FSH concentrations and sensitivity to feedback in infant lambs from breeds differing in prolificacy

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Summary. Plasma FSH concentration was significantly higher in Romanov than Ile-de-France ewe lambs at 5, 6 and 7 weeks of age ($P < 0.001$, $P < 0.02$ and $P < 0.02$, respectively) and at 5, 6 and 7 weeks of age ($P < 0.001$, $P < 0.01$ and $P < 0.05$, respectively) compared to Finn lambs. FSH concentrations were similar and unaffected by time in Ile-de-France and Finn lambs. Ovariectomy at 5 weeks of age produced similar increases in FSH concentrations in Romanov and Ile-de-France ewe lambs, but at 3 months of age the increase in FSH concentrations after ovariectomy was significantly steeper ($P < 0.02$) in Romanov than Ile-de-France lambs.

Sensitivity to oestradiol feedback was related to the age of the lambs. At 5 weeks of age, oestradiol (30 µg in oil per lamb) produced a significant decrease ($P < 0.001$) in FSH concentrations in Romanov and Ile-de-France lambs, demonstrating that negative feedback can be triggered by oestradiol at this age. Positive feedback after an oestradiol challenge was identified in lambs of both breeds at 6 weeks of age. Sensitivity to the negative feedback of follicular fluid compounds was also established at 5–6 weeks and did not differ between breeds. At 9–10 weeks of age, while there was no breed effect of an oestradiol challenge on FSH concentrations, suppression of FSH concentrations by follicular fluid was shorter in Finn than in the other lambs.

As all the feedback mechanisms are functional at 5–6 weeks of age, it is likely that the between breed differences of FSH profile during infancy are linked to differences in gonadal development.

Keywords: FSH; oestradiol; follicular fluid; lambs; prolificacy

Introduction

The maturation of ovarian and pituitary function between birth and puberty is still poorly documented in sheep. Preliminary studies comparing animals of prolific and non-prolific breeds have identified alterations in gonadotrophin secretion and gonadal development associated with high prolificacy. High concentrations of prepubertal plasma FSH were found in sheep of very prolific breeds (Booroola: Findlay & Bindon, 1976; Bindon *et al.*, 1985; Lacaune × Romanov cross: Ricordeau *et al.*, 1984), but not in those of breeds or strains with limited increases in ovulation rate ('T' Merino: Bindon *et al.*, 1985; High line Romney ('Hight' flock): Peterson *et al.*, 1987). Differences in gonadal development have been demonstrated at birth in ewes of the Booroola, Finn cross and Romanov breeds compared to those of non-prolific local controls (Land, 1970; Tassel *et al.*, 1983; Sonjaya & Driancourt, 1987). These differences in ovarian follicular populations could be responsible for the breed differences in circulating FSH if it is assumed that the pituitary of ewe lambs is sensitive to ovarian feedback products (i.e. oestradiol and inhibin). In mature ewes, oestradiol and inhibin interact to control FSH secretion (Martin *et al.*, 1988). In contrast, sensitivity to these compounds is poorly documented in ewe lambs and the relationships between this sensitivity and prolificacy have not been explored.

The aims of this study were therefore: (1) to obtain further insights on the relationships between FSH concentrations during infancy and prolificacy by comparing the patterns of FSH during infancy in two prolific (Romanov and Finn) and one control breed (Ile-de-France), (2) to analyse the effect of ovariectomy on gonadotrophin concentrations at two stages of infancy (5 and 12 weeks), and (3) to measure sensitivity to steroid and follicular fluid feedback and check whether it is related to prolificacy.

Materials and Methods

Animals. Sheep of 3 breeds (Romanov, Finn and Ile-de-France) were used. Romanov and Ile-de-France lambs came from the INRA flocks (Nouzilly, France). Finn lambs were obtained after transfer of embryos from the AFRC flock (Edinburgh, UK) to Romanov ewes and were born at mean $\pm$ s.e.m. birth weight of 2.36 ± 0.1 kg, whereas Ile-de-France and Romanov lambs were born at 4.43 ± 0.27 and 2.51 ± 0.17 kg respectively. The growth rates up to 9 weeks of age were 168.9 ± 23.5 , 226.2 ± 30.3 and 201.1 ± 23.3 g/day for Romanov, Ile-de-France and Finn ewe lambs, respectively. All lambs were artificially reared to minimize maternal effects. The lambs were weaned on Day 5 and received artificial milk during the first 45 days of their life together with concentrates and hay *ad libitum*.

