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Mechanisms governing onset of ovarian cyclicity at puberty in the lamb

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Summary. The immature female sheep is capable of several aspects of adult reproductive endocrine function long before the onset of puberty. The prepubertal female can ovulate in response to an endogenous gonadotropin surge, which can be elicited by an endogenous estradiol rise which, in turn, can be produced by artificially increasing the rate of pulsatile LH discharge to an hourly frequency. Removal of the ovaries can produce an hourly frequency of LH pulses, but this frequency does not normally occur in the presence of the ovary prior to the beginning of puberty. This implies that the sequence of events leading to the first spontaneous gonadotropin surge is being restrained in the immature female by some mechanism inhibiting the onset of hourly LH pulses. Because the same levels of circulating estradiol which are capable of suppressing serum LH in the immature ovariectomized lamb are ineffective in this regard in the same females after the age of puberty, it is postulated that the increase in frequency of LH pulses to hourly at the onset of puberty is due to a decrease in the inhibitory feedback action of estradiol on tonic LH secretion. Thus, the anovulatory condition of the immature lamb can be considered as an active state of repression of reproductive function and puberty as a process of derepression. When viewed in this context, a striking similarity exists between the mechanism proposed for transition into adult-hood in the lamb and the postulated mechanism for transition into the breeding season in the adult. Because the onset of fertility in both the lamb at puberty and the adult at the beginning of the breeding season is associated with a pronounced decrease in response to estradiol negative feedback, it suggests either that an important component of the pubertal mechanism is yet operative during adulthood and regulates seasonal fertility, or that « puberty » is an annual process in the adult female sheep.

Introduction.

In a review of over 50 studies of puberty in the female sheep five years ago, Dyrmundsson (1973) indicated that, « There is neither a fixed age, body weight nor time of the year at which ewe lambs experience their first estrus, owing to the complex interaction between these factors and the time of birth ». While we cannot yet account for how the lamb knows when to begin its amorous activities, we are at least starting to understand some of the physiologic mechanisms which are called upon during its transition into adulthood. We are beginning to discover that the lamb may be quite capable of carrying out most adult reproductive functions long before the actual time of puberty. In this regard, we are starting to view the anovulatory condition of the immature lamb as an active state of inhibition of reproductive function and the onset of puberty as a process of derepression, And finally, we are beginning to learn that a fundamental element of the pubertal mechanism appears to be retained in the mature female and called upon recurrently to regulate fertility during adulthood.

The foregoing conclusions have been formulated primarily from data obtained in our laboratories, both published and unpublished ; the latter will be reported in detail elsewhere. Before presenting some of our recent findings, it is necessary to consider the term « puberty » in the lamb.

Puberty in the female lamb : Event vs. process

A schematic representation of some of the events which occur in the female sheep during its transition into adulthood, beginning at about 30 weeks of age, is presented in figure 1. Most investigators consider « puberty » in the lamb to be an event, « first estrus » because it is a convenient overt sign that the female has achieved maturity. At this time, the lamb will mate and can become pregnant (Watson and Gamble, 1961 ; Dyrmundsson, 1973). However, upon close examination it is evident that two important ovarian cycles are necessary to pave the way for first estrus. In the immediately preceding cycle, the well known « silent cycle » in which a normal luteal phase occurs, ovulation is *not* accompanied by sexual receptivity (Foote *et al.*, 1970 ; Foster *et al.*, 1975*a*). Recently, we have discovered that yet another cycle precedes the « silent cycle » (fig. 1, inset) (Ryan and Foster, 1978). This brief cycle is less than half the length of the normal cycle and it is initiated by the first LH surge, an event which we consider proclaims that puberty has begun. These considerations have led us to regard « puberty » in the lamb to be a *process* consisting of a series of developmental events culminating in first estrus, the final stage of sexual maturation.

