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Involvement of thyroid hormones in seasonal reproduction

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This article reviews experiments performed to investigate the importance of thyroid hormones to the expression of the seasonal reproductive cycle of ewes. Thyroidectomy was found to block the transition from the breeding season to anoestrus and to cause ewes to exhibit oestrous cycles all year round. Mechanistically, thyroidectomy produced this effect by preventing the seasonal increase in responsiveness to the negative feedback action of oestradiol on episodic GnRH secretion, thus interfering with a key neuroendocrine process necessary for anoestrus to develop. This response to thyroidectomy was fully prevented by replacement with physiological concentrations of thyroxine. Furthermore, the reproductive response to thyroidectomy was specific to the mechanisms that lead to anoestrus; other aspects of reproductive neuroendocrine function and seasonal and photoperiodic mechanisms were not affected. Evidence is presented to indicate that thyroid hormones have a permissive mode of action on seasonal reproduction, and that their effect is exerted during a restricted 'window' of time during the year. It is concluded that thyroid hormones play a crucial role of physiological significance to generation of the seasonal reproductive cycle of ewes.

Introduction

Over the past 20 years, many important advances have been made towards a better understanding of the mechanisms that govern seasonal reproduction in domestic ruminants. Among these has been the recognition that thyroid hormones play a key role in expression of the neuroendocrine events that underlie generation of seasonal reproductive cycles. In this article, we briefly trace the development of this concept and review our current understanding of the role played by thyroid hormones in seasonal reproduction in one domestic animal, the ewe.

From a historical perspective, work in the European starling provided the foundation for our understanding of the importance of thyroid hormones in seasonal reproduction. The key finding was made over 50 years ago, when Woitkewitsch (1940) discovered that thyroidectomy of starlings prevented the seasonal decline in testicular activity and sustained the breeding season indefinitely. However, this observation went largely unnoticed for several decades and progress in this area was stalled until the 1970s when Wieselthier and Van Tienhoven re-investigated this phenomenon. They determined that thyroidectomy of starlings prevents the development of photorefractoriness, a spontaneously developing condition that leads to termination of the breeding season (Wieselthier and Van Tienhoven, 1972). Since then, a compelling body of evidence has been gathered in birds to document that thyroid hormones are required for the seasonal decline in secretion of gonadotrophic hormones that causes termination of the breeding season (Jallageas and Assenmacher, 1979; Goldsmith and Nicholls, 1984; Dawson *et al.*, 1985; Follett and Nicholls, 1985; Nicholls *et al.*, 1988a; Goldsmith *et al.*, 1989). More recently, such a role for the thyroid has been extended from birds to certain mammals;

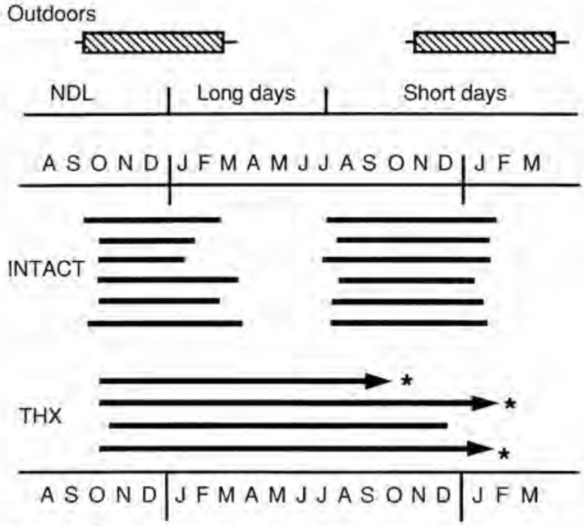

Fig. 1. Effect of thyroidectomy on seasonal occurrence of oestrous cycles in Welsh Mountain ewes either maintained outdoors (top, bars indicate mean ± SEM dates of onset and end of oestrous cycles) or housed in light-control rooms and exposed to the photoperiods indicated (bottom, lines depict interval between first and last oestrous cycle in individual ewes). Asterisks by arrowheads indicate ewes died but exhibited oestrous cycles until death. THX: thyroidectomy; NDL: natural day light. Redrawn from Nicholls *et al.* (1988b).

species studied include golden hamsters, mink, red deer, and both male and female sheep (Vriend, 1985; Jacquet *et al.*, 1986; Nicholls *et al.*, 1988b; Shi and Barrell, 1992; Parkinson and Follett, 1994).

