Gonadotrophin profiles and dioestrous pulsatile release patterns in mares as determined by collection of jugular blood at 4 h intervals throughout an oestrous cycle

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In mares, dioestrous FSH profiles based on once-a-day sampling are variable; however, the pulsatility of plasma FSH, which has been suggested by limited windows of intensive sampling, may contribute to this variability. Jugular blood from six mares was sampled at 4 h intervals throughout an ovulatory cycle to determine cyclic FSH and LH patterns more accurately and to measure gonadotrophin pulse frequency during dioestrus. Synchronous pulses of FSH and LH occurred regularly in all mares between day 4 and day 12 (ovulation = day 0) with a mean ($\pm$ SEM) frequency of 1.9 ± 0.1 (FSH) or 1.6 ± 0.1 (LH) pulses day⁻¹. LH pulse amplitude declined ($P < 0.0001$) between day 4 and day 10, but FSH pulse amplitude remained large and stable, dipping slightly but not significantly on day 6. Daily mean FSH concentrations exceeded ($P < 0.0001$) early oestrous values between day 4 and day 5, and between day 7 and day 10. However, significantly different patterns were obtained when once-a-day sampling was simulated by selecting samples collected at 08:00 h or noon. LH was higher during the periovulatory surge than during dioestrus ($P < 0.0001$) and profiles were similar whether daily means or selected samples were used. It is concluded that: (1) the marked pulsatility of plasma FSH during dioestrus makes once-a-day sampling misleading for determining FSH profiles; (2) the dioestrous pattern of large, slow FSH pulses was consistent among mares, unlike that of the daily mean FSH profiles; and (3) no discrete FSH 'surges' were observed during dioestrus, although FSH pulse amplitude tended to undergo alternate increases and decreases. A period of higher amplitude FSH pulses preceded ovulation by 10.2 ± 0.7 days, which corresponds to the approximate time the ovulatory follicle emerges. Therefore, it is possible that the signal for follicular recruitment in mares is intermittent excursions of plasma FSH above a threshold value.

Introduction

The FSH profile during the ovulatory cycle of the mare remains controversial, despite much research (Ginther, 1992). In the original observations from this laboratory in 1975, two FSH surges, 10 days apart, per 21 day ovulatory cycle were reported. One surge occurred in mid-dioestrus and was followed by a nadir at the onset of oestrus; the second surge accompanied the peak of the ovulatory LH surge in late oestrus–early dioestrus and was followed by another decline before the main dioestrous surge (Evans and Irvine, 1975). These FSH surges were proposed to drive two waves of folliculogenesis, with the mid-dioestrous surge priming the follicle destined to ovulate 10 days later (Evans and Irvine, 1975). Although some workers have replicated our finding of two FSH surges per cycle (Burns and Douglas, 1981; Nett *et al.*, 1979), others have observed different patterns, for example,

one or even three surges per cycle. None the less, all these varied FSH patterns were able to generate normal cyclicity (for review, see Ginther, 1992; Bergfelt and Ginther, 1993) and all studies agree that FSH is suppressed in early oestrus.

The variability in reported dioestrous FSH patterns has been attributed to various factors, including season, age, breed and individual (for review, see Ginther, 1992). The variability may also be artifactual, since windows of intensive jugular blood collection on selected days in dioestrus show that both FSH and LH concentrations fluctuate in low frequency, high amplitude pulses (Thompson *et al.*, 1987; Alexander and Irvine, 1993; Irvine and Alexander, 1993). The FSH profiles reported in mares throughout dioestrus were obtained either from once-a-day or thrice-a-week sampling (for example, Evans and Irvine, 1975; Nett *et al.*, 1979; Miller *et al.*, 1980; Bergfelt and Ginther, 1993). Such bleeding regimens could result in inconsistent plasma FSH patterns, depending on whether or not individual samples were taken at a peak or trough. However, once-a-day sampling has yielded consistent profiles in LH throughout the

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cycle of the mare (for review, see Ginther, 1992) which suggests that bleeding regimen may not be the sole reason for variable FSH patterns, since LH and FSH pulses occur synchronously in mares (Irvine and Alexander, 1993; Alexander and Irvine, 1993).

