

Reproductive hormone profiles in mares during the autumn transition as determined by collection of jugular blood at 6 h intervals throughout ovulatory and anovulatory cycles

C. H. G. Irvine¹, S. L. Alexander¹ and A. O. McKinnon²

¹Animal and Food Sciences Division, Lincoln University, Canterbury, New Zealand; and ²Goulburn Valley Equine Hospital, Shepperton, Australia

The aim was to define precisely the FSH secretion pattern in mares during the two ovulatory cycles before, and for 24 days after, the last ovulation of the season and to compare this with the profiles of other reproductive hormones and follicular growth to identify changes which may lead to the termination of follicular cycles. Jugular blood was collected every 6 h from ten light horse mares for 6 weeks in autumn. Samples were assayed for FSH, LH, prolactin, inhibin, oestrone conjugates and progesterone. Luteolysis occurred earlier and periovulatory oestrone, but not inhibin, concentrations were significantly lower in the last than in the second to last cycles. In ovulatory and anovulatory cycles, daily mean FSH concentrations were low at the expected time of ovulation and high between days 9 and 11 (day 0 = ovulation), which were usually after luteolysis. However, the periovulatory FSH nadir was prolonged in the last compared with the second to last cycles, and the difference between peak and trough values was not significant in anovulatory cycles. Between day 5 and day 8, the FSH interpulse interval was approximately 2 days, and did not vary in successive cycles. The LH profile also showed progressive changes as mares entered acyclicity; the surge terminated sooner in the last than in the second to last cycles, and failed to occur when expected in acyclicity. Sporadic prolactin pulses occurred at luteolysis in a similar proportion of ovulatory and anovulatory cycles. These results indicate that inadequate gonadotrophin stimulation in early dioestrus may be a critical event leading to sub-optimal follicular and luteal development, and eventually acyclicity. Moreover, the time relationships amongst changes in pituitary and ovarian hormones and follicular growth become increasingly disrupted during the autumn transition, which may contribute to the cessation of cyclicity.

Introduction

Mares are seasonal breeders, becoming reproductively inactive during the autumn and resuming ovulatory cycles in the spring. The neuroendocrine mechanisms controlling seasonality are assumed to involve modulation of GnRH pulse frequency, and through this the gonadotrophin signal to the ovaries (Sharp and Davis, 1993). Accordingly, in intact mares, the frequency of FSH and LH pulses increases steadily during the weeks preceding the first ovulation in the spring (Fitzgerald *et al.*, 1987; Alexander and Irvine, 1991). Similarly, in ovariectomized mares, the annual pattern of plasma LH concentrations is a smooth bell-shaped curve, which tracks changing photoperiod and peaks at about the summer solstice (Freedman *et al.*, 1979). This annual LH pattern is underpinned by changes in LH pulse frequency (Fitzgerald *et al.*, 1983). However, the assumption that varying GnRH and gonadotrophin pulse frequency is the

primary regulator of cyclicity has been brought into doubt by work showing that, during the autumn transition out of the breeding season, ovarian steroid hormone secretion decreases before any discernible change in gonadotrophin profile (Nequin *et al.*, 1998). Although the final failure to ovulate is associated with the lack of an LH surge (Snyder *et al.*, 1979; Nequin *et al.*, 1998), corpus luteum function (King *et al.*, 1988) and follicular oestrogen production (Weedman *et al.*, 1993) are compromised several cycles before this point.

It is possible that the FSH priming of the developing follicle becomes inadequate as the season becomes advanced. This could produce some of the seasonal changes in ovarian function, since FSH triggers granulosa cells to express LH receptors and induces the activity of aromatase, the enzyme that converts androgens to oestrogens (reviewed by Alexander and Irvine, 1993). In mares, FSH secretion, like that of LH, is acutely driven by bursts of GnRH (Irvine and Alexander, 1993, 1994). The FSH pulses that are detectable in jugular blood reflect directly the activity of the GnRH pulse generator (Irvine, 1995) and therefore might be expected to

Received 7 April 1999.

show a seasonal change in frequency. However, the FSH profile during the autumn transition in mares is controversial. A study in ponies found a seasonal change in FSH secretion; two surges occurred per cycle early in the breeding season but only one surge occurred late in the season (Turner *et al.*, 1979). However, Snyder *et al.* (1979) reported that FSH patterns are similar during the month after the second to last ovulation and the month after the last ovulation. Similarly, no change in FSH secretion can be detected as ovarian function decreases during the last few cycles of the season (Nequin *et al.*, 1998). These studies have relied on blood sampling once a day, or even less frequently, to determine gonadotrophin profiles. Irvine *et al.* (1998) used sampling at intervals of 4 h to show that, in mid-season cycles, sampling once a day is inadequate to define dioestrous FSH secretion patterns, which consist of high amplitude, infrequent pulses. The aim of the present study was to characterize the FSH profile during the transition out of the breeding season by collecting jugular blood from ten light horse mares at 6 h intervals for 6 weeks in the autumn. Selected samples were also assayed for LH to determine the size and shape of the ovulatory surge, and for prolactin, which has been proposed to play a role in seasonal breeding in horses (for example, see Nequin *et al.*, 1993). Follicular development was monitored once a day, and plasma inhibin, oestrone conjugates and progesterone concentrations were measured to gauge follicular and luteal competence.

Materials and Methods

Horses

Ten regularly cyclic light horse (predominantly Standard-bred) mares aged 5–15 years and weighing 500–550 kg were used. The mares were kept on pasture and supplemented with lucerne hay and grain as needed. Mares were given altrenogest (Regumate, 0.044 mg per kg per day by oral administration; Hoechst-Roussel, Frankfurt) once a day for 15 days to provide some degree of synchronization of cycles. Treatment ceased at least 21 days before the experiment, and all mares had a normal ovulatory cycle before sampling commenced.

Experimental protocol

Blood samples (5 ml) were collected through an indwelling intrajugular cannula (Angiocath 16 g, 5.25 in; Deseret Co., Sandy, UT) into tubes containing 10 mg EDTA, every 6 h from 14 March to 24 April (Southern hemisphere autumn). It is estimated that 80–85% of the research mares become anovulatory each winter, which is similar to the percentage observed in similar breeds pastured locally. Approximately 5% of local mares enter anoestrus in February, 20% in March, 40% in April and the rest in May. The mares stayed in their usual paddock during blood sampling and followed their normal routine. Night samples were collected without light or by torchlight. After each collection, the cannulae were refilled with 3% (w/v) sodium EDTA.

