengths and it is not until about 50 weeks of age that sufficient maturation occurs to cause the decrease in responsiveness to oestradiol inhibition of LH secretion.

Model II, a model based upon a suggestion by Land (1978), proposes that development is not influenced by daylength. In this model, neuroendocrine maturation is sufficient in all lambs



Text-fig. 10. Alternative models for the influence of photoperiod on the decrease in responsiveness to oestradiol inhibition of tonic LH secretion in the lamb. Models I and II are based upon the age and season of the decrease in responsiveness to oestradiol feedback and initiation of ovulation in lambs born in the spring and autumn (Text-figs 6 and 9) in relation to (a) natural photoperiod and (b) annual changes in responsiveness and ovarian cyclicity in the adult (Legan *et al.*, 1977; Legan & Karsch, 1979). Responsiveness is schematically illustrated as the inverse of circulating LH concentrations in chronically oestradiol-treated ovariectomized females. Responsiveness is considered to be high when LH secretion is suppressed and is low when LH secretion is not suppressed; intact females are acyclic during periods of high responsiveness and are cyclic during periods of low responsiveness. (Redrawn from Foster, 1981.)

by about 30 weeks of age to effect the decreased oestradiol negative feedback responsiveness. When this age is achieved during the breeding season under short daylengths (spring-born lamb), reduction in feedback responsiveness is permitted because photoperiod is not inhibitory at this time of year in any female regardless of age. If, however, this final developmental stage occurs during the anoestrous season (autumn-born lamb), the decrease in responsiveness to oestradiol inhibition is not permitted. High responsiveness is maintained, not because of inadequate development, but because of long spring and summer daylengths much the same as in the adult. Thus, the autumn-born female becomes seasonally anoestrous at sexual maturity and the transition into adulthood is 'masked'. In this model, the decrease in feedback responsiveness in an autumn-born female would reflect onset of a breeding season rather than onset of adulthood.

At present, it is difficult to determine which of the two models most appropriately describes the influence of photoperiod on the initiation of cyclicity in the lamb. There is simply not enough information to define the fundamental developmental processes, when such processes are completed and when reproductive neuroendocrine function becomes subservient to length of day.

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