Acceleration of maturation of FSH and LH responses to photostimulation in prepubertal domestic hens by oestrogen

I. C. Dunn¹, P. D. Lewis², P. W. Wilson¹ and P. J. Sharp¹

¹Roslin Institute, Roslin, Midlothian EH25 9PS, UK; and ²Department of Clinical Veterinary Science, University of Bristol, Langford, Bristol BS18 7DU, UK

Egg laying begins in domestic hens, reared on short daylengths, at about day 147 of age and is advanced by photostimulation after but not before about day 42 of age. The development of this response at day 42 may be facilitated by oestrogen. This hypothesis was investigated in prepubertal hens, reared on short daylengths, by comparing the effects of oestrogen treatment on pituitary and plasma FSH and LH responses to photostimulation (16 h light:8 h dark) for 1 week at days 34 and 54 of age. Oestradiol benzoate (0.5 mg kg⁻¹) was injected i.m. on alternate days for 1 week before and after photostimulation. At day 34, pituitary LH content increased after photostimulation but plasma LH and FSH concentrations did not increase. At day 54, pituitary FSH content and plasma FSH and LH concentrations increased after photostimulation, whereas pituitary LH content did not increase. At days 34 and 54, oestrogen treatment decreased

pituitary FSH and LH contents but did not block the stimulatory effect of photostimulation on pituitary FSH. At day 34 but not at day 54, photostimulation combined with oestrogen treatment increased plasma FSH and LH concentrations. Plasma LH but not plasma FSH concentration increased after GnRH-I injection at days 34 and 54. These observations are consistent with the hypothesis that, in prepubertal female chickens, maturation of the neuroendocrine mechanism mediating photoinduced FSH and LH release may be mediated by oestrogen. This effect of oestrogen on photoinduced LH release may be mediated by increased GnRH-I release or enhanced pituitary responsiveness to GnRH-I. It is proposed that neuroendocrine mechanisms controlling photoinduced FSH release may involve oestrogen-responsive interactions between pituitary paracrine factors, including activins and follistatin.

Introduction

The onset of sexual maturity in domestic hens reared on short daylengths, measured as age at production of first egg, occurs at about day 147 of age and is advanced by photostimulation after about day 42. This effect is maximal at about day 63 of age when photostimulation advances the age of production at first egg by approximately 30 days (Lewis *et al.*, 2002). Both plasma FSH (Lewis *et al.*, 1998, 1999) and LH (Dunn *et al.*, 1990; Lewis *et al.*, 1994, 1998, 1999, 2001; Sreekumar and Sharp, 1998; Dunn and Sharp, 1999) concentrations increase in juvenile female chickens after photostimulation. Plasma FSH concentration increases after photostimulation at day 56 of age but not at day 35 of age (Lewis *et al.*, 1999). The development of photoresponsiveness for LH secretion appears to be more progressive: Lewis *et al.* (1998) noted an increase in plasma LH concentration after photostimulation at day 35, which was less robust than that at day 56, and small photoinduced releases of LH have also been reported

at day 21 (Dunn *et al.*, 1990) and day 28 (Sreekumar and Sharp, 1998). Therefore, it appears that in female chickens before about day 42 of age, the neuroendocrine mechanisms controlling photoinduced FSH and LH release are not fully functional. Photostimulation after this age may advance sexual maturation by increasing gonadotrophin secretion, particularly FSH, which stimulates ovarian follicular development (Palmer and Bahr, 1992). However, increased gonadotrophin secretion alone is not the only factor determining age at production of first egg, as somatic maturity, which is not achieved until about day 90–115, is also a critical factor (for examples, see Watson, 1975; Bruggeman *et al.*, 1998a). The response of hypothalamic GnRH neurones to photostimulation is developmentally regulated, as hypothalamic GnRH-I mRNA content increases after photostimulation in peripubertal and somatically mature chickens, but not in juvenile cockerels before day 56 of age (Dunn and Sharp, 1999). However, if juvenile cockerels are treated with oestrogen, hypothalamic GnRH-I mRNA content increases after photostimulation (Dunn and Sharp, 1999). Therefore, it is suggested that the development of the response of the reproductive neuroendocrine system to photostimulation is accelerated by oestrogen. Further

Email: Ian.Dunn@bbsrc.ac.uk

support for this view comes from the observation that photostimulation of day 34 prepubertal hens treated with oestrogen advances age at production of first egg by about 8 days compared with control hens not treated with oestrogen (Lewis *et al.*, 2001).