Ovarian activity was monitored once a day by transrectal ultrasonography from 1 month before to 1 month after the experiment. The diameters of the largest and second largest follicles present, and the occurrence of ovulation were recorded. Seven of the mares entered the non-breeding season during the experiment, a similar percentage to that observed in unsampled mares in the region (C. H. G. Irvine, S. L. Alexander and A. O. McKinnon, unpublished). Samples were collected during most or all of the five second to last (that is, the cycle culminating in the second to last ovulation of the season; mares 1, 2, 5, 7 and 9) and five last cycles (that is, the cycle culminating in the last ovulation of the season; mares 3, 4, 5, 7 and 8) as well as the first 24 days of acyclicity in five mares (mares 3, 4, 6, 8 and 10). Eleven days of the last cycle of mare 9, and the first 14 days of acyclicity in mare 7 were also available.

Sample handling

Blood was refrigerated after collection, and each morning, samples were centrifuged for 5 min at 1400 g and plasma was harvested and stored at -20°C until assayed.

Hormone assays

Pituitary hormones. FSH was measured in all samples using a heterologous radioimmunoassay, that is, anti-human FSH (AFP1391675, Dr AF Parlow, Pituitary Hormones and Antisera Center, UCLA Medical Center, Los Angeles, CA) and equine FSH (Papkoff E276B, Dr H Papkoff, Hormone Research Institute, University of California, San Francisco, CA) as standard and radioligand, which was validated for use on horse plasma (Irvine and Alexander, 1993).

LH was measured in two to four samples per day by heterologous radioimmunoassay, that is, monoclonal antibody against the β -subunit of bovine LH (518B7, Lot 2, Monoclonal Antibodies, Mountain View, CA), and equine LH (Papkoff E263B, Dr H Papkoff) as standard and radioligand, which was validated for use on horse plasma (Shand *et al.*, 1991). Not all samples were assayed since the LH profile during the cycle is defined adequately even by sampling once a day (Irvine *et al.*, 1998).

Prolactin was measured in all samples collected from the day before luteolysis (that is, progesterone concentrations $\leq 1 \text{ ng ml}^{-1}$) to day 17 (ovulation = day 0) of each cycle or of acyclicity using a homologous radioimmunoassay, that is, anti-equine prolactin (No. 4, Dr H Papkoff) and equine prolactin (AFP 7730B, Dr AF Parlow) as standard and radioligand, as described by Evans *et al.* (1991).

Ovarian hormones. Progesterone was measured in one sample per day between day 5 and day 15 of each cycle or of acyclicity using an enzyme immunoassay as described by Irvine *et al.* (1990).

Oestrone conjugates were measured, when possible, in all samples collected between day 8 and the day of ovulation (or 21 days after the previous ovulation in acyclic mares) by enzyme immunoassay as described by Stabenfeldt *et al.*

(1991). The antibody used showed high crossreactivity with oestrone, (269%), oestrone-3-sulphate (100%) and oestrone-3-glucuronide (70%), and reacted to a lesser extent (4–10%) with oestradiol and its conjugates (Stabenfeldt *et al.* 1991). Occasionally, there was insufficient plasma for the assay of oestrone conjugates (for example mare 5); however, at least one measurement per day was always possible.

Inhibin was measured in all samples using a radioimmunoassay (Robertson *et al.*, 1988) that has been used to measure inhibin in horse plasma (Rosser *et al.*, 1994). The assay was based on an antiserum against bovine 31 kDa inhibin A (No. 1989), with bovine inhibin A (BIS 3) as standard and radioligand. Crossreactivity with pro-alpha C (a product of the α -subunit) was 288%. Consequently, the measured amounts comprised both dimeric insulin and free α -subunit.

In each assay, samples were assayed in duplicate. The minimum detectable concentrations (that is zero binding + 2 SD) in each assay were: FSH, 0.4 ng ml⁻¹; LH, 0.2 ng ml⁻¹; prolactin, 2.0 ng ml⁻¹; progesterone, 0.36 nmol l⁻¹; oestrone conjugates, 0.05 ng ml⁻¹ and inhibin, 0.12 ng ml⁻¹. Intra- and interassay coefficients of variation (CV) were: FSH, 6.4, 10.4%; LH, 6.4, 9.8%; prolactin, 7.1, 10.0%; progesterone, 4.4, 13.2%; oestrone conjugates, 10.0, 19.4% and inhibin, 5.7, 5.7%, respectively.

Pulse detection

The Cluster program (Veldhuis and Johnson, 1986) was used to detect FSH pulses between day 4 and day 12 (when the sampling regimen was adequate for this purpose; Irvine *et al.*, 1998). Parameters were set to yield a false positive rate of 2.5% as estimated from data supplied with the program when testing 1 × 1 clusters. The *t* statistics used were asymmetrical (3.15/2.0). The variance model was based on assay replicates. These settings identified pulses that were consistent with visual appraisal. Pulse amplitude was defined as the maximum hormone concentration attained in each Cluster identified pulse.

Normalizing cycles

Because the duration of the cycle was different among mares, data were aligned for comparison with the first 24 days of acyclicity as follows: (i) from day 4 to day 15, which in most ovulatory cycles was the day before the largest follicle attained 35 mm; and (ii) from day 5 before the next ovulation to the following day 3 (that is, day -5 to day +3). The interovulatory interval in the last cycle of mare 7 was only 13 days, and since it could not be normalized successfully in this way, it was included only in the analysis of the progesterone data, which did not require normalization.

Statistical analyses

Daily mean hormone concentrations were calculated for each mare. Because it could not be ensured that complete cycles were observed in each position in all mares, the design

of the experiment was unsuitable for two-way ANOVA with repeated measures. Moreover, the daily data for most hormones did not produce, and could not be transformed to produce, a normal distribution. Therefore, the profile of daily mean hormone concentrations was assessed within each cycle position (that is, second to last, last, or acyclic) by Friedman's non-parametric ANOVA for repeated measures, followed by Dunn's test of selected means when the overall result was significant at the 0.05 level (GraphPad Prism, San Diego, CA). The days compared were chosen on the basis of when high and low concentrations of each hormone are observed during mid-season cycles (Evans and Irvine, 1975; Irvine *et al.*, 1998; Shand *et al.*, 1998) and are noted on the relevant figures. Mean values were calculated for each mare during each period of interest to determine whether hormone concentrations differed with cycle position. These means passed the normality test, and were assessed by one-way ANOVA, followed by Tukey's test when the *F* value was significant (GraphPad Prism).

Other comparisons were made using one-way ANOVA (GraphPad Prism), Spearman's rank correlation test, Fisher's exact probability test (Statistix Analytical Software, St Paul, MN) or Student's *t* test, as noted in the text. Data are given as mean ± SEM.