In the present study, jugular blood was sampled from six mares at 4 h intervals, day and night, throughout an ovulatory cycle. The aim was to determine cyclic FSH and LH patterns more accurately than with once-a-day sampling. A 4 h sampling regimen would also make it possible to measure the slow gonadotrophin pulse frequency during dioestrus; that is, the time that the next ovulatory follicle emerges (Bergfelt and Ginther, 1993). No previous study has attempted to define gonadotrophin pulse frequency on every day of dioestrus. The data generated by this study could then be used to assess whether pulsatile release patterns are similar among mares, even if daily mean concentrations are not.

A further objective of the present study was to distinguish between two possible explanations for the wide range of FSH patterns reported in normally cyclic mares by defining the FSH profile more precisely. The two possible explanations are: (1) the dioestrous pattern of FSH secretion is consistent among mares but the methods used to characterize it have been inadequate, or (2) the exact FSH profile is unimportant for folliculogenesis as long as dioestrous concentrations are increased adequately. Elucidating the method by which mares stimulate folliculogenesis is important for diagnosing and treating reproductive problems and may also lead to improved methods for manipulating reproduction in mares, for example, inducing folliculogenesis and ovulation out of season or superovulating mares for embryo transfer.

Materials and Methods

Animals

Six regularly cycling standardbred (*Equus caballus*) mares (E, G, N, P, S and Y), 5–18 years of age and weighing 450–550 kg were used. The mares were kept on pasture at the Lincoln University Equine Research Unit, across an electric fence from a stallion. The experiment commenced in the middle of the breeding season (late November–mid December, Southern Hemisphere) when mares were given 5 mg i.m. of the prostaglandin $F_{2\alpha}$ analogue, dinoprost (Lutalyse; Upjohn, Auckland) to achieve a degree of oestrus synchronization. Each mare was fitted with a jugular cannula (Angiocath 16 g, 5.25 in, Deseret Co., Sandy, UT) on day 1 or day 2 after ovulation. The study was approved by the Lincoln University Animal Ethics Committee.

Experimental protocol

Behaviour and ovarian changes. The mares were teased and their ovaries were palpated *per rectum* approximately every second day. This study was not intended to determine follicular kinetics precisely but rather to detect the time of ovulation and to assess changes in the diameter and tension of the dominant follicle (Ginther, 1992).

Blood sampling. Blood samples (5 ml) were collected into tubes containing 10 mg EDTA at 4 h intervals, day and night, from day 1 or 2 of one cycle (ovulation = day 0) until noon of day 2 of the following cycle. The choice of sampling interval was a compromise between the depth of detail in the information sought and the practicability of obtaining it. A sample interval of 4 h was chosen on the basis of the long plasma half-life of equine gonadotrophins (5 h; Irvine, 1979) and the paucity of gonadotrophin pulses observed during limited periods of intensive sampling during dioestrus. Sampling windows of 24–48 h have been used previously; however, these have not been adequate to define pulse frequency in all mares studied. For example, Thompson *et al.* (1987) found three, one, zero and zero gonadotrophin pulses in four mares each bled every 15 min for 24 h. Alexander and Irvine (1993) observed four and five pulses in jugular blood from two mares in late dioestrus each bled every 30 min for 48 h. Sampling pituitary venous blood every 5 min for a total of 80.25 h, eight pulses were detected in four mid-dioestrous mares (Irvine and Alexander, 1993). These rather fragmentary data suggest that dioestrous pulse frequency is $\leq 3 \text{ day}^{-1}$, which could be measured by sampling at 4 h intervals. This sampling regimen would not be adequate to define the high frequency, low amplitude gonadotrophin pulses that occur in peripheral blood at oestrus since these are not reliably detected even with 5 min sampling (Fitzgerald *et al.*, 1985; Alexander and Irvine, 1987).