Viewing puberty as a process, rather than a single event, it has become apparent that 2 separate hypotheses are needed to elucidate the *complete* mechanism for the transition of the lamb into adulthood. The first hypothesis must account for the onset

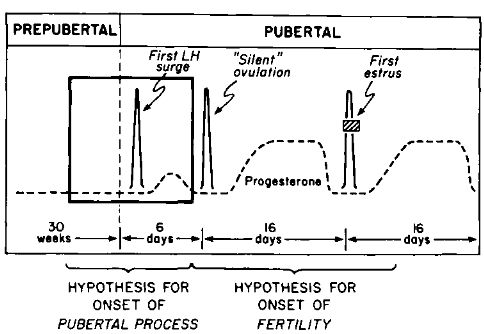

FIG. 1. — *Schematic overview of the pubertal process in female lamb* ; additional details of events leading to the first LH surge (inset) are presented in figure 2.

of the first LH surge because it is one of the earliest identifiable events in the pubertal process (fig. 1, inset). This hypothesis is the subject of the present report. The second hypothesis, which will not be presented herein, is necessary to explain why onset of fertility is not associated with the onset of the first LH surge. A portion of that hypothesis is discussed elsewhere (Ryan and Foster, 1978).

Hypothesis for onset of pubertal process

Central to any hypothesis for the initiation of puberty is the mechanism which leads to the first gonadotropin surge because without it, ovulation is not possible. Our hypothesis has been formulated by working backward in time from the first LH surge in an attempt to clarify several of the major events leading to the onset of the surge mode of gonadotropin secretion. Using this approach, we have constructed a hypothetical model for the initiation of the pubertal process. This model, schematized in figure 2, presents additional details of both known and postulated hormonal relationships which were depicted in figure 1 (inset) during the transition into puberty.

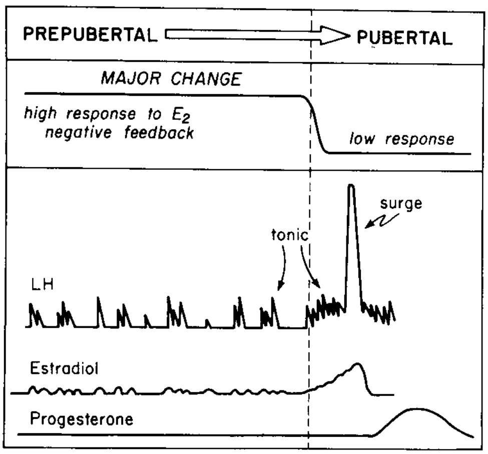

FIG. 2. — *Hypothetical model for onset of the pubertal process in the female lamb.*

In the prepubertal lamb, the gonadotropin surge mechanism is inactive and spontaneous LH surges do not occur (Foster *et al.*, 1975*a*). This mechanism is capable of function, however, since beginning shortly after birth, exogenous estradiol will readily induce an LH surge (Land *et al.*, 1970 ; Squires *et al.*, 1972 ; Foster and Karsch, 1975). If the preovulatory gonadotropin surge system has the *potential* to function in the immature lamb and yet it remains inoperative until the beginning of puberty, it could be postulated that production of a sustained rise in estradiol to activate the surge mechanism is the final development process which times the onset of the first LH surge. To determine which antecedent event causes the sustained estradiol increase, we must turn to the system controlling tonic LH secretion for we feel that it is the responsibility of the mechanism governing pulsatile LH secretion to generate the critical estradiol stimulus at the beginning of puberty.

In contrast to the mechanism governing the surge mode of gonadotropin secretion, the system regulating tonic LH secretion is relatively active throughout the prepubertal period (Foster *et al.*, 1975*a*, 1975*b*). From nearly the time of birth the pattern of basal LH secretion is characterized by marked pulses of LH (fig. 2, left panel) which are about onetenth the size of the preovulatory surge (fig. 2, right panel). Although in general, the frequency of these LH pulses is variable and unpredictable, the number of pulses remains well below one per hour. This low frequency, coupled with the 20- to 30-min halflife of circulating LH in the sheep (Geschwind and Dewey, 1968 ; Kaltenbach *et al.*, 1972), permits the LH baseline to decrease to very low levels between each pulse. We postulate that these infrequent LH pulses cause only *transient* increases in production of ovarian estradiol in the immature lamb, much the same as they do in the seasonally anestrous adult (Scaramuzzi and Baird, 1977), another anovulatory state in which the ovary is steroidogenically active. These resultant short-lived increases in estradiol prior to puberty, however, are not of sufficient magnitude and/or duration to induce a preovulatory surge of LH, and hence, ovulation.