Influence of Thyroidectomy on Seasonal Reproduction in Ewes

Thyroidectomy blocks the transition to anoestrus

The breeding season of ewes begins in late summer or early autumn as photoperiod decreases and, unless pregnancy intervenes, oestrous cycles continue at regular intervals until the following spring when daylength increases (Yeates, 1949; Hafez, 1952). There is now strong evidence that the transitions into and out of the breeding season are driven by an endogenous circannual rhythm, a rhythm that can persist in the absence of changes in relevant environmental time cues (Thwaites, 1965; Ducker *et al.*, 1973; Robinson and Karsch, 1988; Karsch *et al.*, 1989). Photoperiod has long been known to be the primary environmental cue for timing the seasonal reproductive cycle of ewes (Yeates, 1949; Hafez, 1952; Mauleon and Rougeot, 1962). It does so by synchronizing the endogenous rhythm (Woodfill *et al.*, 1991, 1994). In this fashion, the breeding season remains appropriately aligned with the geophysical year. According to this concept, the transition to anoestrus, although regulated in a temporal sense by photoperiod, occurs spontaneously owing to the rhythm. In certain ways, this resembles the photorefractory condition that drives the end of the breeding season of starlings.

Initial evidence implicating a role for the thyroid in seasonal reproduction in ewes was reported by Nicholls *et al.* (1988b), who found that thyroidectomy late in the anoestrous season did not alter the transition into the breeding season but did prevent the subsequent termination of reproductive activity. Thyroidectomized ewes continued to exhibit regular oestrous cycles throughout the entire anoestrous season and remained in breeding condition for more than one year (Fig. 1). This is a remarkable finding

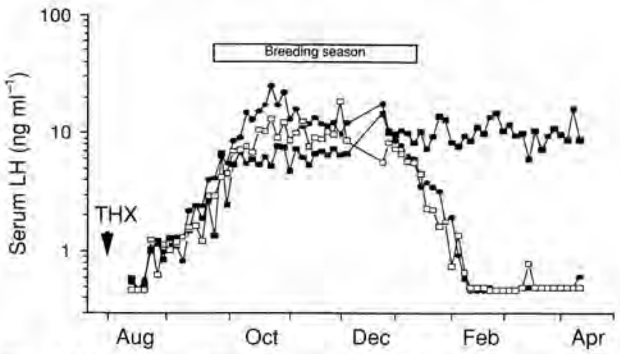

Fig. 2. Mean serum LH concentrations in thyroid-intact control Suffolk ewes (□, $n = 6$) and ewes thyroidectomized (THX) late in anoestrus (arrowhead) and given no replacement (▪, $n = 6$) or thyroxine to maintain a euthyroid state (•, $n = 8$). Each ewe was ovariectomized and treated with an oestradiol implant. Time of breeding season is shown at top. Redrawn from Webster *et al.* (1991a) with permission from The Endocrine Society.

considering the powerful drive to enter anoestrus, as provided by the endogenous rhythm. It is as if thyroidectomy caused the rhythm to stop, or not be expressed. This leads to the question: How does thyroidectomy produce this effect?

Influence of thyroidectomy on the neuroendocrine processes that lead to anoestrus

Before considering how thyroidectomy blocks the transition to anoestrus, the neuroendocrine events that lead to the seasonal pattern of reproduction in ewes will be reviewed. Under normal conditions, the transition to anoestrus is caused by a marked increase in responsiveness to the negative feedback action of oestradiol on LH and FSH secretion; this increased feedback inhibition leads to insufficient gonadotrophin output to support ovarian cyclicity (Legan *et al.*, 1977; Goodman and Karsch, 1980; Legan and Karsch, 1980). This change in feedback responsiveness can be demonstrated by treatment of ovariectomized ewes with implants that release a constant physiological amount of oestradiol, producing a negative feedback clamp on the hypothalamo–pituitary axis. Under these conditions, a high amplitude seasonal cycle of reproductive neuroendocrine activity is expressed, manifested by marked changes in episodic secretion of GnRH from the hypothalamus and gonadotrophic hormones from the pituitary gland (Goodman *et al.*, 1982; Thomas *et al.*, 1988; Karsch *et al.*, 1993). During the breeding season, ewes are rather unresponsive to oestradiol negative feedback; GnRH and LH are thus released as high frequency pulses. At the transition to anoestrus, ewes become very responsive to oestradiol, and episodic discharges of GnRH and LH become very infrequent. It is now well documented that the seasonal changes in responsiveness to oestradiol are driven by the endogenous rhythm; that the timing of these changes is regulated by photoperiod; and that the alteration in response to oestradiol negative feedback constitutes one of the key neuroendocrine determinants of the seasonal waxing and waning of oestrous cyclicity in ewes (Legan *et al.*, 1979; Goodman and Karsch, 1980; Karsch *et al.*, 1984).