Experiment 1: comparative study of the pattern of plasma FSH concentrations during infancy. Ewe lambs born in April–May (14 of each of the Romanov, Finn and Ile-de-France breeds) were bled once a week from 5 to 11 weeks of age and plasma FSH concentrations were measured. No attempt to measure LH secretion was made because of the small size of the lambs and the large number of samples required to measure pulsatile secretion of LH.

Experiment 2: effect of ovariectomy. Lambs at 5 weeks of age (6 Romanov and 6 Ile-de-France) and at 3 months of age (6 Romanov and 6 Ile-de-France) were ovariectomized under general anaesthesia, induced by intravenous injection of thiopentone sodium (10 mg/kg; Abbott Laboratories, 28380 Saint Remy-sur Avre, France) and maintained with halothane in air (3–4% Fluothane; ICI Ltd, Macclesfield, U.K.). After ovariectomy lambs were given an injection of antibiotics (150 000 i.u. procaine penicillin, 5 g dehydrostreptomycin; Gist-Brocades, Netherlands). Blood samples were collected once daily, twice before ovariectomy and 6–7 times after ovariectomy, and assayed for FSH.

Experiment 3: sensitivity to oestradiol and follicular fluid in intact ewe lambs. Romanov ($N = 6$) and Ile-de-France ($N = 6$) lambs of 5–6 weeks of age were used. Treatment 1 (high dose of oestradiol) was achieved by an injection of $30 \mu\text{g}$ oestradiol benzoate in oil per ewe lamb. This dosage was selected because it was close to the dose used by Land *et al.* (1970) to induce an LH surge in 6-week-old lambs. Treatment 2 (low dose of oestradiol together with follicular fluid) was achieved by subcutaneous injection of 1 ml of whole follicular fluid. This follicular fluid was aspirated at slaughter from bovine ovarian follicles excluding obviously cystic ovaries, stored at -20°C and injected without charcoal treatment. Such a choice was made because a low dose of oestradiol sensitizes the pituitary to inhibit in sheep (Martin *et al.*, 1988). Oestradiol concentration of the follicular fluid used was 95 ng/ml . The lambs were bled according to the following schedule: -1 h and -5 min before injection and at $+1 \text{ h}$ and $+2 \text{ h}$, and at 4-h intervals until 30 h after injection. The 6 Romanov and 6 Ile-de-France lambs were randomly divided into 2 groups, (a) to be treated first with oestradiol at 5 weeks of age and then by follicular fluid at 6 weeks, and (b) being first challenged by follicular fluid then by oestradiol.

In the second part of the study, this experiment was repeated with the Romanov, Finn and Ile-de-France lambs used in Exp. 1 but at 9–10 weeks of age. The same doses and bleeding schedules as at 5–6 weeks of age were used. The day of treatment was selected to be at least 5 days before the next day of sampling for Exp. 1 to minimize the interactions between the two experiments. FSH was assayed as described below.

Hormone assays. Plasma FSH was measured using the homologous NIAMDD assay kit previously described by McNatty *et al.* (1987) and validated in our laboratory. The sheep (o)FSH for iodination was NIAMDD-oFSH I-1, the oFSH reference preparation was NIAMDD-oFSH-RP-I (biopotency $75 \times \text{NIH FSH-S1}$) and the oFSH antiserum was NIAMDD-anti-oFSH-I (AFP-C5288113). At a final FSH antiserum dilution of 1:80 000 this homologous assay had a working range of 0.1 to 15.5 ng per assay tube. The volume of plasma which was assayed was $100 \mu\text{l}$ and each sample was assayed in duplicate. The intra-assay coefficients of variation were 5.81%, 5.09% and 2.84% for 14.37, 3.30 and 1.62 ng FSH respectively. The inter-assay coefficients of variation were 7.25% and 9.07% for 14.37 and 1.62 ng FSH respectively. Results are expressed in terms of $\text{ng NIAMDD-oFSH-RP-I/ml}$ plasma. An FSH rebound was said to occur at the time when FSH concentrations first exceeded the mean of all previous samples plus three standard deviations.

Statistical analysis. The effects of breed or of treatment on changes in hormone concentrations were analysed by two-way analysis of variance (Exps 1, 2 & 3b) or three-way analysis of variance (Exp. 3a) which takes into account repeated measurements in the same animal as described by Gill & Hafs (1971). In Exp. 1, data were transformed to $(\sqrt{x} + 1)$ to obtain equality of variances. In Exps 2 and 3, the FSH concentration after treatment was expressed as a percentage of the mean of the 2 pretreatment values and the percentage data were transformed by $\log(x + 1)$ before analysis to homogenize the variances. Once significant main effects were detected, differences between means were identified using Tukey's procedures (Lellouch & Lazar, 1974). Results are expressed as means $\pm$ s.e.m.