The aim of the present study was to determine whether the effect of oestrogen on advancing photoperiodically induced sexual maturation in domestic hens might be explained by a facilitatory effect of oestrogen on the responses of the reproductive neuroendocrine system to photostimulation. The effects of oestrogen treatment on the photoperiodic responses were assessed in juvenile hens by measuring pituitary and plasma FSH and LH concentrations, and by measuring plasma FSH and LH responses to GnRH-I injection.

Materials and Methods

Animals

Female ISA Brown chickens (ISA Poultry Services Ltd, Peterborough) were placed at 1-day-old in a floor pen exposed to a short daily photoperiod of 8 h light:16 h dark.

Experiment 1

At day 27 of age the birds were assigned randomly by body weight to eight groups each consisting of eight birds ($n=8$). The birds were moved into two rooms at day 27 or day 47 depending on the photoperiodic treatment while maintained on a 8 h light:16 h dark photoperiod. Treatments were given in a balanced design: (i) photostimulation (transfer to 16 h light:8 h dark); (ii) age at time of photostimulation (34- or 54-days old); and (iii) oestradiol treatment. Oestrogen was administered by i.m. injection of 0.5 mg oestradiol benzoate kg^{-1} body weight on alternate days at 1 week before and 1 week after photostimulation. Comparable dose regimens of oestradiol benzoate have been found to result in plasma concentrations of oestradiol equivalent to those of sexually mature birds (Laugier *et al.*, 1978) and previous studies have found that this dose can reverse neuroendocrine changes after gonadectomy that lower doses cannot (Sun *et al.*, 2001). Control groups for photostimulation were birds retained on short daylengths at days 34 and 54 of age, and control groups for oestrogen injection were long- and short-day birds at days 34 and 54 injected with arachis oil vehicle only. At 1 week after photostimulation, blood samples were collected for FSH and LH measurements at 3 h after dawn from the brachial vein into heparinized syringes, and the birds were killed by cervical dislocation. Pituitary glands were removed and frozen immediately in liquid nitrogen, and were stored at -80°C for measurement of FSH and LH content.

Experiment 2

In a complimentary experiment the plasma FSH and LH responses of pullets to i.v. injections of chicken GnRH-I (Bachem (UK) Ltd, St Helens) were determined at days 34 and 54 of age. The treatments used were identical to those in Expt 1 except that the birds were not photostimulated, and were maintained on 8 h light:16 h dark. Each bird was injected with 0 (phosphate-buffered saline vehicle), 0.1, 1.0 or 10 μg chicken GnRH-I peptide kg^{-1} body weight in a Latin square design at 1 week after the start of treatment with oestradiol benzoate ($n=8$) or arachis oil vehicle ($n=8$). The injections were performed on consecutive days and blood samples were collected at -2 and $+2$ and $+20$ min relative to the time of injection. The 24 h 'washout' period between treatments was sufficient to remove the possibility of carryover effects, as the duration of effect of GnRH-I injection on plasma LH is not > 2 h (Sharp *et al.*, 1986) and the half-life of GnRH-I is < 4 min in birds (Sharp *et al.*, 1987).

Assay of FSH and LH

FSH and LH were measured by homologous radioimmunoassays (FSH: Krishnan *et al.*, 1993; LH: Sharp *et al.*, 1987). FSH and LH concentrations were each assayed in a single assay; the intra-assay coefficients of variation were 6.2 and 5.9%, respectively. Pituitary glands were prepared for assay by homogenization in 0.5 ml chilled assay buffer (40 mmol phosphate buffer l^{-1} , pH 7.4, 2% horse serum, 150 mmol NaCl l^{-1} , 8 mmol EDTA l^{-1} , 0.3% (w/v) sodium azide) using an IKA Ultra Turrax S8N-5G homogenizer (Fisher Scientific, Loughborough) and subsequent centrifugation at 13 000 g for 10 min. The supernatant was separated, stored at -80°C and diluted before the assays were performed.