In our laboratory, we have focused on how thyroidectomy influences the increased responsiveness to oestradiol negative feedback associated with termination of the breeding season. Initially, we tested the hypothesis that the thyroid gland, through its secretion of thyroxine, is required for the heightened response to oestradiol (Moenter *et al.*, 1991; Webster *et al.*, 1991a). Ovariectomized ewes treated with constant-release implants of oestradiol were thyroidectomized late in the anoestrous season (July), and changes in LH secretion were monitored well beyond the end of the subsequent natural breeding season. We found that thyroidectomy did not alter the pronounced increase in LH secretion at the onset of the breeding season, but it completely obliterated the subsequent fall in LH at the time of the transition to

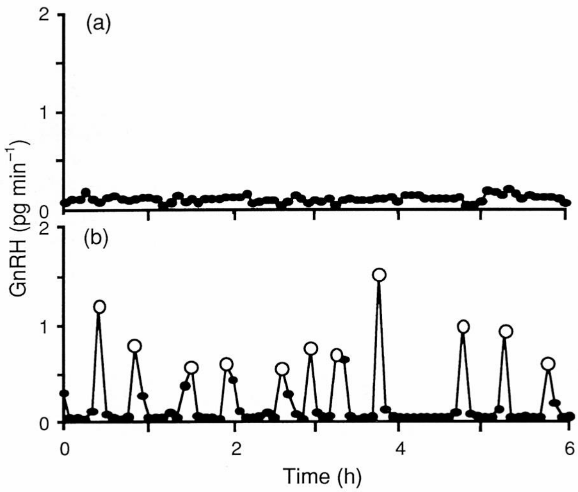

Fig. 3. Patterns of GnRH in pituitary portal blood of (a) a control and (b) a thyroidectomized Suffolk ewe during the natural anoestrous season (May). Each ewe was ovariectomized and treated with an oestradiol implant. Samples were obtained at intervals of 5 min. (○) peaks of GnRH pulses. Redrawn from Webster *et al.* (1991b) with permission from the Endocrine Society.

anoestrus (Fig. 2). This effect of thyroidectomy was manifested through the system that generates the pulsatile discharges of LH (Moenter *et al.*, 1991), and it was fully prevented by replacement of thyroxine at a dose that restored a physiological serum concentration of that hormone (Fig. 2). These findings indicate that thyroid hormones are obligatory for the increased responsiveness to oestradiol negative feedback that causes termination of the breeding season.

Because the seasonal increase in responsiveness to oestradiol negative feedback leads to a marked reduction in GnRH pulse frequency (Karsch *et al.*, 1993), we tested whether the effect of thyroidectomy on the transition to anoestrus is due to an effect on the GnRH neurosecretory system. We monitored the episodic pattern of GnRH in pituitary portal blood obtained at intervals of 5 min from both thyroid-intact ewes that had entered anoestrus and thyroidectomized ewes that had failed to do so (Webster *et al.*, 1991b). Typical GnRH profiles are illustrated in Fig. 3. GnRH secretion in thyroid-intact controls was extremely low with no evidence of episodic release (Fig. 3a). In marked contrast, high frequency pulses of GnRH characteristic of the breeding season were evident in thyroidectomized ewes (Fig. 3b).

Physiological Role for Thyroid Hormones

The foregoing observations demonstrate that the neuroendocrine processes that lead to the end of the breeding season of ewes do not occur in the absence of thyroid hormones. It must be stressed, however, that these findings do not distinguish a specific physiological role of the thyroid in seasonal reproduction from a non-specific pathological or general metabolic consequence of thyroidectomy. In this regard, we have observed that some thyroidectomized ewes become rather lethargic, although most do not exhibit a major debility. Intuitively, health-related problems arising from the long-term absence of thyroid hormones would seem to favour premature termination of the breeding season rather than its extension. Despite this presumption, the potential importance of non-specific effects of thyroidectomy needed to be evaluated experimentally to address the physiological relevance of the thyroid to the seasonal reproductive process. We therefore studied a variety of phenomena to assess the possible non-specific neuroendocrine consequences of the long-term absence of thyroid hormones. These studies are now summarized.

Steroid metabolism

Because thyroid hormones play an important role in metabolism, thyroidectomy might affect the rate of breakdown of gonadal steroids. This could change the balance of negative feedback signals that are important to the manifestation of seasonal shifts in neuroendocrine activity. To examine this possibility, we determined the effect of thyroidectomy on serum concentrations of oestradiol and progesterone in ewes treated with constant-release implants of these steroids. If steroid metabolism were markedly altered in the absence of thyroid hormones, we reasoned that, given the constant steroid input, the circulating concentrations of oestradiol and progesterone should differ in thyroidectomized and thyroid-intact ewes. However, we observed that thyroidectomy had no effect on circulating concentrations of oestradiol and progesterone; altered metabolic clearance of steroids is therefore unlikely to account for continuation of the breeding season in the absence of thyroid hormones (Moenter *et al.*, 1991; Karsch and Dahl, 1994). [It is important to note at this point that sheep do not appear to have a high-affinity, sex-steroid-binding protein in plasma (Legan *et al.*, 1977). Thus, altered transport proteins for circulating steroids are unlikely to account for the influence of thyroidectomy.]