With the onset of puberty, a sustained rise in the LH baseline occurs over a brief period of a few days (fig. 2, right panel). This sustained rise, we feel, reflects an increase in rate of pulsatile LH discharges to about one per hour, a frequency which does not allow the LH baseline between pulses to return to extremely low levels as did the lower rates of pulsatile release prior to puberty. As a consequence of this frequent and relentless bombardment of the ovary by LH, one or more follicles begin to develop toward the preovulatory stage and the transient rises in estradiol (fig. 2, left panel) become transformed into a steady increase (fig. 2, right panel). Now for the first time, the ovary through its persistent signal, a sustained rise in circulating estradiol, calls upon the dormant preovulatory surge mechanism to evoke the first massive discharge of LH.

What major change takes place at the start of puberty to cause an increase in frequency of LH pulses, and thus, a sustained elevation in the LH baseline to drive estradiol production ? We postulate that an « escape » from inhibitory feedback of ovarian steroids on gonadotropin secretion occurs, more specifically *a marked decrease in response of the negative feedback action of estradiol on tonic LH secretion* (fig. 2, upper panel). The concept of a decrease in feedback of ovarian steroids on gonadotropin secretion is not novel since this was proposed several years ago as the mechanism for onset of puberty in the female rat (Fevold, 1939 ; Donovan, 1963). As indicaded by recent publications on the developing rat (Odell and Swerdloff, 1974 ; Ojeda *et al.*, 1976 ; Rabii and Ganong, 1976), however, the evidence to support this concept remains controversial. Particularly problematic has been the failure to demonstrate a rise in basal levels of gonadotropins immediately preceding the first gonadotropin surge and the failure to provide strong evidence that the postulated increased sensitivity of the immature rat to estradiol negative feedback cannot be explained equally well by a decreased metabolic clearance rate of this steroid. Because of this, some investigators are now seeking alternative explanations for the mechanism leading to puberty in the rat, one of which is an increasing ovarian sensitivity to low levels of circulating gonadotropins (Odell and Swerdloff, 1974 ; Advis and Ojeda, 1978). Nevertheless, we feel the long-held popular theory of puberty, although now being questioned in the rat by some workers, may well be valid when applied to the maturing lamb and that a neuroendocrine, rather than a pelvic (ovarian) « clock » is responsible for the initiation of puberty in the female sheep.

Evidence for hypothesis

Evidence for the pronounced decrease in feedback inhibition of LH secretion at the beginning of puberty in the female sheep is presented in figure 3. Patterns of circulating LH are illustrated for lambs in which the ovaries were removed several weeks before the expected age for onset of puberty (about 30 weeks of age). In some of these lambs, a small Silastic capsule containing estradiol was inserted (SC) at ovariectomy. This treatment produced low concentrations of estradiol in the circulation (4 $\pm$ 1 pg/ml, lower panel), levels which are comparable to those present in the adult during the luteal phase of the cycle (Hauger *et al.*, 1977). Ovariectomy without estradiol replacement therapy prior to puberty evoked a rapid, several-fold increase in serum LH (fig. 3, middle panel). Treatment with estradiol beginning at the time of ovariectomy, not only prevented the postcastration LH rise, but suppressed circulating LH to undetectable levels (fig. 3, middle panel). This high degree of suppression of LH secretion by exogenous estradiol was not maintained indefinitely and mean levels of serum LH, by 28 weeks of age, began to rise. The subsequent 20-fold increase in circulating LH concentrations occurred in the presence of constant levels of serum estradiol. It is important to note that this increase in circulating LH in estradiol-treated ovariectomized lambs (fig. 3, middle panel) was coincident with the onset of ovulations in intact lambs (fig. 3, histogram, upper panel). Whereas ovarian cyclicity was not initiated in any intact lamb during the period when estradiol was highly effective in suppressing serum LH levels in ovariectomized females (weeks 19-27), first ovulation occurred in 4 of 9 lambs during the same week (week 28) when LH became detectable in estradiol-treated ovariectomized lambs.