Results

At the ovary

Follicles. The diameter of the largest follicle differed with day in the second to last and last cycles ($P < 0.0001$ for each), growing to preovulatory size after luteolysis and then ovulating (data not shown). These changes did not occur in acyclicity.

Progesterone and the interovulatory interval. Plasma progesterone increased after each ovulation and each corpus luteum was subsequently lysed, as shown by a decrease in progesterone to undetectable concentrations (Fig. 1). However, the day that progesterone decreased below 1 ng ml⁻¹ (indicating luteolysis) was later ($P < 0.05$; one-way ANOVA) in the second to last cycles (day 11.4 ± 0.8) than in the last cycles (day 9.5 ± 0.4) or acyclicity (day 8.5 ± 0.8). The mean interovulatory interval was greater ($P < 0.02$, Student's *t* test) in the last cycles (22.3 ± 0.3 days, excluding the 13 day cycle of mare 7) than in the second to last (19.6 ± 0.7 days) cycles. The combination of a decrease in the duration of the luteal phase and an increase in duration of the interovulatory interval in the last cycles resulted in a prolonged follicular phase compared with the second to last cycles (second to last, 8.2 ± 0.5 days; last, 12.8 ± 0.5 days; $P < 0.001$, Student's *t* test).

Oestrone conjugates. Daily mean oestrone conjugate concentrations varied during the second to last ($P < 0.02$) and the last ($P < 0.05$) cycles; the concentrations were higher immediately before ovulation than during mid-dioestrus, but did not change in acyclicity (Fig. 2). The magnitude of the surge in oestrone conjugates at about the time of ovulation was influenced by cycle position ($P < 0.005$); higher mean

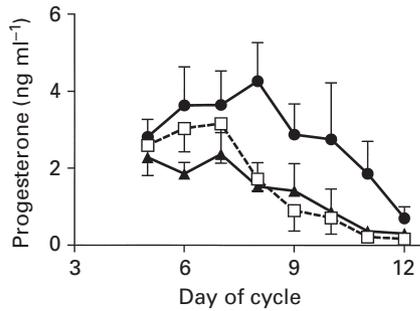


Fig. 1. Mean (\pm SEM) concentrations of progesterone in second to last (\bullet , $n = 5$) or last (\square , $n = 6$) cycles of the season, or the first 12 days of acyclicity (\blacktriangle , $n = 6$) in mares. The number of mares in the last cycle and acyclic groups is greater than in other hormone comparisons, because part cycles could be included since normalization to a succeeding ovulation was not required. Within each group, progesterone concentrations on day 7 were compared with those on days 9–12 using Dunn's test at the 0.05 level. In second to last cycles, day 7 > day 12; in last cycles, day 7 > days 11 and 12; in acyclicity, day 7 > days 10, 11 and 12.

values were observed in the second to last than in the last cycles or in acyclicity (Fig. 2).

Inhibin. The pattern of daily mean inhibin concentrations paralleled that of follicular growth, increasing after luteolysis in ovulatory ($P < 0.0001$ for both the second to last and the last cycles) but not anovulatory cycles (Fig. 2). Mean periovulatory inhibin concentrations varied with cycle position ($P < 0.05$) and were higher in second to last cycles than in acyclicity.

At the pituitary

FSH. Daily mean FSH concentrations varied with day in all cycle positions (second last, $P < 0.001$; last, $P < 0.002$; acyclic, $P < 0.005$); however, only in ovulatory cycles were values between day 9 and day 11 significantly greater than those at about the time of ovulation or expected time of ovulation (Fig. 3). In the second to last but not the last cycles, FSH concentrations tended to increase after ovulation and did not differ from mid-dioestrous values by day +3 (Fig. 3). Neither FSH pulse frequency nor amplitude between day 5 and day 12 differed with cycle position (Fig. 4). In most mares, the duration of the interpulse interval was large between day 5 and day 8 (Fig. 5) and was slightly but not significantly shorter in the second to last cycles than in the last cycles or acyclicity (second to last, 1.6 ± 0.2 days; last, 2.0 ± 0.8 days; acyclic, 1.9 ± 0.5 days).

LH. Daily mean LH concentrations increased in an ovulatory surge in second to last ($P < 0.0001$) and last ($P < 0.001$) cycles, but not in acyclicity (Fig. 3). Accordingly, mean periovulatory LH concentrations were affected by cycle position ($P < 0.02$), and were higher in second to last ($P < 0.001$) and last ($P < 0.05$) cycles than in acyclicity. The