Gonadal steroid feedback

We next tested the possibility that thyroidectomy leads to a general perturbation of all gonadal steroid-feedback effects on the hypothalamo–hypophyseal axis. We examined both the positive feedback action of oestradiol in eliciting the preovulatory LH surge and the negative feedback effects of oestradiol and progesterone during both the breeding and anoestrous seasons (Webster *et al.*, 1991a). All of these feedback responses (except oestradiol negative feedback during anoestrus) were comparable in thyroid-intact and thyroidectomized ewes. As expected, an increased responsiveness to the negative feedback action of oestradiol during anoestrus was not evident in thyroidectomized ewes. In conjunction with the demonstration that thyroidectomized ewes can exhibit normal oestrous cycles, become pregnant and bear offspring (Falconer, 1963; Nicholls *et al.*, 1988b), these findings are consistent with the view that the removal of thyroid hormones does not cause a general compromise of neuroendocrine responses to the feedback actions of gonadal steroids.

Seasonal processes and photoperiodic signal transduction

We then considered the possibility that thyroidectomy causes a non-specific blockade of all seasonal processes or a generalized disruption of the photoperiodic signal transduction pathway. Thyroidectomized ewes were found to exhibit a seasonal cycle of circulating prolactin (Moenter *et al.*, 1991). Furthermore, when exposed to a photoperiodic challenge consisting of an abrupt switch from a short to a long daylength, thyroidectomized ewes exhibited a normal resetting of the circadian rhythm of melatonin secretion and a typical increase in prolactin secretion (Dahl *et al.*, 1994a). In marked contrast, thyroidectomized ewes failed to exhibit a reproductive neuroendocrine response to this photoperiodic challenge. Specifically, LH secretion remained high despite transfer to the inhibitory long day photoperiod (Dahl *et al.*, 1994a). These findings indicate that the influence of thyroidectomy on seasonal reproduction cannot be accounted for by a general disturbance of seasonal phenomena or photoperiodic responses. Moreover, since the melatonin response to photoperiod was normal in thyroidectomized ewes, these observations provide evidence that, mechanistically, the functional deficit produced by thyroidectomy lies somewhere between the pineal gland and the GnRH neurosecretory system.

Thyrotrophin-releasing hormone

Another possible non-specific effect of thyroidectomy that might account for the blockade of anoestrus relates either to an altered secretion of thyrotrophin-releasing hormone (TRH) or to a change in the activity of neurotransmitter systems that modulate TRH release. For example, it has been suggested that hypothalamic dopaminergic systems participate in both the regulation of TRH release and the negative feedback action of oestradiol during anoestrus (Joseph-Bravo *et al.*, 1979; de Greef and Visser, 1981; Meyer and Goodman, 1985; Thiery *et al.*, 1989). Our interest in this possibility was intensified by three recent observations. First, thyroid hormones do exert feedback inhibition of TRH secretion in ewes (Dahl *et al.*, 1994b). Second, TRH secretion in ewes increases 2–3 weeks after withdrawal of thyroxine negative feedback (Dahl *et al.*, 1994b). Third, this 2- to 3-week interval is consistent with the time required for reactivation of reproductive neuroendocrine function of rams following thyroidectomy during the non-breeding season (Parkinson and Follett, 1994).

To test the hypothesis that the blockade to anoestrus is linked to increased TRH secretion, we manipulated TRH experimentally and monitored GnRH release during the anoestrous season in two groups of thyroidectomized ewes. The control group received a physiological replacement of thyroxine; as expected both TRH and GnRH secretion were suppressed. The experimental group also received thyroxine replacement, but this treatment was terminated to withdraw thyroid-hormone negative feedback. Once the consequences of this withdrawal were evident (judged by an increase in circulating TSH), pituitary portal blood was collected to monitor TRH and GnRH release. As expected, the withdrawal of thyroxine allowed TRH secretion to increase, but GnRH secretion remained suppressed, and was virtually undetectable in all samples (Dahl *et al.*, 1994c). This dissociation of TRH and GnRH secretion following the withdrawal of thyroxine provides initial evidence that the blockade of anoestrus in thyroidectomized ewes may not result from either an increased secretion of TRH or altered activity of neurotransmitter systems that modulate TRH release.

The influence of thyroidectomy on seasonal reproduction does not therefore appear to be attributed to altered metabolism of ovarian steroid hormones, to a generalized perturbation of reproductive neuroendocrine feedback mechanisms, to a disruption of all seasonal processes, to a disturbance of the photoperiodic signal transduction pathway, or to an alteration in TRH secretion. Rather, the effect of thyroidectomy on reproduction seems to be quite specific, being restricted to the seasonal increase in responsiveness to oestradiol negative feedback. Although we still cannot discount the possibility that a general influence on metabolism accounts for the reproductive effects of thyroidectomy, the high degree of specificity of the response lends credence to the view that thyroid hormones play a physiological role in seasonal reproduction and that their action is obligatory for the transition to anoestrus. It thus becomes of interest to consider the mode of action of thyroid hormones in this system.