Results

Pattern of FSH secretion (Exp. 1)

As shown in Fig. 1, there were significant breed ($P < 0.001$) and age effects ($P < 0.005$) and a significant breed $\times$ age interaction ($P < 0.02$) for FSH concentrations. Romanov ewe lambs had elevated FSH concentrations at 5, 6 and 7 weeks of age compared to Ile-de-France and Finn ewe lambs. In contrast, FSH concentrations were similar in Finn and Ile-de-France lambs and were unaffected by time.

Fig. 1. FSH concentrations of the ewe lambs of the 3 breeds, during the post-natal period. Between-breed differences are shown by **** $P < 0.001$, *** $P < 0.01$, ** $P < 0.02$, * $P < 0.05$, NS = not significant; A, Romanov vs Ile-de-France, B, Romanov vs Finn, C, Ile-de-France vs Finn. Within-breed differences are shown by 1 vs 2 ($P < 0.05$) and 1 vs 4 ($P < 0.01$).

Effect of ovariectomy at 5 weeks and 3 months of age (Exp. 2)

There was a time effect ($P < 0.001$), but no breed effect or breed $\times$ time interaction on FSH concentrations after ovariectomy at 5 weeks of age. The increase in FSH concentrations was similar in Romanov and Ile-de-France ewe lambs (Fig. 2a). In contrast, at 3 months of age (Fig. 2b), there was a breed effect, a time effect and a breed $\times$ time interaction on FSH concentrations after ovariectomy. This was linked to a steeper rise in FSH concentrations after ovariectomy in Romanov than Ile-de-France lambs.

Sensitivity to oestradiol and follicular fluid feedback in 5–6-week-old intact ewe lambs (Exp. 3a)

The 3-way ANOVA taking into account age, time, breed and their interactions (for overall data obtained at 5 and 6 weeks) showed that there was an age effect ($P < 0.05$) and a time effect ($P < 0.01$) on FSH concentrations after the oestradiol injection. Hence, subsequent analysis was made for each age. At 5 weeks of age, there was a clear time effect as evidenced by reduced FSH values as early as 6–10 h after treatment ($P < 0.001$) (Fig. 3a), but no breed effect and no

Fig. 2. FSH concentrations (% of pre-ovariectomy values) after ovariectomy in Romanov and Ile-de-France lambs at 5 weeks (a) and 3 months (b) of age. Between-breed differences are shown by *** $P < 0.01$, ** $P < 0.02$, NS = not significant. Within breed differences, compared to pre-ovariectomy values are shown by 1 vs 2 ($P < 0.05$), 1 vs 3 ($P < 0.02$), 1 vs 4 ($P < 0.01$) and 1 vs 5 ($P < 0.005$).

breed $\times$ time interaction for FSH concentrations after oestradiol administration. One of the 3 Ile-de-France lambs showed a positive feedback at 18 h after treatment, while in Romanov lambs the FSH concentration remained low. In contrast, at 6 weeks of age there were no significant time ($P < 0.1$) or breed effects and no time $\times$ breed interactions for FSH concentrations following treatment (Fig. 3b), since 2 out of 3 Ile-de-France and 1 out of 3 Romanov lambs exhibited a positive feedback between 16 and 20 h after injection following a short-lived negative feedback. The 3-way ANOVA did not reveal a significant age effect on FSH concentrations after injection of follicular fluid. In contrast, a significant time effect ($P < 0.001$) was detected as FSH concentrations decreased as early as 10 h after treatment in the two breeds at 5 and 6 weeks of age (Fig. 4a, b). The duration of FSH suppression was similar in the two breeds, with concentrations starting to decrease at 10 h and remaining low until 14 h after treatment. There was no breed effect or breed $\times$ time interaction. An FSH rebound starting at 26 h after treatment was detected in 1 out of

Fig. 3. FSH concentrations (% of pre-injection values) after oestradiol injection to intact Romanov (▲), and Ile-de-France (■) at 5 weeks (a) and at 6 weeks (b), and these two breeds plus Finn (●) lambs at 9–10 weeks (c) of age. Compared to pre-injection levels (1): 1 vs 2 ($P < 0.05$), 1 vs 3 ($P < 0.02$), 1 vs 4 ($P < 0.01$), 1 vs 5 ($P < 0.005$), 1 vs 6 ($P < 0.001$).

3 Ile-de-France and 1 out of 3 Romanov lambs at 5 weeks and in 3 lambs (1 out of 3 Ile-de-France and 2 out of 3 Romanov lambs) at 6 weeks of age.