Statistical analysis

ANOVA was performed on log-transformed data to normalize variance and, where appropriate, differences were identified by least significant differences. The responses to injections of cGnRH-I were analysed by ANOVA using the difference between the log-transformed values for plasma hormones measured 2 min before and 2 or 20 min after injection. In addition, analysis of concentrations of plasma FSH in response to injection of cGnRH-I injection was also performed using all the available data, with bird as block in an ANOVA. As a result of the large effect of oestrogen on some measurements it was felt appropriate to analyse oestrogen-treated animals separately in some cases. Significant differences between means were identified by least significant differences. All analyses were carried out using Genstat version 4.2 (VSN International Ltd, Oxford).

Fig. 1. The effect of photostimulation (short days (SD; □): 8 h light:16 h dark; long days (LD; ■): 16 h light:8 h dark), age at photostimulation (34 or 54 days) and oestradiol (arachis oil: veh; oestradiol benzoate: oest) treatment on (a) mean plasma FSH and (b) mean plasma LH concentrations, and (c) pituitary FSH and (d) pituitary LH contents in domestic hens. Values are mean $\pm$ SEM. Statistical analysis was performed on the log-transformed values shown within the bars ($n=8$). Least significant difference for log-transformed values at the level of significance given are: plasma FSH concentration: 0.143, $P < 0.05$; pituitary FSH content: 0.26, $P < 0.05$; plasma LH concentration: 0.215, $P < 0.05$; 0.287, $P < 0.01$; pituitary LH content: 0.30, $P < 0.05$; 0.39, $P < 0.01$. * $P < 0.05$ and ** $P < 0.01$.

Results

Experiment 1

Effects of photostimulation and oestrogen treatment on pituitary FSH content and plasma FSH concentration at days 34 and 54 of age. ANOVA demonstrated significant effects of age and significant interactions of age and oestradiol treatment and age, photoperiod and oestradiol treatment on plasma FSH concentrations ($P < 0.001$). Plasma FSH concentration increased after photostimulation at day 54 but not at day 34 of age, and increased significantly after photostimulation combined

with oestrogen treatment at day 34, but not at day 54 of age (Fig. 1a). Oestrogen treatment at day 54 blocked photoinduced FSH release (Fig. 1a). At days 34 and 54 of age, pituitary FSH content increased after photostimulation and was markedly decreased by oestradiol treatment ($P < 0.001$). However, oestrogen treatment did not block a significant increase in pituitary FSH content after photostimulation at day 54 (Fig. 1c).

Effects of photostimulation and oestrogen treatment on pituitary LH content and plasma LH concentration at days 34 and 54 of age. ANOVA demonstrated significant

Table 1. Effects of photostimulation, age at photostimulation and oestradiol treatment on ovary mass, pituitary mass and body weight in domestic hens

Age at photostimulation		Ovary mass (g)		Pituitary mass (mg)		Body weight (g)	
		Oestradiol	Vehicle	Oestradiol	Vehicle	Oestradiol	Vehicle
34 days	8 h light:16 h dark	0.21	0.28	5.56	5.23	480	498
	16 h light:8 h dark	0.23	0.27	5.69	5.76	484	482
54 days	8 h light:16 h dark	0.42	0.41	6.65	6.47	1015	1005
	16 h light:8 h dark	0.41	0.45	6.53	6.15	1024	976
Least significant difference (LSD: $P < 0.05$, $P < 0.01$)		0.07, 0.10		0.77, 1.03		64, 85	

Each group contained $n = 8$ hens.

effects of photoperiod ($P < 0.05$) and oestrogen treatment ($P < 0.001$) on plasma LH concentration, and significant interactions for age and steroid treatment ($P < 0.001$), and age, photoperiod and steroid treatment ($P < 0.01$). Plasma LH concentration increased after photostimulation at day 54 of age but not at day 34 (Fig. 1b). Oestrogen treatment decreased plasma LH concentration at day 54, but not at day 34, and, at day 54, blocked the photoinduced release of LH (Fig. 1b). When oestrogen-treated animals were analysed separately the increase in plasma LH concentration at day 34 of age was significant.