The mares were kept in their usual paddock throughout the experiment 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. Blood was refrigerated after collection and, each morning, samples were centrifuged at 1400 g for 5 min at 4°C. The plasma was harvested and stored at -20°C until assayed.

Hormone assays

Gonadotrophins were measured in duplicate using established radioimmunoassays (FSH: Irvine and Alexander, 1993; LH: Shand *et al.*, 1991). The FSH radioimmunoassay was based on an antiserum against human FSH (AFP1391675; gift from A. F. Parlow, UCLA, Torrance, CA) and used equine FSH (E276B; gift from H. Papkoff, UC, San Francisco, CA) as standard and radioligand. The LH radioimmunoassay was based on a monoclonal antibody against the β -subunit of bovine LH (gift from J. F. Roser, UC Davis, CA) and used equine LH (E263B; gift from H. Papkoff) as standard and radioligand. The detection limits of the assays were 0.04 ng FSH or 0.24 ng LH per tube. All samples from a given mare were assayed together. Mean intra-assay coefficients of variation (CV) were 5.9% (FSH) or 6.1% (LH) in the concentration range in which most samples were diluted to fall. When potency estimates fell outside this optimal range, assays were repeated at a more favourable dilution. Mean interassay CVs were 10.7% (FSH) or 9.8% (LH) within the optimal range.

Pulse detection

Pulses in LH or FSH were detected by the Cluster programme (Veldhuis and Johnson, 1986). Programme parameters

were set to yield a false positive rate of 2.5% as estimated from data supplied with the programme 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 in each hormone that were consistent with visual appraisal. Pulse amplitude was defined as the maximum hormone concentration attained in each Cluster-identified pulse. The Cluster programme, set as described above, was also used to detect significant increases in daily mean FSH concentrations in each mare.

Statistical analyses

Daily mean LH or FSH concentrations and the ratio between daily mean FSH and LH concentrations were calculated for each mare. Because the length of each mare's cycle differed, data were aligned for analysis in two ways: (1) from day 2 through to day 17, which in four of six mares was the second day of oestrus, and then (2) from the day before ovulation (day -1) through to the following day 2. The effect of day of cycle on hormone parameters was assessed by ANOVA for repeated measures, followed by the Newman-Keuls multiple comparison test at the 0.05 level when the F value was significant (GraphPad Prism, San Diego, CA). For each mare, the frequency and mean amplitude of FSH and LH pulses was determined between day 4 and day 12 (that is, when the sampling regimen was adequate to detect pulses). The effect of day of cycle on pulse frequency or amplitude was tested by ANOVA, as described above. The 08:00 h sample on each day was selected to simulate once-a-day sampling and analysed using ANOVA, as described above, to assess whether the sampling regimen affected the FSH or LH profiles. This process was repeated using the noon sample.

The relationship between daily mean concentrations of FSH and LH was tested by cross correlation analysis. The significance of r values was assessed as described by Iranmanesh *et al.* (1989). Data are given as means $\pm$ SEM.

Results

Ovarian changes

Interovulatory intervals in the six mares varied from 17 to 24 days (mean = 20 ± 1 days) and were in the range considered to be normal (Ginther, 1992). Development of the dominant follicle for each mare is shown (Fig. 1). The follicle diameter at ovulation ranged from 30 to 50 mm, and the tension ranged from soft to very soft.