Although somewhat obscured by the logarithmic scale, a 50 p. 100 increase in serum LH occurred in *untreated* ovariectomized lambs during the rise in circulating LH in estradiol-treated lambs. Such a secondary increase in LH secretion has also been observed in agonadal children at the age of puberty (Winter and Faiman, 1972 ; Conte *et al.* 1975) ; at present it is difficult to evaluate what contribution, if any, this non-steroid-mediated increase in LH secretion makes at the time of puberty.

If a marked decrease in response to estradiol occurs at the onset of puberty, then a rise in the LH baseline should be evident in the intact female shortly before the first LH surge. Due to the marked pulsatile pattern of LH in the developing female, however, the nature of the critical increase in circulating LH at the beginning of puberty has been difficult to establish. This is illustrated in figure 4 which depicts changes in serum LH immediately before the first preovulatory LH surge in two individual intact lambs where blood samples were collected at 4-hr intervals during the peripubertal period. In the female shown in the left panel, circulating LH, although variable, returned to low levels on several occasions prior to 6 days before the first LH surge. Thereafter, the baseline of LH remained elevated until the time of the massive preovulatory discharge. In contrast, the profile of LH secretion for the lamb on the right illustrates the other extreme observed, namely the failure of circulating LH to remain increased

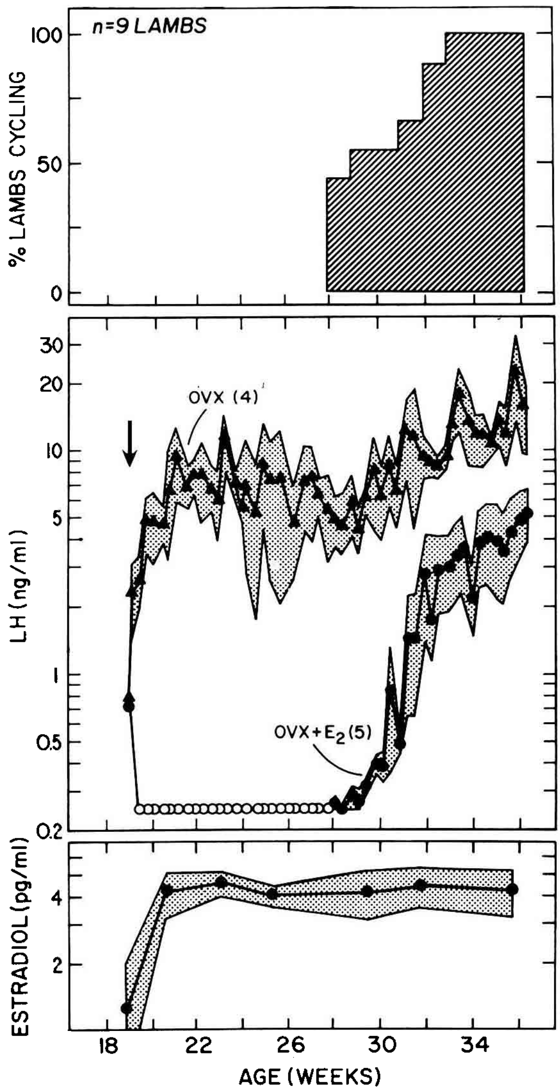

FIG. 3. — *Relationship between decrease in response to estradiol on tonic* LH *secretion* (ovx + E_2, middle panel) *and initiation of ovulation* (histogram, upper panel) *in the lamb.* (First ovulation determined by the appearance of luteal phase levels of serum progesterone.) Vertical arrow indicates time of ovariectomy and beginning of estradiol treatment ; resultant levels of serum estradiol are presented in lower panel. Data are depicted as means ($\pm$ SE, shaded area) of circulating hormone concentrations obtained thrice weekly. Values for LH below assay detectability are indicated by open circles. Note logarithmic scales are used. (From Foster and Ryan, 1978.)

until one and one-half days before the first surge. In both cases, however, mean levels of serum LH (shaded horizontal bars) increased 2-fold before the beginning of the LH surge.