Sensitivity to oestradiol and follicular fluid feedback in 9–10-week-old intact ewe lambs (Exp. 3b)

There was a time effect ($P < 0.005$), but no breed effect or breed $\times$ time interaction on FSH concentrations after oestradiol injection. Subsequent analysis within breed showed that oestradiol

Fig. 4. FSH concentrations (% of pre-injection values) after injection of bovine follicular fluid to intact Romanov (▲) and Ile-de-France (■) at 5 weeks (a) and at 6 weeks (b) and these two breeds plus Finn (●) lambs at 9–10 weeks (c) of age. Compared to pre-injection values (1): 1 vs 2 ($P < 0.05$), 1 vs 3 ($P < 0.02$), 1 vs 4 ($P < 0.01$), 1 vs 5 ($P < 0.005$), 1 vs 6 ($P < 0.001$).

was responsible for a marked reduction in FSH values in all 3 breeds. Significantly reduced concentrations compared to the pretreatment values were observed at 6 and 10 h after treatment in Ile-de-France ($P < 0.05$ and $P < 0.02$; respectively), at 10 h ($P < 0.05$) in Romanov and at 10 h ($P < 0.005$) in Finn ewe lambs (Fig. 3c). Oestradiol induced an FSH surge at 22–24 h after treatment in 4/5 Ile-de-France, 3/5 Romanov and in 2/5 Finn ewe lambs.

There was a time effect ($P < 0.001$) and a significant breed $\times$ time interaction ($P < 0.005$), but no breed effect on FSH concentrations after injection of follicular fluid (Fig. 4c). Plasma FSH

concentrations were significantly decreased in all breeds after the injection of follicular fluid ($P < 0.001$) compared to the pretreatment values and the magnitude of this response was different between breeds. The duration of FSH suppression was shorter in Finn and longer in Romanov ewe lambs. A decrease in plasma FSH concentrations was noted from 6 to 10 h after treatment in Finn lambs ($P < 0.05$ and $P < 0.01$; respectively), from 6 to 18 h in Ile-de-France lambs ($P < 0.005$, $P < 0.001$, $P < 0.01$ and $P < 0.02$; respectively) and from 2 to 18 h ($P < 0.05$, $P < 0.01$, $P < 0.005$, $P < 0.01$ and $P < 0.01$; respectively) in Romanov ewe lambs. Hence, at 18 h after injection, plasma FSH concentrations were significantly higher in Finn lambs than in the other breeds ($P < 0.01$ for Finn vs Ile-de-France; $P < 0.005$ for Finn vs Romanov). All 5 Finn lambs demonstrated an FSH rebound at 32 h after treatment, and concentrations were significantly higher ($P < 0.05$) than pretreatment values.

Discussion

The main conclusions of this study are that: (1) high prolificacy when mature is not always associated with high FSH concentrations during infancy, (2) after ovariectomy, the negative feedback regulation of FSH secretion is functional in ewe lambs of both breeds as early as 5 weeks of age, and (3) the hypothalamo-pituitary unit is able to exhibit negative and positive feed-back as early as 5–6 weeks of age in response to oestradiol or follicular fluid.

The high FSH concentrations found in Romanov ewe lambs at 5–7 weeks are in good agreement with previous results also demonstrating higher FSH concentrations during the early postnatal weeks in Lacaune $\times$ Romanov cross (Ricordeau *et al.*, 1984), Booroola (Bindon *et al.*, 1985), while the low FSH values in Finn lambs are in agreement with the results of Bindon *et al.* (1985) and Peterson *et al.* (1987), reporting that plasma FSH concentrations were low in 'T' Merino and Romney lambs selected for increased prolificacy ('Hight' flock).

Three possibilities (i.e. higher endogenous activity of the hypothalamus-pituitary unit and/or lower sensitivity to ovarian feedback products and/or low production of these products) could explain the high FSH concentrations at 5–7 weeks in Romanov lambs. It is unlikely that these different FSH patterns are produced by a higher endogenous activity of the hypothalamo-pituitary unit of Romanov lambs, since Exp. 2a demonstrated that, at 5 weeks of age, the increase in FSH concentrations in response to ovariectomy was similar between the two breeds. Furthermore, differences in the sensitivity of the hypothalamo-pituitary unit to ovarian feedback products are also unlikely to be causing these different FSH patterns, since there was no difference in the sensitivity to ovarian feedback (i.e. oestradiol and inhibin in follicular fluid) between intact Romanov and Ile-de-France lambs at 5 and 6 weeks of age (Exp. 3a). Hence, the high FSH concentration in the Romanov lambs up to 7 weeks of age may be associated with their retarded ovarian development. The ovary of Romanov lambs at birth is almost devoid of antral follicles and at 4 weeks of age demonstrates very few large antral follicles exceeding 2 mm in diameter (Sonjaya & Driancourt, 1987), the size at which follicles start to secrete actively oestradiol and inhibin (Carson *et al.*, 1981; Tsonis *et al.*, 1983, 1984).