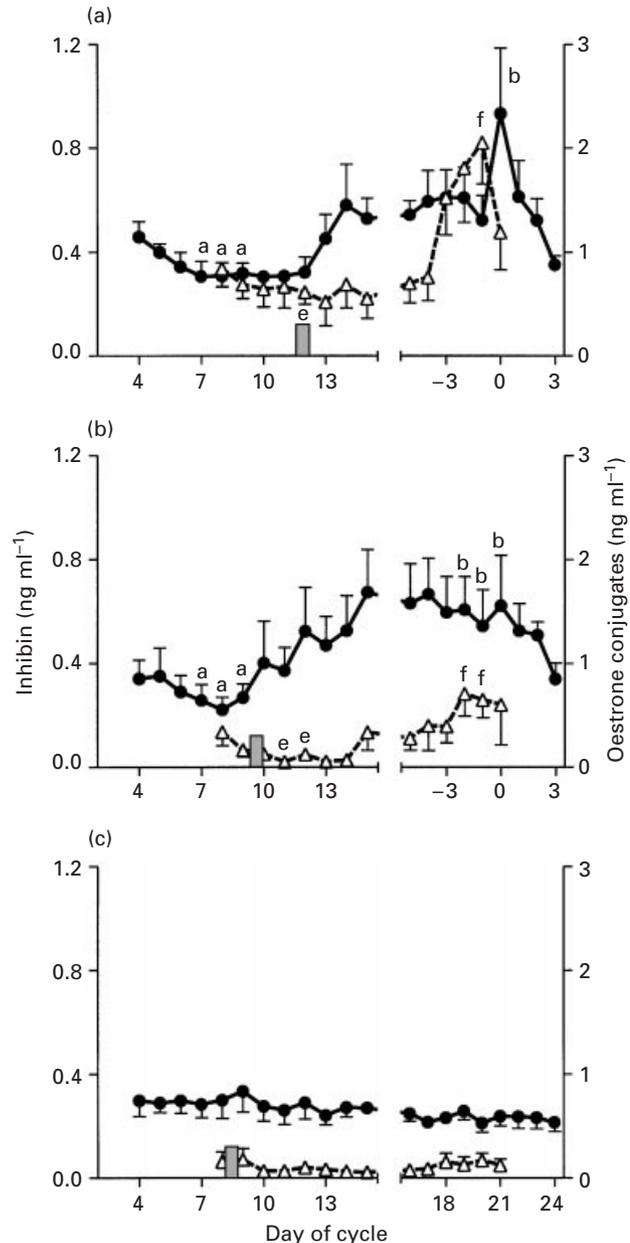


Fig. 2. Daily mean (\pm SEM) concentrations of oestrone conjugates (Δ) and inhibin (\bullet) during the (a) second to last ($n = 5$) or (b) last ($n = 4$) cycles of the season, or (c) the first 21 or 24 days of acyclicity ($n = 5$) in mares. Cycles have been aligned from the day of ovulation (day 0) to day 15, then from 5 days before to 3 days after the next ovulation. The grey vertical bars show the mean day of luteolysis (that is, progesterone concentration < 1 ng ml^{-1}). Within each group, data for oestrone conjugates or inhibin were analysed by non-parametric ANOVA for repeated measures and, when the F value was significant, by Dunn's comparison of selected days as noted on the figure. The days for comparison were chosen on the basis of when high and low concentrations of each hormone are observed during mid-season cycles. Daily means that differed at the 0.05 level are marked with different letters. Days compared: inhibin, days 7, 8 and 9 versus days -2, -1 and 0 (a,b); oestrone conjugates, days 9, 10, 11 and 12 versus days -2, -1 and 0 (e,f).

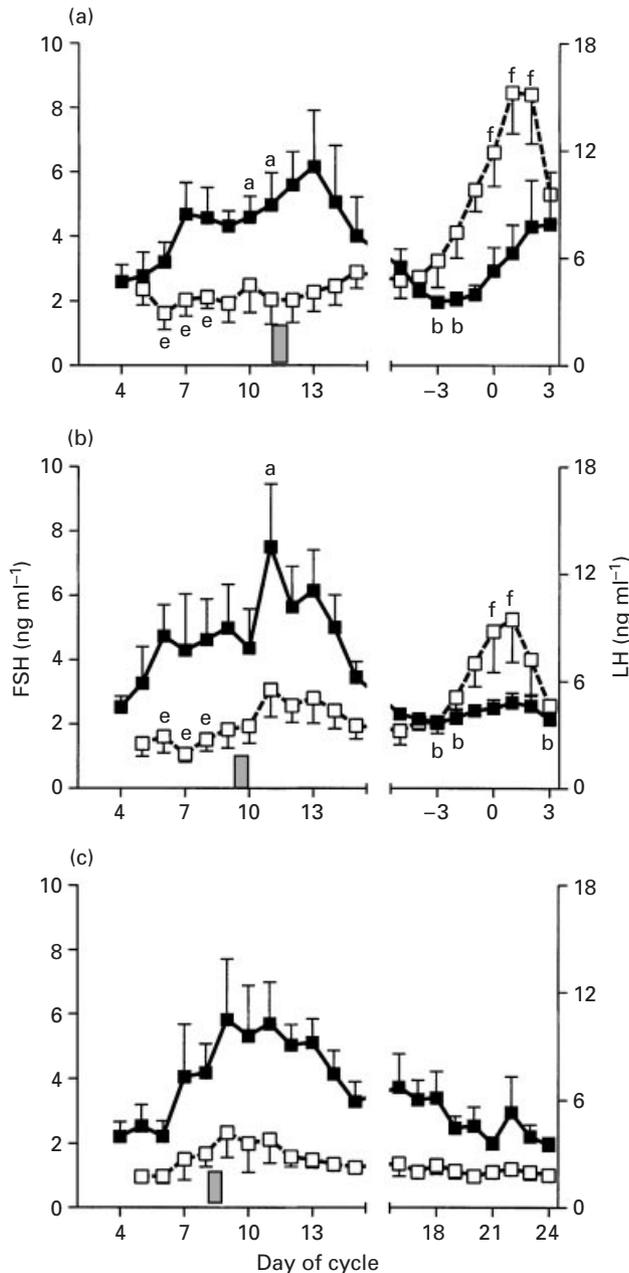


Fig. 3. Daily mean (\pm SEM) concentrations of FSH (■) and LH (□) during the (a) second to last ($n = 5$) or (b) last ($n = 4$) cycles of the season, or (c) the first 24 days of acyclicity ($n = 5$) in mares. The grey vertical bars show the mean day of luteolysis. Within each group, LH and FSH data were analysed as described in the legend to Fig. 2, and daily means that differ at the 0.05 level are marked with different letters. Days compared: FSH, days 4, 5, 9, 10 and 11 versus days -3, -2 and +3 (a,b); LH, days 6, 7 and 8 versus days 0, 1, 2 and 3 (e,f).

duration of increased LH concentrations was greater in second to last than in last cycles, extending the second day after ovulation in second to last cycles compared with the day after ovulation in last cycles (Fig. 3). Overall, the maximum daily LH mean was positively ranked with the maximum concentration of oestrone conjugates attained (Spearman's rank correlation $r = 0.75$; $P < 0.005$).

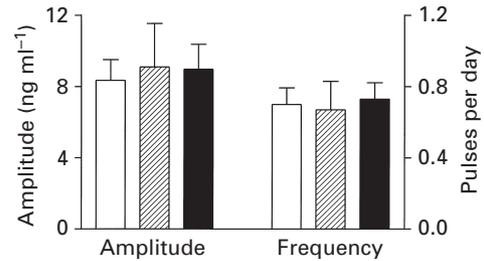


Fig. 4. Mean (\pm SEM) frequency and amplitude of FSH pulses detected by the Cluster program between days 5 and 12 in the second to last (□, $n = 6$) or last (▨, $n = 5$) cycles of the season, or in acyclicity (■, $n = 6$) in mares. Values did not differ in successive cycles.

At 2 days after luteolysis, LH concentrations had increased by $\geq 50\%$ in four of five second to last, three of five last cycles and in three of six acyclic periods. However, in all cases, except the last cycle of mare 7, there was subsequently a delay, sometimes lasting more than 1 week, before further LH increments indicated the onset of the LH surge in ovulatory cycles (Figs 3 and 5). When luteolysis occurred on day 9 of the last cycle of mare 7, a follicle 40 mm in diameter was present, which ovulated in response to the post-luteolytic LH increase, producing a 13 day interovulatory interval. In contrast, the diameters of the largest follicle at luteolysis in the second to last and last cycles and in acyclicity were 21 ± 2 , 14 ± 3 and 17 ± 2 mm, respectively. In ovulatory cycles, there was a tendency for the duration of the follicular phase to be inversely related to the diameter of the largest follicle at luteolysis (Spearman's rank correlation $r = -0.62$).