Hormone changes

Pulse characteristics. Using sampling intervals of 4 h, clear-cut pulses in FSH and LH concentrations were detected in all mares from day 4 until 2 ± 0.6 (FSH) or 4 ± 0.6 (LH) days before the onset of oestrus (that is, approximately days 4–12) (Fig. 1). FSH pulses occurred at least once per day in all mares during this period. The mean pulse frequency was 1.9 ± 0.1 and

1.6 ± 0.1 pulses day⁻¹ for FSH and LH, respectively. The frequency of LH pulses did not change ($P = 0.1166$) during this period (data not shown), whereas FSH pulse frequency varied with day ($P = 0.022$), being slightly higher on day 12 (2.7 ± 0.2 pulses day⁻¹) than on day 4 or day 6 (both 1.5 ± 0.2 pulses day⁻¹). LH pulse amplitude declined ($P < 0.0001$) steadily throughout dioestrus, whereas FSH pulse amplitude was stable between day 4 and day 10, decreasing slightly but not significantly on day 6 (Fig. 1). Pulses in LH and FSH were coincident between day 4 and day 12, with 95% (82 of 86 observed) of LH pulses accompanied by an FSH pulse. However, only 81% (82 of 101 observed) of FSH pulses were concurrent with LH pulses. The extra 'solo' FSH pulses occurred most often after day 10, when LH pulse amplitude had often decreased below the cutoff point for pulse detection (Fig. 1). After day 12, FSH pulses appeared to broaden and merge in three of the six mares (E, N and Y; Fig. 1) before becoming undetectable as FSH decreased towards its oestrous nadir (Fig. 1).

There was some evidence for a circadian rhythm in the timing of gonadotrophin pulses ($P = 0.0083$), with pulses less likely to occur at 08:00 h than at any other sampling time except 20:00 h.

Cyclic FSH profiles: daily means. Using daily means, there was an effect of day of cycle on FSH concentrations ($P < 0.0001$) (Fig. 2a). Values were increased above those in oestrus between day 4 and day 5, and again between day 7 and day 10. Individual mares varied in the extent to which they matched the overall mean pattern; however, four of six mares did show a decrease in FSH pulse amplitude and, consequently, in mean FSH concentrations around day 6 (E, N, P and S; Fig. 1). A late dioestrous increase in daily mean FSH concentrations was seen in the three mares in which FSH pulses broadened at this time (E, N and Y; Fig. 1). Ovulation followed the maximum of a Cluster-detected increase in the daily mean FSH concentrations of each mare by 10.2 ± 0.7 days. In five mares, this FSH increase occurred in mid-dioestrus (day 9 ± 1) while, in mare E, it was in late dioestrus (day 15).

Effect of sampling regimen on dioestrous FSH profiles. When once-a-day sampling was simulated by selecting the 08:00 h sample, FSH concentrations varied with day ($P = 0.0043$), with the day 4 value higher than those in oestrus (Fig. 2b). When the 12:00 h sample was selected, FSH concentrations varied with day ($P = 0.0012$), with the values on day 7 and day 8 higher than those in oestrus (Fig. 2c). Combined ANOVA showed that the three dioestrous FSH profiles (that is, those derived from the daily means, the 08:00 h or the 12:00 h samples) were different ($P = 0.038$).

Cyclic LH profiles: daily means and effect of sampling regimen. When daily means were analysed, there was an effect of day on LH concentrations ($P < 0.0001$). Values were lower than the ovulatory surge maxima between day 6 and day 16. Concentrations first exceeded the dioestrous nadir on the day before ovulation (Fig. 2a). The profiles drawn from simulated once-a-day sampling did not differ from those drawn using daily means ($P = 0.3365$; Fig. 2a–c).

Fig. 1. Jugular venous FSH and LH concentrations (—) in six mares (a) mare E, (b) mare G, (c) mare N, (d) mare P, (e) mare S, (f) mare Y bled at 4 h intervals throughout an ovulatory cycle. □, maxima of pulses detected by the Cluster programme; ■, daily mean concentration of each hormone; ●, diameter of ovulatory follicle; ■, diameter of nonovulatory follicle. The day of ovulation is marked by arrows and the duration of behavioural oestrus is shown by the solid bar.