Permissive Mode of Action of Thyroid Hormones

A seasonal cycle of circulating thyroxine has been observed in a broad spectrum of seasonal breeders ranging from fish to mammals (Maurel *et al.*, 1977; Jallageas *et al.*, 1978; Maurel and Boissin, 1981; Dawson, 1984; Licht *et al.*, 1985; Lea *et al.*, 1986). The peak of this cycle is attained at about the time that the breeding season ends. We have found that this holds true for our Suffolk ewes; minimal concentrations of total thyroxine circulate in late summer shortly before the onset of the breeding season and maximal values are achieved in late winter near the transition to anoestrus (Webster *et al.*, 1991a). This temporal relationship between thyroxine and reproductive activity led to the hypothesis that the transition to anoestrus is actively driven by the seasonal increase in thyroid hormone secretion. Some support for this hypothesis is provided by recent observations that the breeding season of ewes can either be extended by lowering thyroxine secretion (Follett and Potts, 1990) or terminated prematurely by injecting large doses of thyroxine (O'Callaghan *et al.*, 1993). However, a certain degree of caution must be exercised in interpreting these findings relative to the hypothesis that the seasonal increase in circulating thyroid hormone drives the transition to anoestrus. First, the extension of the breeding season was by only one or two oestrous cycles. Second, the dose of thyroxine that shortened the breeding season was overtly pharmacological, causing a marked increase in respiration rate and excessive weight loss. Third, the temporal association of circulating thyroxine and reproductive activity, as described above, does not prove causality.

Because of these uncertainties in the previous studies, we took a more direct approach to test the hypothesis that the seasonal increase in circulating thyroxine actively drives the transition to

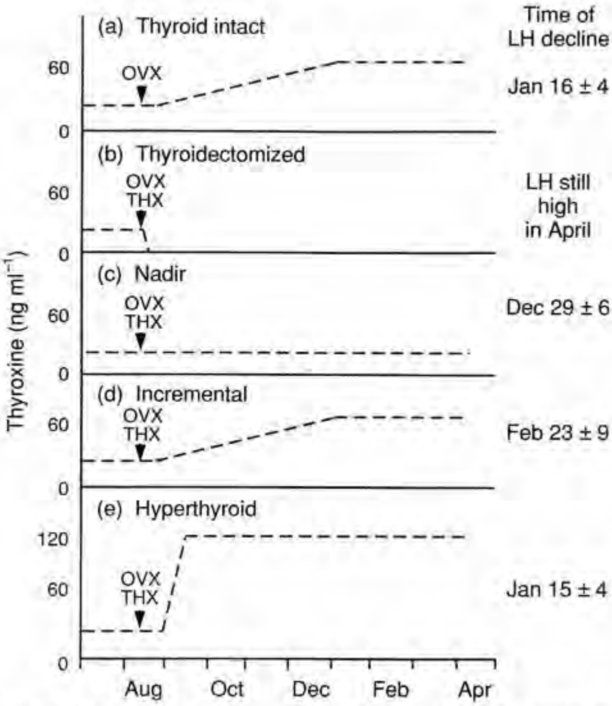

Fig. 4. Design of experiment to test the hypothesis that a rise in the serum concentration of thyroxine is required to drive the onset of anoestrus in ewes. Animals were thyroidectomized (THX, arrowhead), simultaneously ovariectomized (OVX) and treated with a constant-release implant of oestradiol, and assigned to one of four treatment groups as follows: no thyroxine replacement (b); thyroxine replacement either to maintain the summertime nadir of the seasonal thyroxine cycle (c), to reproduce the incremental thyroxine concentrations of autumn and winter (d), to render animals mildly hyperthyroid (e). Animals not thyroidectomized served as controls (a). Dashed lines schematically represent the actual serum concentrations of thyroxine achieved by various treatments. Mean ± SEM date that LH declined to baseline is indicated at right. Modified from Dahl *et al.* (1995).

anoestrus (Fig. 4). Ewes thyroidectomized late in the anoestrous season were not treated with thyroid hormone (b), treated with a low dose of thyroxine to maintain a serum thyroxine concentration equivalent to the summertime nadir of 20–30 pg ml^{-1} (c), treated with increasing doses of thyroxine to reproduce the naturally occurring gradual increase during autumn and winter (d), or made mildly hyperthyroid by treatment with a higher dose of thyroxine to sustain a serum thyroxine concentration of about 120 ng ml^{-1} (e). We reasoned that, if the seasonal increase in circulating thyroxine normally drives the transition to anoestrus, then this transition should not occur in the nadir group, or it should be delayed, whereas the transition to anoestrus should be advanced in the mildly hyperthyroid group. Our observations did not support this contention. The LH decline was not delayed in the nadir group, nor was it advanced in the mildly hyperthyroid ewes relative to that in the thyroid-intact controls (compare (c) and (e) with (a), thyroid-intact control). As expected, LH declined during the winter in thyroidectomized ewes receiving the incremental dose of thyroxine (d), whereas LH remained high until the end of the study in thyroidectomized controls not given thyroxine replacement (b).