ANOVA demonstrated significant effects of age ($P < 0.001$) and oestradiol treatment ($P < 0.001$) on pituitary LH content. Pituitary LH content at day 34 and at day 54 was decreased ($P < 0.001$) by oestrogen in short daylength control and photostimulated groups (Fig. 1d). With the exception of vehicle-treated groups at day 34, there were no significant effects of photostimulation on pituitary LH content (Fig. 1d).

Pituitary mass, ovary mass and body weights. Pituitary mass, ovarian mass and body weight were influenced only by age ($P < 0.001$) (Table 1) and were not affected by photostimulation or oestrogen treatment.

Experiment 2

Effects of GnRH-I injection on plasma FSH and LH concentrations. GnRH-I injection did not alter the concentration of plasma FSH after 2 or 20 min at any of the doses used at either day 34 or day 54, irrespective of whether the birds were treated with vehicle or oestrogen. Hence, only data for birds injected with $10 \mu\text{g}$ GnRH-I kg^{-1} body weight are shown (Fig. 2). Although there was no effect of GnRH on plasma FSH concentrations, the experimental design revealed effects on plasma FSH that were not obvious in Expt 1 because the sampling repetition gave greater precision. The plasma FSH concentrations were lower in oestrogen-treated birds than in control birds ($P < 0.001$), and were lower in birds at day 34 compared with day 54 of

age ($P < 0.001$) (Fig. 3). Plasma LH concentration was measured only in control birds and birds treated with $10 \mu\text{g}$ GnRH-I kg^{-1} body weight to ensure that the injection of GnRH in Expt 2 was effective. At days 34 and 54, GnRH-I injection increased the concentration of plasma LH in vehicle-treated birds, whereas oestrogen treatment significantly attenuated this response ($P < 0.001$) (Fig. 2). At day 34 of age the concentration of plasma LH in oestrogen-treated birds assessed before GnRH-I injection ($2.08 \pm 0.42 \text{ ng ml}^{-1}$, $n = 8$) was significantly lower ($P < 0.05$) than in vehicle-injected birds ($3.66 \pm 0.71 \text{ ng ml}^{-1}$, $n = 8$). Similarly, at day 54 the plasma LH concentration in oestrogen-treated birds assessed before GnRH-I injection ($2.25 \pm 0.56 \text{ ng ml}^{-1}$, $n = 8$) was significantly lower ($P < 0.01$) than in vehicle-injected birds ($6.08 \pm 1.29 \text{ ng ml}^{-1}$, $n = 8$).

Discussion

The results of the present study demonstrate an interaction of age, photostimulation and oestradiol treatment on plasma FSH concentrations that are consistent with the hypothesis that oestrogen promotes maturation of the FSH axis at about day 42 of age.

The increase in plasma FSH concentration between long- and short-day groups was significant only in oestrogen-treated birds at day 34; however, the difference approaches significance at day 54 in vehicle-treated birds. Furthermore, the highest concentrations of circulating FSH were evident in vehicle-treated birds after photostimulation at day 54, followed by birds photostimulated at day 34 and treated with oestrogen. This hypothesis had been supported by previous experiments which suggested that FSH was a candidate to explain the differences in photostimulation in peripubertal chickens (Lewis *et al.*, 1998, 1999, 2001; Dunn and Sharp, 1999). The observation in untreated juvenile female chickens that there was no photoinduced FSH and LH release at day 34 is consistent with earlier observations on photoinduced FSH (Lewis *et al.*, 1998) and LH release (Sreekumar and Sharp, 1998). In agreement with Lewis