Prolactin. Prolactin concentrations in most samples were beneath the detection limit of the assay. Sporadic pulses (that is peak $> 3 \times$ the assay detection limit) occurred in three of five second to last cycles, one of five last cycles (including mare 7) and in two of five acyclic mares (Fig. 5a). These distributions were not different. Prolactin spikes were clustered just before or soon after luteolysis; the first measurable value was observed 0.4 ± 0.6 days after progesterone had decreased to ≤ 1 ng ml $^{-1}$. This was equivalent to day 10 ± 0.8 of the cycle.

Discussion

The profiles of daily mean FSH concentrations as defined by sampling at 6 h intervals in the second to last and last cycles and in early acyclicity were qualitatively but not quantitatively similar. In general, profiles consisted of a single period of increased concentration in mid-cycle followed by a trough at ovulation or when ovulation was expected to occur. However, the periovulatory nadir was prolonged in the last compared with the second to last cycles, and the difference between peak and trough values was reduced in anovulatory compared with ovulatory cycles. In mid-summer, two 'surges' of FSH occur in each cycle: one in late oestrus or early dioestrus and the other in mid-dioestrus

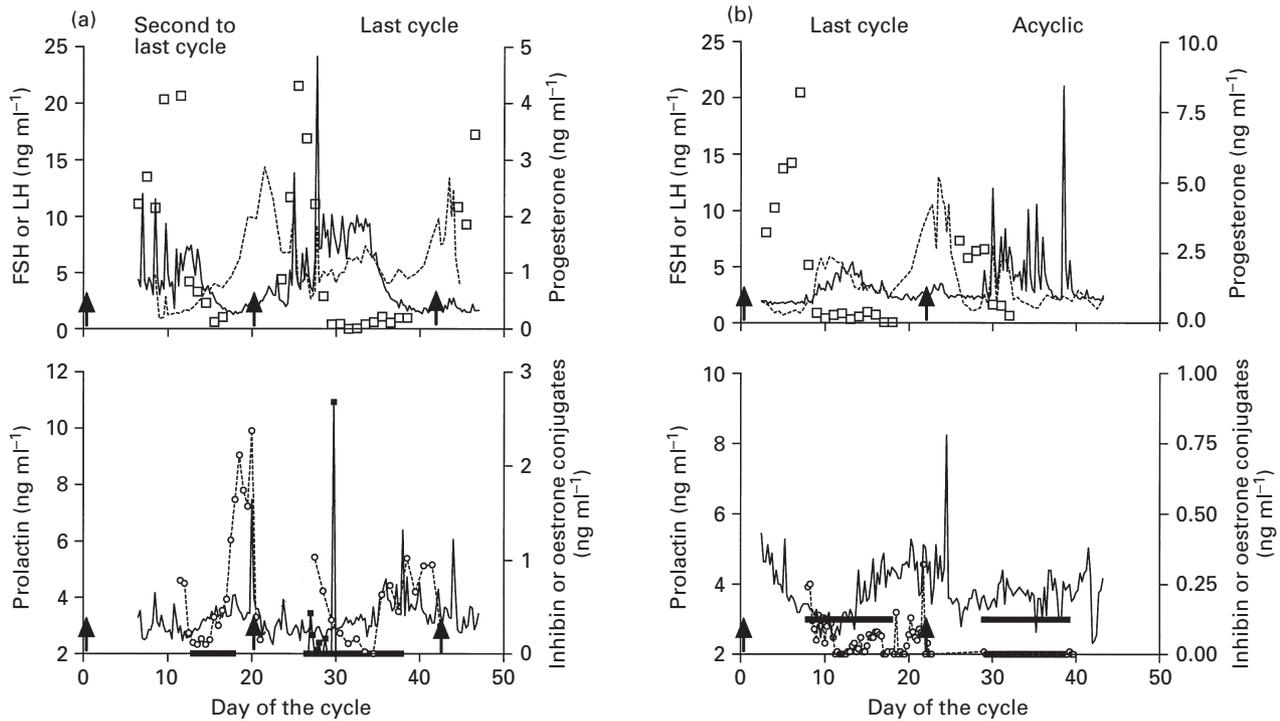


Fig. 5. Hormone profiles during the transition out of the breeding season in (a) mare 5 and (b) mare 8. Top panels: FSH (—), LH (---), progesterone (□); bottom panels: inhibin (—), oestrone conjugates (---○---), prolactin (■). Prolactin and oestrone conjugates were not assayed on every day. Most prolactin concentrations were at the detection limit of the assay (that is 2–3 ng ml⁻¹) and therefore the data points appear to form a solid horizontal bar at this value. The days are numbered from the ovulation preceding the start of sampling, with the days of ovulation marked by arrows. The position of each cycle during the transition is shown at the top of each graph.

(Evans and Irvine, 1975; Nett *et al.*, 1979; Irvine *et al.*, 1998). The small postovulatory increase in FSH observed only in the second to last cycles of mares in the present study might therefore represent the last trace of the early dioestrous surge. The apparent change in the FSH profile during the autumn transition from two surges to one surge per cycle is similar to that observed in ponies when blood was sampled once every 3 days (Turner *et al.*, 1979). The present study was not expected to replicate the finding of Turner *et al.* (1979) since, in mid-season cycles, even sampling once a day is inadequate to define dioestrous FSH secretion patterns, which consist of high amplitude, infrequent pulses (Irvine *et al.*, 1998).

The LH profile also showed progressive changes as mares entered acyclicity; the surge terminated sooner in the last than in the second to last cycles and failed to occur at the expected time in acyclicity. Similarly, other studies have reported that the first failure to ovulate in the autumn is associated with the absence of an LH surge (Turner *et al.*, 1979; Nequin *et al.*, 1998) and that the last LH surge is smaller than the second to last in most mares studied (Nequin *et al.*, 1998). In the last cycles of mares in the present study, the LH surge was curtailed at the same time as FSH concentrations were suppressed compared with the second to last or summer cycles. The most likely explanation for these congruent changes in gonadotrophin profile is reduced frequency of pulsatile release, since, in horses, FSH and LH are almost always secreted together (see for example Irvine

and Alexander, 1993, 1994, 1997), although the ratio of FSH:LH within pulses can vary markedly (Turner and Irvine, 1991). In the present study, from day 5 (when pulses could be detected reliably by sampling at intervals of 6 h) to day 8, the average interval between FSH pulses in autumn cycles was approximately 2 days (that is, 0.5 pulses per day). Although this interpulse interval is similar to that observed in deeply anoestrous mares (Alexander and Irvine, 1991), it is in marked contrast to summer cycles in which the FSH and LH pulse frequency as determined by sampling at intervals of 4 h ranges from 1.5 ± 0.2 per day in early dioestrus to 2.7 ± 0.2 per day on day 12 (Irvine *et al.*, 1998). It is unlikely that this difference in pulse frequency between summer and autumn cycles is due to the sampling regimen (that is, sampling at 4 h versus 6 h intervals). Although the reduced sampling frequency could lead to some pulses being missed, it would certainly have been adequate to detect daily pulses, since the hormone has a half-life of 5 h (Irvine, 1979) and dioestrous FSH pulses are usually large (Irvine *et al.*, 1998). In summer cycles, dioestrous FSH surges can be resolved into periods of high amplitude FSH pulses (Irvine *et al.*, 1998). It is probable that the absence of the early dioestrous FSH surge in autumn cycles is due to the extremely slow pulse frequency at this time.