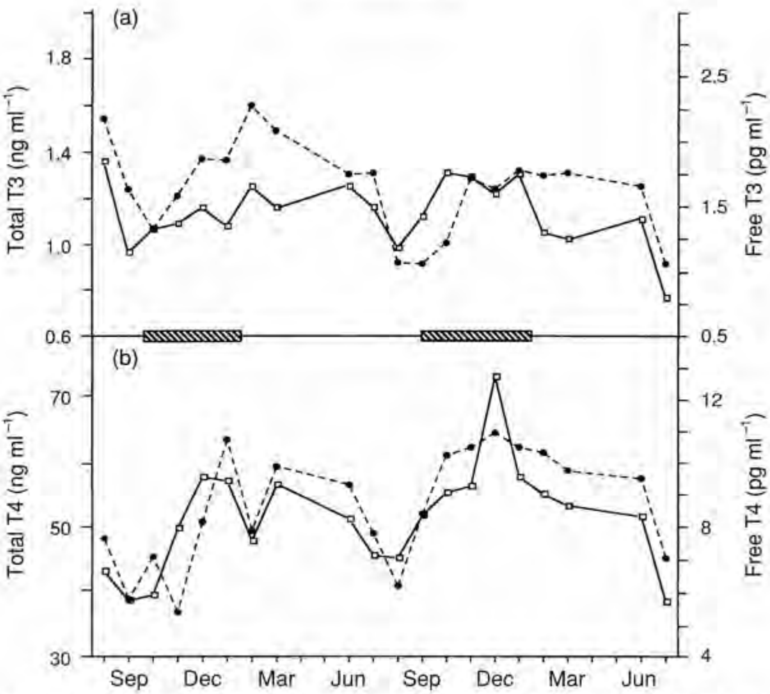

Fig. 5. Serum concentration of (a) total and free triiodothyronine (T3) and (b) thyroxine (T4) in a Suffolk ewe over 2 years. Samples were taken twice a week and combined into monthly pools for assay. Data from April and May of each year are omitted as it had been determined that the springtime shearing of the ewes in this study caused an increase in thyroid hormone secretion (Webster *et al.*, 1991a). The ewe was ovariectomized and treated with an estradiol implant. (•): Total T3 and T4; (□): free T3 and T4. Times of high serum LH concentrations indicative of the breeding season are depicted by hatched bars.

These data refute the hypothesis that the increase in circulating thyroxine during the course of the breeding season actively drives the onset of anoestrus. Rather, our findings provide strong evidence that thyroid hormones act permissively, their mere presence being needed to end the breeding season. A similar permissive role has recently been postulated for thyroid hormones in the seasonal reproductive cycle of red deer stags (Shi and Barrell, 1994). Such a permissive mode of action is in keeping with the nature of thyroid hormone effects in other systems (Rothwell and Stock, 1981; Martin, 1985).

There is, however, a caveat to this conclusion. It is known that only a small portion of the total circulating thyroxine is in the 'active' or 'free' form; the remainder is bound to high-affinity plasma proteins (Martin, 1985). In some species, these proteins appear to vary seasonally (Young *et al.*, 1986; Licht *et al.*, 1991). Thus, the amount of thyroid hormone available for exerting action might change with season, even under conditions in which total circulating thyroxine concentration remains constant as, for example, in the nadir group of our study. We are currently testing this possibility by examining seasonal changes in circulating free, as well as total, thyroxine and tri-iodothyronine, and by testing for seasonal changes in thyroid-hormone binding activity in serum (T. M. Hachigian, G. E. Dahl and F. J. Karsch, unpublished). Although our results are still incomplete and preliminary, they suggest that the concentrations of circulating free thyroxine and tri-iodothyronine (T3) vary in parallel to the total circulating concentrations of these hormones, albeit at a much lower range of absolute values (Fig. 5a, T3; b, thyroxine). We therefore do not favour a seasonal alteration in the amounts of free thyroid hormones as an explanation for our results and expect thyroid hormones do act permissively.