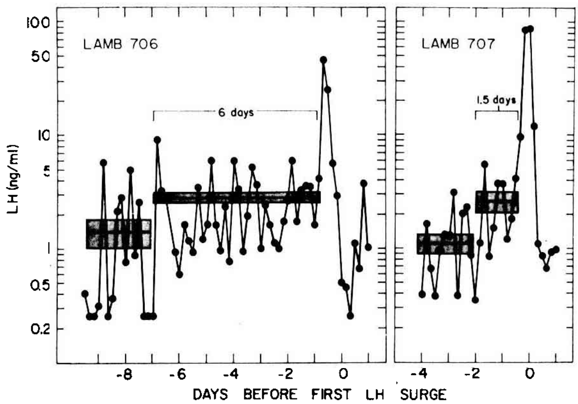

FIG. 4. — *Concentrations of circulating* LH *determined at 4-hour intervals in two female lambs during the peripubertal period.* Horizontal bars indicate means (± SE, shaded area) of individual data points. Note logarithmic scales are used. (From Ryan and Foster, 1978*b*.)

Such findings indicate that the time course of this increase in the LH baseline may differ somewhat between individuals. Nevertheless, one thing seems evident and that is — the rise is relatively abrupt beginning only a few days before the first LH surge. Although direct observation of patterns of circulating LH at much closer intervals than 4 hrs is necessary to resolve clearly the issue of whether the pubertal LH rise reflects an increase in rate or amplitude of LH release, data presented below (fig. 5) point to an increase in frequency.

Figure 5 illustrates the pattern of circulating LH, monitored at 20-min intervals over a 6-hr period, in individual lambs before (upper panel) and after (lower panel) puberty. It can be seen that the height of pulsatile LH discharges, although variable ranging from 5-20 ng/ml, is similar at both times. By contrast, whereas the number of LH pulses observed in the immature female was 1-3 per 6-hr period, the number of pulses during the follicular phase of the *first* estrous cycle, when rapid follicular development and its accompanying rise in serum estradiol occur, ranged from 5-7 per 6 hrs. This frequency of pulsatile LH release of about one per hour in the postpubertal lamb does not permit circulating LH to return to the undetectable levels as does the lower rate of pulses in the prepubertal female.

It is noteworthy that in other studies, ovariectomy of the immature lamb increases the LH pulse rate to hourly (Foster *et al.*, 1975*b*). Furthermore, if such a rate of LH pulses is artificially produced in immature females, by acute intravenous injections of purified ovine LH each hour, an LH surge and ovulation occur (Foster and Ryan, 1979). These latter data suggest that the immature female is readily capable of producing the hourly pulses if inhibitory steroids are removed and that if the ovary is exposed to such a frequency of LH pulses, it is capable of producing the required estradiol stimulus for the LH surge.

All of the foregoing observations are consistent with the hypothesis that an increase in LH pulse frequency to hourly is the necessary trigger for a sustained estradiol rise and that this frequency of tonic LH secretion first occurs at the onset of puberty. It is apparent, however, that observations of transient rises in circulating estradiol during periods of infrequent LH pulses and a sustained estradiol increase during periods of frequent LH pulses (fig. 5) are essential to confirm our hypothesis. Such data remain to be obtained. A more difficult technical challenge will be demonstration of the transition from low frequency to hourly LH pulses because such a change, if it occurs, may be evident over a period of only a few days before the first LH surge (fig. 4) when no external signs have yet appeared to herald that the female is approaching puberty. Finally, we must account for the role of other pituitary hormones in the pubertal process, particularly FSH. Although extensive measurements of this gonadotropin during maturation have not been reported for the lamb, the data available indicate that circulating FSH levels do not increase at the beginning of puberty (Foster *et al.*, 1975*a* ; Fitzgerald and Butler, 1978). Such observations, when combined with those demonstrating that an LH-like stimulus alone produces an increase in estradiol secretion, raise the possibility that FSH is important to maintenance of ovarian function in the prepubertal lamb, but that a rise in circulating LH is the necessary stimulus leading to final follicular development to the preovulatory stage.