Fig. 2. The effect of age (34 or 54 days), oestradiol treatment (arachis oil: vehicle; oestradiol benzoate: oestrogen) and injection of GnRH-I (V: 0 $\mu\text{g kg}^{-1}$; G: 10 $\mu\text{g kg}^{-1}$) on the change in plasma FSH (■) and LH (□) concentrations in domestic hens at 2 min after injection in Expt 2. Values are mean $\pm$ SEM. Log-transformed values on which ANOVA was performed are within the bars ($n=8$). Least significant difference for log-transformed values at the level of significance given are: change in plasma FSH concentration: 0.15, $P < 0.05$; plasma LH concentration: 0.26, $P < 0.05$; 0.35, $P < 0.01$. ** $P < 0.01$.

Fig. 3. The effect of age (34 or 54 days) and oestradiol treatment (arachis oil: veh (□); oestradiol benzoate: oest (■)) on plasma FSH concentration in samples from domestic hens in Expt 2. Values are mean $\pm$ SEM. Log-transformed values on which ANOVA was performed are within the bars ($n=8$). Samples were analysed using bird as block giving effectively 12 replicates per bird. Least significant difference for log-transformed values was 0.07 at $P < 0.01$. ** $P < 0.01$.

et al. (1998), photore sponsiveness for both FSH and LH secretion was fully developed by day 54.

The current observations extend earlier findings by showing that oestrogen treatment for a week before and after day 34 of age also advances maturation of the photoperiodic response for FSH release. This effect of oestrogen on the development of photoperiodic responses for FSH and LH release indicates that oestrogen treatment at day 34 may induce photoperiodic responsiveness for gonadotrophin secretion and, hence, may advance age at production of first egg. These findings indicate that the effect of oestrogen on photostimulation at day 54 may have become inhibitory, so that an increase in plasma FSH concentration is not observed.

A novel and striking finding of the present study is that an increase in photoperiod, irrespective of treatment, can increase the content of FSH in the pituitary, although the magnitude of the effect appears to be modified by treatment. This finding is in contrast to the situation for LH, where pituitary content at day 7 after photostimulation increased only in vehicle-treated birds at day 34.

The stimulatory effect of long days on pituitary FSH content and the absence of an interaction of age, photostimulation and oestradiol treatment indicate that although photostimulation increases FSH synthesis it may fail to stimulate release of FSH before day 42. The oestrogen-dependent acquisition of capacity to release

FSH and LH in response to increased photoperiod was not dependent on a simple facilitatory effect on LH or FSH synthesis because oestrogen treatment reduced pituitary LH and FSH content.

In domestic hens, plasma oestradiol concentration increases markedly at day 90–100, approximately 42 days before the onset of egg laying (Senior, 1974). This increase in plasma oestradiol concentration coincides with the rapid growth of white yolky follicles, which are a major source of oestrogen production (Robinson and Etches, 1986), and in a rapid increase in pituitary FSH (Bruggeman *et al.*, 1998a) and plasma FSH (Vanmontfort *et al.*, 1995; Bruggeman *et al.*, 1998a; Lewis *et al.*, 2001). Pituitary and plasma LH also increase at about this age (Bruggeman *et al.*, 1998a), although an increase in plasma LH concentration is not consistently observed (Lewis *et al.*, 1998). Therefore, it is possible that the maturational effects of oestrogen on photoinduced FSH and LH release may be physiologically relevant during the peripubertal period when rapid ovarian follicular growth is initiated. This contention is consistent with the observation that oestrogen treatment at day 34 of age enhances the maturation of the mechanism controlling photoinduced FSH and LH release.

The development of photoinduced release of LH and FSH at day 54 could be a consequence of a decrease in circulating inhibin, because in turkeys active immunization against inhibin stimulates expression of FSH β and LH β subunit genes (Ahn *et al.*, 2001). However, this possibility is unlikely as plasma inhibin concentration is very low throughout prepubertal development in chickens and does not increase until after the development of yellow yolky ovarian follicles (Vanmontfort *et al.*, 1995; Lovell *et al.*, 2001).