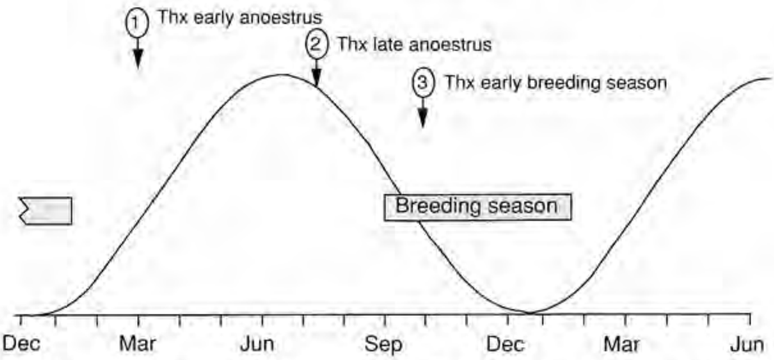

Fig. 6. Design of study to determine the effects of thyroidectomy of ewes at different times of year on subsequent seasonal changes in reproductive neuroendocrine activity. Animals were thyroidectomized (Thx, arrows) at one of three times of year: early anoestrous season (mid-March), late anoestrous season (late July), or early breeding season (late October). Ewes were ovariectomized and treated with constant-release implants containing oestradiol; seasonal changes in reproductive neuroendocrine activity were assessed from serum concentrations of LH. The time of the natural breeding season and the annual photoperiodic cycle are shown schematically.

'Window' of Time for Thyroid Hormone Action

The observation that an increase in circulating thyroid hormones is not needed to drive the transition to anoestrus raises questions related to the nature of the response mechanism for thyroid hormone action on seasonal reproduction. Of particular interest in this regard is the possibility that thyroid hormones may act permissively during a specific stage of the seasonal reproductive cycle, as they do during a critical stage of fetal life to permit normal development of the brain (Dussault and Ruel, 1987). We have thus investigated when during the year thyroid hormones exert their influence on seasonal reproduction.

Time of year of thyroidectomy

In the studies described thus far, the influence of thyroidectomy on seasonality in ewes was confined to an effect on the transition to anoestrus in late winter; onset of the breeding season in late summer was not significantly altered (Nicholls *et al.*, 1988b; Moenter *et al.*, 1991; Webster *et al.*, 1991a; Dahl *et al.*, 1994a, 1995). Two explanations may account for this observation. First, thyroid hormones may not influence the neuroendocrine changes that lead to the onset of the breeding season. Second, thyroid hormones may be required for normal onset of the breeding season, but thyroidectomy was performed too close to the onset of the breeding season for that reproductive transition to be affected. Specifically, ewes were thyroidectomized at the end of July and early August; this was only 1–2 months before the transition and after the time that the photoperiodic cues for onset of the breeding season would have been perceived (Woodfill *et al.*, 1994). Thus, before thyroidectomy, the neuroendocrine processes that cause entry into the breeding season may have been irrevocably set in motion by the endogenous rhythm. Some support for this contention is provided by the recent finding that thyroidectomy of rams much earlier in the year (March) caused premature entry into the next breeding season (Parkinson and Follett, 1994).

To gain insight into when during the year thyroid hormones exert their action in ewes, we are conducting a series of studies to determine the response to thyroidectomy at different times of year. In one experiment, ewes were thyroidectomized in March, that is, early anoestrus (Fig. 6, Group 1). This allowed a full 6 months to elapse between thyroidectomy and the expected onset of the breeding season (i.e. similar to the interval between thyroidectomy in July–August and the blocked transition to anoestrus; Fig. 6, Group 2, late anoestrus). We observed that the transition to the breeding season was

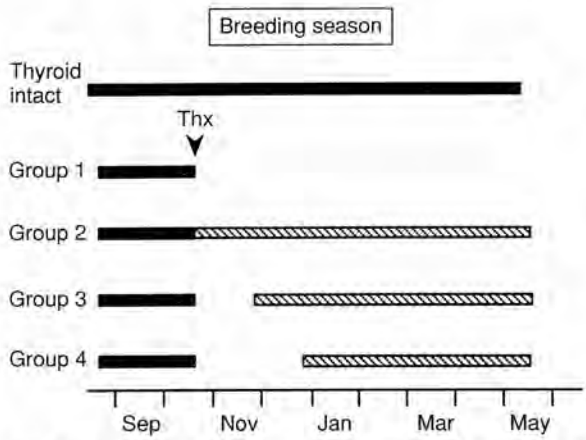

Fig. 7. Design of study to test the hypothesis that there is a 'window' of time when thyroid hormones act to cause the end of the breeding season of ewes. Animals were thyroidectomized (Thx, arrow) early in the breeding season (October) and treated as follows: no thyroxine replacement (Group 1); replacement of thyroxine from the time of thyroidectomy (Group 2); replacement of thyroxine beginning either one (Group 3) or two (Group 4) months after thyroidectomy. (■): Times when endogenous thyroid hormones were present; (▧): times when exogenous thyroxine was present. Each ewe was ovariectomized and treated with a constant-release implant of oestradiol; seasonal changes in reproductive neuroendocrine activity were assessed from serum LH concentrations. Time of natural breeding season is indicated.

unaffected by thyroidectomy early in anoestrus (Thrun *et al.*, 1994). This finding suggests that, unlike the situation in rams (Parkinson and Follett, 1994), the influence of thyroid hormones on the reproductive axis of ewes is restricted to those neuroendocrine mechanisms that lead to the transition from the breeding season into anoestrus.

