Seasonal changes in FSH and LH concentrations in ewes with gonadal hypoplasia: Evidence that steroid-independent mechanisms control seasonality in sheep?

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ABSTRACT

Current hypotheses for the control of seasonality are based on the premise that seasonal changes in sensitivity to ovarian steroid hormones drive changes in reproductive status. Most studies have used the ovariectomised, oestrogen-treated ewe as an experimental model, but such animals may be influenced by previous entrainment or exposure to gonadal steroids. This study uses an infertile animal - the homozygous Inverdale ewe - as a unique 'intact' animal model in which to study seasonal patterns of gonadotrophin release.

Blood samples were collected from 11 homozygous Inverdale ewes, twice weekly over an 18 month period. There were marked seasonal changes in FSH concentrations consistent between all animals. In both years, mean plasma FSH levels fell from a mean maximum concentration (25.0 $\pm$ 3.08 ng/ml, 1992 and 27.7 $\pm$ 2.82 ng/ml, 1993) to reach a nadir (7.8 $\pm$ 0.95 ng/ml, 1992 and 7.5 $\pm$ 1.4 ng/ml, 1993) on 5 or 11 November, respectively. Although seasonal changes in LH were less obvious, a significant fall in mean plasma concentration was associated with the decline in plasma FSH concentrations in both years. The high level of synchrony between animals in changes in FSH concentrations, suggests a major component of the mechanism controlling seasonality may be under non-steroidal control.

Keywords: sheep; seasonal breeding; FSH, LH; non-steroidal control.

INTRODUCTION

Many studies have demonstrated that photoperiod is the primary cue controlling seasonal breeding in sheep (Yeates, 1949; Ducker and Bowman, 1970; Legan and Karsch, 1980). A key observation has been that there are seasonal changes in the effectiveness of exogenous oestradiol in suppressing the release of gonadotrophic hormones (Legan *et al.*, 1977). During the breeding season, oestradiol has only weak negative feedback activity, so plasma concentrations of LH and FSH are elevated, whereas during seasonal anoestrus it is a potent suppressor of gonadotrophin secretion. To date, changes in sensitivity to oestradiol have been monitored using a 'standard' animal model - the ovariectomised, oestrogen-implanted ewe in which abrupt changes in feedback potency coincide temporally with the onset and cessation of breeding in intact ewes of the same breed (Karsch *et al.*, 1980; Webster and Haresign, 1984).

The observation that large seasonal changes in plasma concentrations of LH occur in the face of exposure to a constant level of exogenous oestradiol whereas only small changes occur in the absence of oestradiol (Legan *et al.*, 1977), has provided strong support for the hypothesis that photoperiod controls the annual reproductive cycle by modulating the inhibitory actions of oestradiol. However, there are some limitations in using the oestradiol-implanted ovariectomised ewe as an experimental model. For example, entrainment to environmental cues may have occurred in these animals prior to ovariectomy, as they would have already undergone several breeding and anoestrus seasons and previously been exposed to high concentrations of gonadal steroid hormones. Animals with congenital ovarian hypoplasia may be more appropriate for the investigation of mechanisms controlling seasonality, as these infertile animals do not exhibit oestrous cyclicity and their ovaries do not secrete steroid hormones. Sheep carrying the Inverdale gene ($FecX^I$), a major gene for prolificacy in Romney sheep (Davis *et al.*, 1991), may provide such a model. As a consequence of the lack of antral follicle development in homozygous carriers of the gene, oestradiol is not produced and plasma concentrations of LH and FSH are elevated - comparable to those recorded in ovariectomised ewes (McLeod *et al.*, 1995). The present investigation was undertaken to determine if seasonal changes in plasma gonadotrophin concentrations occur in homozygous Inverdale ewes.

MATERIALS AND METHODS

Animals and management

The ewes monitored were all putative homozygous carriers of the Inverdale gene ($FecX^I$ $FecX^I$), classified as such on the basis of bilateral ovarian hypoplasia (streak ovaries, Davis *et al.*, 1992). These animals were all offspring of dams known to be heterozygous for the Inverdale gene, that had been mated to progeny-tested heterozygous Inverdale sires. The ovaries in homozygous Inverdale ewes, which are about one third the size of normal ovaries (Braw-Tal *et al.*, 1993), are typically flattened, streak-like organs on which follicles are not visible. In a proportion of homozygous ewes, large (10-20 mm diameter) abnormal structures develop spontaneously on the ovaries and these may be associated with the secretion of inhibin (McLeod *et al.*, 1995). Any animals in which ovarian structures were recorded, or in which plasma inhibin concentrations were

detectable at any time, were excluded from this study. The occurrence of abnormal ovarian structures was monitored by routine laparoscopic observation. Animals first underwent laparoscopy at approximately 7 months of age, and thereafter at 6 to 12 week intervals throughout the experimental period. The ewes were maintained on pasture (latitude 45° S) as a single group and were kept isolated from rams.

Experimental

Blood samples (5 ml) were collected twice weekly by jugular venepuncture from 11 homozygous Inverdale ewes, over an eighteen month period from July 1992. All samples were centrifuged at 400 g at 4°C for 20 mins immediately after collection, and plasma was stored at -20°C until assayed for hormone content. All samples were analysed for plasma concentrations of FSH, LH and inhibin.

Hormone Assays

Plasma LH concentrations were determined using a previously published homologous double-antibody radioimmunoassay (Meikle and Fisher, 1996). Sensitivity of the assay was 0.1 ng/ml, the intra-assay and inter-assay coefficients of variation were less than 11.0%. Plasma FSH concentrations were determined using the homologous double-antibody RIA of McNatty *et al.* (1989). Within this study the limit of sensitivity was 1.0 ng/ml and the intra-assay and inter-assay coefficients of variation were both < 10%. Inhibin concentrations were measured using the radioimmunoassay procedure described by McNatty *et al.*(1992). Sensitivity of this assay, which recognises inhibin a-subunit, was < 3.0 IU/ml.