In the present study, the changes in gonadotrophin profile were accompanied by steadily diminishing ovarian function, that is, the periovulatory oestrogen surge decreased in magnitude from the second to last to the last cycles and did

not occur at all in acyclicity, and the duration of the luteal phase was progressively decreased. Even in the second to last cycles, luteolysis occurred earlier (day 11.4 ± 0.8) than in summer cycles (day 15; Shand *et al.*, 1998) and earlier than is considered to be typical of horse mares (days 14–15; reviewed by Ginther, 1992; Daels and Hughes, 1993). Although the ability of follicles to secrete oestrogen decreases during the autumn transition (Weedman *et al.*, 1993), it is controversial whether corpus luteum activity changes with season. Early studies detected no seasonal fluctuation in the duration of the luteal phase or in peak progesterone concentrations (reviewed by Daels and Hughes, 1993). More recently, average dioestrous plasma progesterone concentrations in light horse mares have been reported to decrease steadily from the summer solstice (King *et al.*, 1988; King *et al.*, 1993; Nequin *et al.*, 1998). The reduced progesterone average was produced either by lower peak concentrations (King *et al.*, 1993) or decreased duration of the luteal phases (Weedman *et al.*, 1993), as in the present study. There is no ready explanation for the discrepancy among studies, other than the small sample sizes used and the well known variability among mares in reproductive endocrinology.

Could the changes in ovarian function observed in the present study be produced by reduced gonadotrophin secretion in early dioestrus? In ruminants, an inadequate corpus luteum can arise after ovulation of a follicle that has not attained its full complement of LH receptors or the ability to synthesize oestradiol maximally (Hunter, 1991). This could reflect inadequate FSH priming since in many species including horses FSH induces LH receptors on, and aromatase activity in, granulosa cells (reviewed by Alexander and Irvine, 1993). It has been proposed that exposure to two periods of increased FSH concentrations is needed to mature follicles in mares (Evans and Irvine, 1975), and it may be that the absence of the early dioestrous surge in autumn cycles is a critical event leading to sub-optimal follicular development. The reduction of the LH surge in autumn cycles may also affect corpus luteum function. High concentrations of LH after ovulation and the sporadically occurring LH pulses in dioestrus are also important for luteal development in mares (Ginther, 1992). This has been determined by passively immunizing mares on days 1–10 of the cycle against a gonadotrophin-rich extract of equine pituitary and observing smaller or regressing corpora lutea (Pineda *et al.*, 1972, 1973). Moreover, when seasonally acyclic mares are induced to ovulate by GnRH administration, a corpus luteum forms only when high LH concentrations are maintained for several days after ovulation (Evans and Irvine, 1977).

In most of the mares in the present study, both ovulatory and anovulatory, LH (and FSH) increased after luteolysis, presumably due to the removal of progesterone negative feedback and the consequent increase in the frequency of the GnRH and gonadotrophin pulses (Silvia *et al.*, 1995; Irvine and Alexander, 1997). However, this LH increase did not progress immediately into an LH surge as in the summer cycles (see for example Irvine *et al.* 1998), but remained at a moderate plateau, sometimes for more than 1 week in ovulatory cycles, or declined quickly to baseline values in

anovulatory cycles. This is a novel observation, which may be explained by the fact that luteolysis occurred early in autumn cycles. When the corpus luteum is lysed by prostaglandin administration on day 8 of summer cycles, an LH pattern similar to that in ovulatory autumn cycles follows (Nett *et al.*, 1979). In the present study, the removal of progesterone often preceded the mid-dioestrous FSH surge. This finding, together with the absence of an early dioestrous FSH surge, means that follicles had received little (second to last and last cycles) or virtually no (acyclicity) FSH priming at luteolysis and almost certainly could not respond immediately to increased LH with either growth or oestradiol synthesis. Oestradiol is a key factor in generating a full LH surge in mares. It increases LH synthesis (Sharp *et al.*, 1991; Robinson *et al.*, 1995), induces GnRH receptors on gonadotrophs in other species (Clayton, 1989) and probably in mares (Silvia *et al.*, 1986) and may augment GnRH secretion (Sharp and Davis, 1993). Administration of oestradiol to ovariectomized mares increases LH concentrations (Aurich *et al.*, 1995), whereas ovariectomy on day 14 does not increase LH to the concentrations of the ovulatory surge (Ginther, 1992), indicating that an ovarian hormone, presumably oestradiol, enhances LH secretion during the surge. In the mares in the present study, the magnitudes of the oestrogen and LH surges were correlated. These observations indicate that the LH surge cannot commence until the dominant follicle secretes sufficient oestradiol and cannot occur when oestrogen positive feedback is inadequate.

In the present study, plasma inhibin concentrations did not increase after luteolysis in the first anovulatory 'cycle'. This is a novel observation. How inhibin secretion is regulated in mares is not known. Because inhibin suppresses release of FSH in many species (Taya *et al.*, 1996), it might be expected that FSH stimulates inhibin production, thus forming a regulatory negative feedback loop. There is some evidence for such a feedback loop, particularly in women (Muttukrishna *et al.*, 1997; Welt *et al.*, 1997). However, in sheep, inhibin secretion does not appear to be controlled acutely by either FSH or LH, and plasma concentrations may simply reflect the number and size of follicles (Baird *et al.*, 1991). This may also be the case in horses, since in the present study inhibin patterns paralleled follicular growth and were not related to previous FSH exposure.