The decrease in FSH concentrations in Romanov lambs between 5 and 8 weeks of age could be linked to their ovarian development since laparoscopic examination of the ovaries demonstrates follicles ≥ 2 mm and ≥ 4 mm in 4/6 and 3/6 Romanov ewe lambs at 8 weeks of age (H. Sonjaya & M. A. Driancourt, unpublished results) and at 12 weeks of age the ovary of Romanov lambs is mature in terms of its ability to shed a number of ovulations typical of the breed in response to hCG (Sonjaya & Driancourt, 1988). This is supported by data for rats in which high FSH concentrations during infancy have been measured in rats of strains having a retarded follicular development (de Reviers & Mauléon, 1979) and showing that the increase in the numbers of antral follicles with ageing and the decrease in FSH concentrations are temporally related (de Reviers & Mauléon, 1979).

In contrast to the Romanov lambs, Finn ewe lambs had low FSH concentrations during infancy. This could be related to the similar structure of ovaries of Finn and Ile-de-France lambs at birth (Land, 1970; Sonjaya & Driancourt, 1987) which contrasts with the retarded ovarian development of Romanov lambs (Sonjaya & Driancourt, 1987). It can be concluded from the different patterns of FSH secretion during infancy in Finn and Romanov lambs that high FSH concentrations during infancy are not an absolute requirement for high prolificacy when mature. They might be needed in some breeds or simply associated with high adult prolificacy. This is supported by the observation in rats that the suppression of the high FSH concentrations noticed during infancy by androgen treatment does not alter ovulation rate when the rats are mature (Uilenbroek *et al.*, 1979).

The second important result of this study is that ovariectomy as early as 5 weeks of age produces an increase in FSH concentrations which is similar in Romanov and Ile-de-France lambs. That the negative feedback regulation of FSH secretion has appeared as early as 5 weeks of age is in agreement with previous reports (Foster *et al.*, 1975a; Bremmer *et al.*, 1981) also demonstrating an increase in FSH concentrations at 5 weeks of age after ovariectomy at 120 days *post coitum* or at 2 weeks of age. The period during which ovariectomy does not result in increased gonadotrophin secretion is therefore much shorter in lambs than in pigs (Colenbrander *et al.*, 1987) or mares (Wesson & Ginther, 1979), but similar to that found in calves (Odell *et al.*, 1970). Worthy of note is the steeper increase in FSH concentrations after ovariectomy in Romanov lambs at 3 months of age, a feature which suggests a higher endogenous pituitary activity and/or a higher sensitivity of the pituitary to ovarian feedback products in this breed at this age. Whether a similar breed difference exists in mature ewes is unknown. The third important feature of this study is that the differentiation of feedback to oestradiol and follicular fluid in Romanov and Ile-de-France was completed in both breeds as early as 5–6 weeks of age. That negative feedback in response to oestradiol is functional at this age is in good agreement with the results of Liefer *et al.* (1972) and Gluckman *et al.* (1983), also demonstrating that negative feedback response to oestradiol can be detected in fetal and infant lambs. That the mechanisms involved in the positive feedback response to oestradiol start to be functional around 6 weeks of age fits with previous data from Land *et al.* (1970) and Foster & Karsch (1975). The lack of breed difference in the responses to oestradiol needs however, to be confirmed with different doses of oestradiol and more physiological delivery systems. In contrast, no information on sensitivity to inhibin feedback was available in lambs. Even at 5–6 weeks of age, lambs were able to demonstrate a reduction in FSH concentrations after injection of follicular fluid, followed by a rebound 26 h later in some of the lambs. An early differentiation of inhibin feedback, similar to that found in this study for the lamb, has been reported for pigs (Colenbrander *et al.*, 1987). At 9 weeks of age, a breed effect was detected in the response to follicular fluid. Whether a similar breed difference exists in mature ewes is unknown.

In conclusion, this study established that the mechanisms involved in cyclicity are all functional well before the time of puberty. Feedback mechanisms are established at 5–6 weeks of age and large mature follicles are present in the ovaries at 12 weeks of age (Sonjaya & Driancourt, 1988). The limiting factor blocking the onset of puberty might therefore be an insufficient oestradiol production by the follicles, possibly as a consequence of the low pulsatility of LH occurring in prepubertal lambs (Foster *et al.*, 1975b; Kinder *et al.*, 1987).

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