Analysis of results

Changes in hormone concentrations with time were assessed by analysis of variance and the relationship between FSH and LH concentrations was analysed by linear regression. All values are presented as means ± s.e.m.

RESULTS

Profiles of mean plasma concentrations of FSH and LH are shown in Fig. 1. In all ewes, there was a consistent seasonal pattern in plasma concentrations of FSH. Plasma FSH concentrations were elevated between January and August, after which they fell significantly ($P < 0.001$) to reach a nadir in late October/early November in both years of the study (see Table 1 and Fig. 1). Neither the median date at which they were recorded, nor mean values of the minimum and maximum FSH concentrations differed significantly ($P > 0.05$) between years (Table 1).

Seasonal patterns in LH concentrations were less well-defined due to greater sample-to-sample variation (Fig. 1). However, mean plasma LH concentrations at the time of the nadir in FSH were significantly lower ($P < 0.01$) than those during the breeding season (recorded in heterozygous Inverdale flockmates). In addition, the nadir in LH concentrations occurred within 5 sample dates of that for FSH in 9/11 ewes. Over the whole sample period, there was a positive correlation ($P < 0.001$, $r^2 = 0.39$) between FSH and LH concentrations.

Inhibin was not detectable in any sample taken from any of the animals in this study.

FIGURE 1. Seasonal changes in mean (± s.e.m.) plasma FSH (l) and LH (o) concentrations in homozygous Inverdale ewes (N=11). Blood samples were collected twice-weekly over an 18 month period.

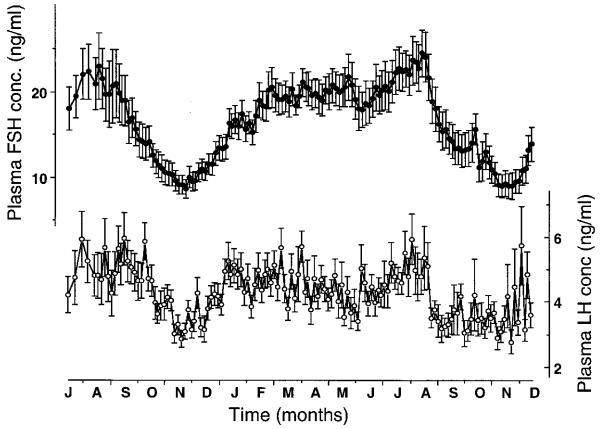

TABLE 1: Maximum and minimum plasma FSH concentrations (mean ± s.e.m.) in homozygous Inverdale ewes (N=11) recorded over an 18 month sampling period. Blood samples were collected twice-weekly.

	Peak	Nadir
1992		
Median date	3 Aug	12 Nov
(range)	27 Jul - 27 Aug	19 Oct - 19 Nov
FSH (ng/ml)	26.4 + 4.08	7.7 + 0.98
1993		
Median date	22 Jul	21 Oct
(range)	28 Jun - 8 Aug	24 Sept - 11 Nov
FSH (ng/ml)	25.4 + 2.99	7.8 + 1.44

DISCUSSION

Inverdale ewes with gonadal hypoplasia show strong seasonal patterns of FSH release in the absence of ovarian steroid hormones, with the period of elevated FSH corresponding closely to the breeding season for fertile ewes of this breed (heterozygous Inverdales) at this location (from March/April to July/August). Seasonal changes in FSH concentrations are not evident in ovariectomised control ewes that have a previous history of breeding seasons and exposure to ovarian steroids, unless they are treated with oestradiol (Legan and Karsch, 1980; Webster and Haresign, 1984).

The pattern of FSH concentrations recorded in this study, is different from that seen in ovariectomised, oestradiol-treated ewes. In oestradiol-treated animals, in which both the fall and the increase in FSH concentrations are much more abrupt, and the period for which FSH concentrations remain low is much longer (Legan and Karsch, 1980). However, that pattern of FSH release observed in homozygous Inverdale ewes more closely fol-

lows those reported for LH concentrations in ovariectomised red deer hinds in the absence of oestradiol (Meikle and Fisher, 1996). The rate of the decrease and subsequent increase in FSH seen in homozygous Inverdale ewes coincided with those for LH in non-treated hinds, suggesting the possibility that a steroid-independent control mechanism may be involved in these animals. Both studies were carried out at the same location so both species would have been exposed to the same changes in photoperiod.

In all types of animals studied, evidence of steroid-independent seasonal changes in gonadotrophin release has been found. However, the magnitude of these changes varies widely between species. Goodman and Karsch (1981) suggested that this reflected species differences in the relative degree of steroid dependency for the control of seasonality. They hypothesised that seasonal changes in gonadotrophin concentrations were predominantly under steroid-independent control in birds, that hamsters and Soay sheep were influenced by both steroid-independent and steroid-dependent systems, but that domestic breeds of sheep relied primarily on steroid-dependent control. The strong seasonal changes in gonadotrophin concentrations recorded in infertile domestic sheep in the present study, would contest that view. In these animals that have never been exposed to ovarian steroid hormones (at least, not since birth), there are major steroid-independent changes in gonadotrophin concentrations. We would suggest that in some species (e.g. domestic sheep) steroid-independent control may be dominated by steroidal control, and that this imposes greater synchrony between individual animals. Under the influence of ovarian steroids, gonadotrophin concentrations change much more abruptly, and these changes are more closely aligned to the onset and cessation of the breeding season.

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