An additional finding from the present study is that oestrogen has a profound influence on FSH content in the pituitary. Previous studies had indicated that oestradiol could influence circulating FSH concentration in embryos (Rombauts *et al.*, 1993), but the magnitude of the effect of oestrogen administration on pituitary FSH content was unexpected. The decrease in pituitary FSH content is about 97% and contrasts with a maximum decrease in the concentration of FSH in the plasma of about 45% between vehicle- and oestrogen-treated groups. The relatively small decrease observed in circulating concentrations indicates that this may reflect a reduction in storage of FSH rather than necessarily a decrease in its synthesis. This inhibitory effect of oestrogen on pituitary FSH and LH content could be mediated, at least in part, by suppression of hypothalamic GnRH-I synthesis and release (Lal *et al.*, 1990; Dunn and Sharp, 1999), or by suppression of expression of pituitary GnRH receptors, or both. In chickens, oestrogen treatment *in vivo* decreases hypothalamic GnRH-I mRNA content (Dunn and Sharp, 1999; Sun *et al.*, 2001), GnRH release (Lal *et al.*, 1990) and anterior pituitary GnRH receptor mRNA content (Sun *et al.*, 2001). Indirect evi-

dence in chickens comes from the observation that gonadectomy increases hypothalamic GnRH synthesis and LH release (Knight *et al.*, 1983), and increases pituitary LH β and common α -subunit mRNAs that are suppressed by oestrogen treatment (Terada *et al.*, 1997).

These different pituitary FSH and LH responses to photostimulation indicate that FSH synthesis may be more dependent on photoinduced regulatory factors than is LH. In mammals the synthesis of FSH and LH is GnRH dependent but FSH synthesis is additionally regulated by the paracrine interactions between pituitary activin and follistatin (Padmanabhan *et al.*, 2002). Activin stimulates FSH synthesis, whereas follistatin inhibits FSH synthesis by blocking activin binding to its receptor; therefore, the ratio of pituitary activin and follistatin may determine basal FSH synthesis (Robertson, 1992; DeWinter *et al.*, 1996). Oestradiol has been observed to decrease FSH expression and secretion in mammals and it is possible that a substantial component of its effect may be mediated through an inhibition of activin (Baratta *et al.*, 2001). Given that there are synergies between the effects of activin and GnRH on FSH gene transcription (Huang *et al.*, 2001), possibly through expression of GnRH receptor, activin appears to be a good candidate for the control of secretion of FSH and may be responsible for some of the effects observed.

GnRH stimulates the synthesis of pituitary activin and follistatin, and follistatin synthesis is more dependent on increased GnRH pulse frequency in rats (Besecke *et al.*, 1996; Burger *et al.*, 2002). In chickens, concentrations of FSH in plasma and in the pituitary gland do not show significant changes during prepubertal development, and then increase to a peak at about 3 weeks before the first oviposition (Vanmontfort *et al.*, 1995; Bruggman *et al.*, 1998a), a time when oestrogen concentration is increasing. In ewes, oestrogen alters the frequency of GnRH pulsatility (Evans *et al.*, 1995), which, in turn, may alter the profile of FSH storage and release. Assuming the presence of activin and follistatin in the chicken pituitary gland, it is possible that the photoinduced increase in pituitary FSH content observed at days 34 and 54 might be a consequence of increased pituitary activin relative to follistatin, in response to a change in the pattern of GnRH release. LH release and, by implication, GnRH release, is pulsatile in adult male and gonadectomized chickens (Wilson and Sharp, 1975). Information on the pattern of LH release in female birds after photostimulation is restricted to turkeys (Chapman *et al.*, 1994), in which photostimulation results in an increase in basal plasma LH concentration associated with a decrease in LH pulse amplitude and frequency. In hamsters, which are photoperiodic rodents, the photostimulation is followed by a more rapid increase in FSH than LH that is mimicked by slow GnRH pulses (Meredith *et al.*, 1998) and, in rats, low GnRH pulse frequency correlates with persistent FSH β gene transcription and low follistatin mRNA content (Burger *et al.*, 2002). Therefore, it is