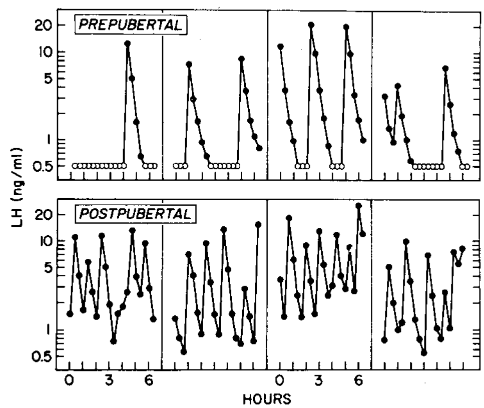

FIG. 5. — *Concentrations of circulating* LH *determined at* 20-*min intervals over a* 6-*hour period in individual female lambs before onset of puberty* (25 weeks of age, upper panel) *and during the follicular phase of the first estrous cycle* (lower panel). Values for LH below assay detectability are indicated by open circles. Note logarithmic scales are used. (Replotted from Foster *et al.*, 1975*a*.)

Similarity of puberty in the lamb and onset of the breeding season in the adult

Recent collaborative studies with S. J. Legan and F. J. Karsch in our program have revealed that the adult female during its annual transition from anestrus to the breeding season in the early fall (August-September) is associated with a marked reduction in response to estradiol inhibitory feedback on LH secretion (Legan *et al.*, retained during adulthood to serve a new purpose — to regulate seasonal fertility — or that « puberty » is a recurrent process in females of seasonally breeding species.

To understand more clearly the similarities and differences between the *attainment of maturity* and the *onset of seasonal breeding* will require additional comparative studies of the *stimulus* which initiates the « escape » from negative feedback in the lamb and in the adult. In the adult female, an external cue, photoperiod, appears to be the primary stimulus. In the lamb, the nature of the stimulus for the critical decrease in response to estradiol feedback at puberty remains to be determined.

Conclusion.

The scheme of interrelated events which has been presented to account for the transition of the immature female sheep into adulthood is by no means complete. As such it should be regarded as a working hypothesis, which in turn, provides the framework upon which an overall concept of the pubertal process can eventually be built. Although our hypothesis has been formulated from the observation of a marked decrease in estradiol negative feedback on LH secretion during puberty, many questions remain to be answered, both to support this hypothesis and to account for the role of other hormones in the pubertal process. Armed with this working hypothesis, hopefully we can now proceed more rapidly to sort out which factors (age, body weight, environmental cues) initiate the decrease in estradiol negative feedback, and presumably, the onset of adulthood in the female sheep.

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Résumé. L'agnelle immature peut manifester plusieurs aspects du fonctionnement endocrinien de la reproduction de l'adulte. L'agnelle prépubère peut ovuler en réponse à une décharge gonadotrope endogène, induite par une élévation du niveau endogène d'estradiol, elle-même artificiellement obtenue en augmentant le nombre de décharges de LH jusqu'à une par heure. Après castration, les décharges de LH peuvent se produire toutes les heures. Mais en présence des ovaires une telle fréquence des décharges n'existe pas spontanément avant le début de la puberté. Ceci implique que, chez la femelle immature, la séquence des événements amenant à la première décharge gonadotrope spontanée est réprimée par un mécanisme qui empêche l'apparition des décharges de LH toutes les heures. Comme des taux d'estradiol circulant capables de supprimer la LH sérique chez l'agnelle immature ovariectomisée sont inefficaces chez les mêmes femelles après la puberté,

on peut penser que, au début de la puberté, l'augmentation de la fréquence des décharges de LH résulterait d'une diminution de la rétroaction négative exercée par l'estradiol sur la sécrétion tonique de LH. Ainsi, l'absence d'ovulation chez l'agnelle immature serait le résultat de l'existence d'une répression active de la fonction de reproduction et la puberté résulterait d'un processus de dérépression. Le mécanisme proposé pour expliquer le début de la saison sexuelle chez l'adulte est très comparable. Dans les deux cas on observe une diminution importante de l'efficacité de la rétroaction négative exercée par l'estradiol sur la sécrétion de LH. Ceci suggère qu'une composante importante du mécanisme pubertaire est toujours efficace chez l'adulte ou que la « puberté » est un processus annuel chez la brebis.

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