Prolactin may also play a role in seasonal breeding: concentrations are high in the summer and low in the winter (Johnson, 1986; Worthy *et al.*, 1987; Evans *et al.*, 1991) and treating mares with prolactin (Nequin *et al.*, 1993) or drugs that stimulate prolactin secretion, for example sulpiride (Besognet *et al.*, 1997), can hasten the first ovulation in spring. In summer cycles, sharp pulses in plasma prolactin concentrations occur shortly after luteolysis and are closely followed by an increase in oestrone concentrations (Shand *et al.*, 1998). The proximity of these events implies that prolactin is involved in follicular maturation. However, prolactin pulses were observed at luteolysis not only in autumn cycles, which is consistent with other studies (Worthy *et al.*, 1987), but also in early acyclicity. This finding indicates that prolactin pulses *per se* are insufficient to mature follicles. Nevertheless, if prolactin does affect ovarian function, it is

probable that it acts in concert with other hormones, for example LH, FSH and oestrogens. During autumn, the time relationships among changes in these hormones, follicular growth and prolactin peaks appear to become disturbed and this may perturb the action of prolactin. This possibility deserves further investigation.

In summary, sampling of jugular blood at intervals of 6 h showed that progressive changes in FSH and LH profiles accompanied decreasing ovarian function during the autumn transition in mares. These results contradict recent work (reviewed in the Introduction) which found that diminished ovarian function preceded altered LH or FSH, implying that other factors must be important in regulating the ovaries in the autumn. Although the possibility that this discrepancy is due to breed or individual variation in the mares studied cannot be excluded, it seems likely that it could arise from differences in sampling frequency; the more intensive regimen used in the present study allowed more precise definition of the FSH profile as shown for summer cycles (Irvine *et al.*, 1998). Understanding how the ovaries are controlled is central to the science of equine reproduction. The results indicate that the gonadotrophins continue to regulate ovarian function in the autumn as at other times of the year and, specifically, that inadequate gonadotrophin stimulation in early dioestrus may be a critical event leading to sub-optimal follicular and luteal development, and eventually acyclicity.

The authors thank Monika Baffy and Nigel Perkins for assisting with field work, and Julie Turner and Natalie Shand for performing hormone assays. Inhibin measurements were made under the direction of D. M. de Kretser, Monash University, Clayton, Australia. We are grateful to A. F. Parlow, H. Papkoff and J. F. Roser for generously donating assay reagents. This study was supported in part by the New Zealand Equine Research Foundation.

References

- Alexander SL and Irvine CHG (1991) Control of onset of breeding season in the mare and its artificial regulation by progesterone treatment *Journal of Reproduction and Fertility Supplement* **44** 307–318
- Alexander SL and Irvine CHG (1993) FSH and LH. In *Equine Reproduction* pp 45–56 Eds AO McKinnon and JL Voss. Lea and Febiger, Philadelphia
- Aurich C, Daels PF, Ball BA and Aurich JE (1995) Effects of gonadal steroids on the opioid regulation of LH and prolactin release in ovariectomized pony mares *Journal of Endocrinology* **147** 195–202
- Baird DT, Campbell BK, Mann GE and McNeilly AS (1991) Inhibin and oestradiol in the control of FSH secretion in the sheep *Journal of Reproduction and Fertility Supplement* **43** 125–138
- Besognet B, Hansen BS and Daels PF (1997) Induction of reproductive function in anestrus mares using a dopamine antagonist *Theriogenology* **47** 467–480
- Clayton RN (1989) Gonadotrophin-releasing hormone: its actions and receptors *Journal of Endocrinology* **120** 11–19
- Daels PF and Hughes JP (1993) The normal estrous cycle. In *Equine Reproduction* pp 121–132 Eds AO McKinnon and JL Voss. Lea and Febiger, Philadelphia
- Evans MJ and Irvine CHG (1975) Serum concentrations of FSH, LH and progesterone during the oestrous cycle and early pregnancy in the mare *Journal of Reproduction and Fertility Supplement* **23** 193–200
- Evans MJ and Irvine CHG (1977) Induction of follicular development, maturation and ovulation by gonadotropin releasing hormone administration to acyclic mares *Biology of Reproduction* **16** 452–462
- Evans MJ, Alexander SL, Irvine CHG, Livesey JH and Donald RA (1991) *In vitro* and *in vivo* studies of equine prolactin secretion throughout the year *Journal of Reproduction and Fertility Supplement* **44** 27–35
- Fitzgerald BP, Anson H, Loy RG and Legan SJ (1983) Evidence that changes in LH pulse frequency may regulate the seasonal modulation of LH secretion in ovariectomized mares *Journal of Reproduction and Fertility* **69** 685–692
- Fitzgerald BP, Affleck KJ, Barrows SP, Murdoch WL, Barker KB and Loy RG (1987) Changes in LH pulse frequency and amplitude in intact mares during the transition into the breeding season *Journal of Reproduction and Fertility* **79** 485–493
- Freedman LJ, Garcia MC and Ginther OJ (1979) Influence of photoperiod and ovaries on seasonal reproductive activity in mares *Biology of Reproduction* **20** 567–574
- Ginther OJ (1992) *Reproductive Biology of the Mare: Basic and Applied Aspects* 2nd Edn pp 135–172 Equiservices, Cross Plains, WI
- Hunter MG (1991) Characteristics and causes of the inadequate corpus luteum *Journal of Reproduction and Fertility Supplement* **43** 91–99
- Irvine CHG (1979) Kinetics of gonadotrophins in the mare *Journal of Reproduction and Fertility Supplement* **27** 131–141
- Irvine CHG (1995) The nonpregnant mare: a review of some current research and of the last 25 years of endocrinology *Biology of Reproduction Monograph* **1** 343–360
- Irvine CHG and Alexander SL (1993) Secretory patterns and rates of gonadotrophin-releasing hormone, follicle-stimulating hormone, and luteinizing hormone revealed by intensive sampling of pituitary venous blood in the luteal phase mare *Endocrinology* **132** 212–218
- Irvine CHG and Alexander SL (1994) The dynamics of gonadotrophin-releasing hormone, LH and FSH secretion during the spontaneous ovulatory surge of the mare as revealed by intensive sampling of pituitary venous blood *Journal of Endocrinology* **140** 283–295
- Irvine CHG and Alexander SL (1997) Patterns of secretion of GnRH, LH and FSH during the postovulatory period in mares: mechanisms prolonging the LH surge *Journal of Reproduction and Fertility* **109** 263–271
- Irvine CHG, Sutton P, Turner JE and Mennick PE (1990) Changes in plasma progesterone concentrations from days 17 to 42 of gestation in mares maintaining or losing pregnancy *Equine Veterinary Journal* **22** 104–106
- Irvine CHG, Turner JE, Alexander SL, Shand N and van Noordt S (1998) Gonadotrophin profiles and dioestrous pulsatile release patterns in mares as determined by collection of jugular blood at 4 h intervals throughout an oestrous cycle *Journal of Reproduction and Fertility* **113** 315–322
- Johnson AL (1986) Serum concentrations of prolactin, thyroxine and triiodothyronine relative to season and the estrous cycle in the mare *Journal of Animal Science* **62** 1012–1020
- King SS, Nequin LG, Drake S, Hebner TS, Roser JF and Evans JW (1988) Progesterone levels correlate with impending anestrus in the mare *Journal of Equine Veterinary Science* **8** 108–111
- King SS, Neumann KR, Nequin LG and Weedman BJ (1993) Time of onset and ovarian state prior to entry into winter anestrus *Journal of Equine Veterinary Science* **13** 512–515
- Muttukrishna S, Groome N and Ledger W (1997) Gonadotropic control of secretion of dimeric inhibins and activin A by human granulosa-luteal cells *in vitro*. *Journal of Assisted Reproduction and Genetics* **14** 566–574
- Nequin LG, King SS, Johnson AL, Gow GM and Ferreira-Dias GM (1993) Prolactin may play a role in stimulating the equine ovary during the spring transition *Journal of Equine Veterinary Science* **13** 631–635
- Nequin LG, King SS, Roser JF, Soderstrom BL, Carnevale EM and Renaas KG (1998) Uncoupling of the equine reproductive axes during transition into anestrus *Proceedings of the 7th International Symposium on Equine Reproduction* Pretoria, South Africa p. 41
- Nett TM, Pickett BW and Squires EL (1979) Effects of equimate (ICI-81008) on levels of luteinizing hormone, follicle-stimulating hormone and progesterone during the estrous cycle of the mare *Journal of Animal Science* **48** 69–75
- Pineda MH, Ginther OJ and McShan WH (1972) Regression of corpus luteum in mares treated with an antiserum against an equine pituitary fraction *American Journal of Veterinary Research* **33** 1767–1773
- Pineda MH, Garcia MC and Ginther OJ (1973) Effect of antiserum against an equine pituitary fraction on corpus luteum and follicles in mares during diestrus *American Journal of Veterinary Research* **34** 181–183
- Robertson DM, Tsonis CG, McLachlan RI *et al.* (1988) Comparison of inhibin immunological and *in vitro* biological activities in human serum *Journal of Clinical Endocrinology and Metabolism* **67** 438–444
- Robinson G, Porter MB, Peltier MR, Cleaver BC, Farmerie TA, Wolfe MW, Nilson JH and Sharp DC (1995) Regulation of luteinizing hormone β and α