possible that the increase in pituitary FSH content observed after photostimulation at days 34 and 54 in hens is a consequence of a decrease in follistatin synthesis induced by a reduction in GnRH pulse frequency. Unlike in mammals it has been reported that in chickens the expression of FSH occurs in separate cells to that of LH (Proudman *et al.*, 1999), which provides an obvious mechanism for the existence of a separate mechanism influencing the release of each gonadotrophin. It is also possible that a specific unidentified factor may release FSH (Padmanabhan and McNeilly, 2001).

These explanations of how GnRH-I may influence FSH secretion may in part explain why there was no evidence of any effect of GnRH injection on plasma FSH values; this finding is in agreement with previous studies using this assay indicating that acute injection of GnRH stimulates LH but not FSH release (Krishnan *et al.*, 1993; Bruggeman *et al.*, 1998b). However, these results are in contrast to results obtained using an assay developed by Sakai and Ishii (1983). Studies using this assay for both quail and chicken plasma have shown that GnRH has similar potencies on the release of FSH and LH (Hattori *et al.*, 1986b). The assay of Krishnan *et al.* (1993) shows no apparent crossreactivity with chicken LH preparations and has been checked, as far as is possible in birds, for the absence of crossreactivity with thyroid stimulating hormone (TSH). The Sakai and Ishii (1983) assay does crossreact with LH, as did previous assays. Therefore, it is possible that some of the observations made with the latter assay do not reflect secretion of FSH only.

The lack of observable FSH secretion in response to GnRH indicates that the secretion of FSH may not be tightly coupled to GnRH stimulation; this finding implies that FSH synthesis is constitutively linked to release. This view is supported by Hattori *et al.* (1986a) who found in quail that FSH, unlike LH, appears to be released constitutively, and also by observations in mammals, which show that FSH release is predominantly constitutive (Farnworth, 1995; Padmanabhan and McNeilly, 2001).

If FSH release is mediated by an increase in activin-stimulated FSH synthesis, it is difficult to explain how the photoinduced increase in pituitary FSH content at days 34 and 54 was associated with increased FSH release at day 54 but not at day 34. Therefore, it appears that FSH release is regulated independently of synthesis before day 54.

The development of a suppressive action of oestrogen treatment on photoinduced FSH and LH release between day 34 and day 54 implies a developmentally regulated change in the response of the anterior pituitary gland to oestrogen.

There is no information available for chickens on the effect of oestrogen treatment *in vitro* on FSH release. However, in juvenile rats, *in vitro*, oestrogen stimulates FSH secretion (Wilson and Handa, 1998), which may

be mediated by the oestrogen receptor β (ER β) that predominates in gonadotrophs at this age (Wilson *et al.*, 1998; Nishihara *et al.*, 2000). It is possible that a shift from the expression of ER β to ER α in the anterior pituitary gland between juvenile and adult chickens, as observed in rats (Nishihara *et al.*, 2000), may underlie the switch from a facilitatory to an inhibitory effect of oestrogen on photoinduced FSH and LH release. This effect could be mediated by changes in gonadotrophin Ca²⁺ signalling pathways without affecting gonadotrophin synthesis (King *et al.*, 1989).

In conclusion, the results of the present study demonstrate that expression of FSH in hens is influenced by both photoperiod and oestradiol. In addition, photostimulation can increase the amount of FSH in either the blood or the pituitary even in the presence of inhibitory concentrations of oestrogen. The interaction of photoperiod and oestradiol on FSH may provide the endocrine basis for the observation that photostimulation before day 42 has a different outcome on puberty to photostimulation after day 42. However, as the highest concentrations of both gonadotrophins in the plasma occurred in control birds after photostimulation at day 54 of age, it is possible that in oestrogen-treated birds that had been photostimulated at 34 days of age the effect is not one of FSH alone. As has been observed previously, GnRH does not have an acute effect on secretion of FSH into the plasma.

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