In another experiment (Fig. 6, Group 3), we tested the response to thyroidectomy in late October. This was 1–2 months after the onset of the breeding season and beyond the period of exposure to the photoperiodic cues that time the transition to anoestrus (Wayne *et al.*, 1990; Woodfill *et al.*, 1994). In this case, the subsequent transition to anoestrus was blocked (Webster *et al.*, 1991a; Thrun *et al.*, 1993). Thus, exposure to thyroid hormones up to the time of the early breeding season is not sufficient for the transition to anoestrus. Rather, exposure to thyroid hormones after this time is relevant. Collectively, these findings lead to the hypothesis that, for anoestrus to develop, thyroid hormones need to be present only at some point during the last 3–4 months of the breeding season. This hypothesis suggests that there may be a 'window' of time for thyroid hormone action.

Is there a 'window' of time for thyroid hormone action?

As an initial test of this hypothesis, the transition to anoestrus was examined in ewes thyroidectomized early in the breeding season and replaced with a physiological dose of thyroxine beginning either at the time of thyroidectomy, one month later, or two months later (design shown in Fig. 7). The last two treatments produced gaps when circulating thyroxine was not present, the most extreme case being elimination of thyroxine for all but the last 1–2 months of the breeding season (Group 4). We observed that, in contrast to thyroidectomized controls which remained reproductively active (Group 2), all groups replaced with thyroxine ended their breeding season at about the same time as did the thyroid-intact controls (Thrun *et al.*, 1993). This was observed even for ewes that received thyroxine replacement during only the last 1–2 months of the breeding season (Group 4). In conjunction with the studies involving thyroidectomy at different times of the year, these observations suggest that the only time when thyroid hormones are needed for the normal progression of the seasonal reproductive rhythm is during a relatively brief 'window' of time during which seasonal reproductive shutdown occurs. We are continuing to test this hypothesis in ongoing studies.

Site and Mechanism of Thyroid Hormone Action

A permissive action of thyroid hormone during a restricted stage of the underlying circannual rhythm of reproductive neuroendocrine activity is in keeping with its nature and mode of action during development. In that case, thyroid hormones must be present during a critical period of fetal life to permit normal maturation of the brain (Greenberg *et al.*, 1974; Dussault and Ruel, 1987). Given this analogy and the observation that thyroid hormone receptors are present in neuroendocrine tissues and notably in GnRH neurones (Jansen *et al.*, 1994), it is possible that the primary site of action for thyroid hormone in regulating seasonality is within the central nervous system. However, it is important to emphasize that such a central site has not been tested definitively, and so a primary action on metabolism or some other peripheral effect cannot be ruled out at this time.

We are still far from understanding the fundamental change(s) above the level of the GnRH neurone that underlies the seasonal switches in reproductive neuroendocrine activity. Thus, any consideration of how thyroid hormones act must be highly speculative. Nevertheless, thyroid hormones may act within the brain to promote morphological changes in the GnRH neurosecretory system, a type of neuronal differentiation that recurs on an annual basis. This line of inquiry arose from observations in the avian brain, for which a major seasonal plasticity has been described in morphology of the neuronal centres that control singing (Nottebohm, 1981; DeVoogd *et al.*, 1985). A structural plasticity associated with functional alterations has been described in certain mammalian neurosecretory systems, including the GnRH neurosecretory system (Theodosis and Poulain, 1984; Tweedle and Hatton, 1987; Witkin, 1987; Xiong *et al.*, 1992). Of particular interest in this regard is the finding of a seasonal plasticity of synaptic input to GnRH neurones in ewes. Specifically, the density of synaptic inputs onto fibres and perikarya of GnRH cells was found to be approximately twice as great during the breeding season than during anoestrus (Karsch *et al.*, 1987). The hypothesis that seasonal changes in the activity of the GnRH neurosecretory system result, in part, from a thyroid hormone-dependent seasonal rearrangement of synaptic inputs onto GnRH neurones should be tested.

Conclusions

Strong evidence has been gathered to support the concept that thyroid hormones play an integral role in expression of the seasonal reproductive cycle in a broad spectrum of species ranging from birds to rodents to domestic ruminants. Evidence has been presented that, in ewes, thyroid hormones must be present to permit the seasonal changes in neuroendocrine activity that lead to a reduction of GnRH secretion and the consequent transition from the breeding season to anoestrus. In the absence of thyroid hormones, the seasonal reproductive rhythm seems to stop, or not be expressed. The action of thyroid hormones on the reproductive axis of ewes appears to be confined to those neuroendocrine changes that lead to the end of the breeding season and this action appears to be restricted to a limited 'window' of time during the seasonal reproductive rhythm. Further studies are needed to define this window, to assess the primary site and mechanism of thyroid hormone action, and to begin to explore the possibility that this phenomenon may be exploited in a practical sense to manipulate the occurrence or timing of seasonal reproduction in domestic ruminants.

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