- messenger ribonucleic acid by estradiol or gonadotropin-releasing hormone following pituitary stalk section in ovariectomized pony mares *Biology of Reproduction Monograph* **1** 373–383
- Roser JE, McCue PM and Hoyer E** (1994) Inhibin activity in the mare and stallion *Domestic Animal Endocrinology* **11** 87–100
- Shand N, Alexander SL and Irvine CHG** (1991) Comparison of the microheterogeneity of horse LH and FSH in the pituitary with that secreted into pituitary venous blood at oestrus *Journal of Reproduction and Fertility Supplement* **44** 1–11
- Shand N, Irvine CHG, Turner JE and Alexander SL** (1998) A detailed study of hormonal profiles at the time of luteolysis *Proceedings of the 7th International Symposium on Equine Reproduction* Pretoria, South Africa p. 71
- Sharp DC and Davis SD** (1993) Vernal transition. In *Equine Reproduction* pp 133–143 Eds AO McKinnon and JL Voss. Lea and Febiger, Philadelphia
- Sharp DC, Grubbaugh WR, Weithenauer J, Davis SD and Wilcox CJ** (1991) Effects of steroid administration on pituitary luteinizing hormone and follicle-stimulating hormone in ovariectomized pony mares in the early spring: pituitary responsiveness to gonadotropin-releasing hormone and pituitary gonadotropin content *Biology of Reproduction* **44** 983–990
- Silvia PE, Squires EL and Nett TM** (1986) Changes in the hypothalamo-hypophyseal axis of mares associated with seasonal reproductive recrudescence *Biology of Reproduction* **35** 897–905
- Silvia PJ, Meyer SL and Fitzgerald BP** (1995) Pulsatile gonadotropin secretion determined by frequent sampling from the intercavernous sinus of the mare: possible modulatory role of progesterone during luteolysis *Biology of Reproduction* **53** 438–446
- Snyder DA, Turner DD, Miller KE, Garcia MC and Ginther OJ** (1979) Follicular and gonadotrophic changes during transition from ovulatory to anovulatory seasons *Journal of Reproduction and Fertility Supplement* **27** 95–101
- Stabenfeldt GH, Daels PE, Munro CJ, Kindahl H, Hughes JP and Lasley B** (1991) An oestrogen conjugate immunoassay for monitoring pregnancy in the mare: limitations of the assay between days 40 and 70 of gestation *Journal of Reproduction and Fertility Supplement* **44** 37–44
- Taya K, Kaneko H, Takedomi T, Kishi H and Watanabe G** (1996) Role of inhibin in the regulation of FSH secretion and folliculogenesis in cows *Animal Reproduction Science* **42** 563–570
- Turner DD, Garcia MC and Ginther OJ** (1979) Follicular and gonadotrophic changes throughout the year in pony mares *American Journal of Veterinary Research* **40** 1694–1700
- Turner JE and Irvine CHG** (1991) The effect of various gonadotrophin-releasing hormone regimens on gonadotrophins, follicular growth and ovulation in deeply anoestrous mares *Journal of Reproduction and Fertility Supplement* **44** 213–225
- Veldhuis JD and Johnson ML** (1986) Cluster analysis: a simple, versatile and robust algorithm for endocrine pulse detection *American Journal of Physiology* **250** E486–E493
- Weedman BJ, King SS, Neumann BJ and Nequin LG** (1993) Comparison of relationships between estradiol and folliculogenesis during the autumn transition and breeding season in the mare *Journal of Equine Veterinary Science* **13** 502–505
- Welt CK, Martin KA, Taylor AE, Lambertmessenger GM, Crowley WE, Smith JA, Schoenfeld DA and Hall JE** (1997) Frequency modulation of follicle-stimulating hormone (FSH) during the luteal-follicular transition – evidence for FSH control of inhibin B in normal women *Journal of Clinical Endocrinology and Metabolism* **82** 2645–2652
- Worthy K, Colquhoun K, Escreet R, Dunlop M, Renton JP and Douglas TA** (1987) Plasma prolactin concentrations in non-pregnant mares at different times of the year and in relation to events in the cycle *Journal of Reproduction and Fertility Supplement* **35** 269–276