Fig. 2. Daily mean ($\pm$ SEM) LH ($\bullet$) and FSH ($\square$) concentrations in jugular blood in six mares calculated from samples collected (a) at 4 h intervals, night and day, (b) at 08:00 h, or (c) at 12:00 h. Cycles have been aligned from one ovulation (day 0 on the left of the graph) through to day 17, and again from the day before the next ovulation (day 0 on the right of the graph) through to day 2 of the following cycle. Means that differ ($P < 0.05$) from respective nadir values are marked by asterisks (LH) or plus signs (FSH).

Comparison of mean LH and FSH profiles. In five of six mares, daily mean FSH and LH concentrations were negatively correlated ($r = -0.54$ to -0.72), with changes in FSH preceding those in LH by 1–4 days. The exceptional mare (Y) had the shortest cycle length and her daily mean FSH and LH concentrations were positively correlated ($r = 0.8$, with LH leading FSH by 2 days). The ratio between daily mean FSH and LH concentrations varied with cycle stage ($P < 0.0001$) and was more than 15-fold higher between day 7 and day 15 than at oestrus (Fig. 3). The nadir in the FSH:LH concentration occurred on the day after ovulation (Fig. 3), which reflects the fact that, in all mares, the lowest value of FSH detected by measurements made at 4 h intervals occurred on the day after ovulation, 8 h before the peak of the LH surge (for example, see Fig. 1).

Discussion

Blood sampling at 4 h intervals throughout an ovulatory cycle showed that FSH and LH pulses occurred regularly in all mares between day 4 and day 12 (ovulation = day 0). During this period, mean pulse frequencies were 1.9 ± 0.1 or 1.6 ± 0.1 pulses day^{-1} for FSH and LH, respectively. These frequencies are consistent with estimates based on 24–48 h windows of more intensive (5–30 min) sampling of jugular blood from cyclic mares in dioestrus (Alexander and

Fig. 3. Mean ($\pm$ SEM) ratio between daily mean FSH and LH concentrations in six mares. Asterisks mark means that differ ($P < 0.05$) from nadir values.

Irvine, 1986, 1993; Thompson *et al.*, 1987; Irvine and Alexander 1993).

The similarity in pulse frequency obtained with samples taken at 4 h intervals and estimates from intensive sampling suggests that the 4 h regimen was adequate to detect dioestrous gonadotrophin pulses. In late dioestrus, FSH pulses in some mares appeared to broaden and merge. More intensive

sampling shows that gonadotrophin pulse frequency starts to increase as progesterone starts to decrease in late dioestrus (Alexander and Irvine, 1993) and it is likely that the individual pulses could no longer be distinguished by sampling at 4 h intervals. The pulsatility in peripheral FSH concentrations at the time of follicular recruitment in mares contrasts with observations in other species. For example, FSH is not clearly pulsatile at any stage of the cycle in ewes (Findlay and Clarke, 1987) or women (Filicori *et al.*, 1986), although deconvolution analysis shows that, in women, small secretory episodes do occur approximately once per hour in the follicular phase (Booth *et al.*, 1996).

When daily mean FSH concentrations were calculated from samples collected at 4 h intervals, values were low at oestrus and high during dioestrus, a trend that has been observed by previous workers (for review, see Ginther, 1992). During dioestrus, daily mean FSH concentrations were significantly increased above oestrous concentrations between day 4 and day 5 and, again, between day 7 and day 10; however, not all mares matched this mean profile. Furthermore, significantly different patterns could be obtained when once-a-day sampling was simulated by selecting samples collected at noon or 08:00 h. This variability in outcome reflects that of other reports based on once-a-day sampling (for review, see Ginther, 1992; Bergfelt and Ginther, 1993). Moreover, the finding that different FSH patterns could be produced using selected points from the same data set showed that the variability does not stem solely from factors such as season, age or breed, as has been suggested (Ginther, 1992). Rather, it appears that the markedly pulsatile secretion of FSH during most of dioestrus is a major source of variation in profiles derived from once-a-day sampling.

In contrast to the variability in daily mean FSH concentrations, the dioestrous pattern of slow, high amplitude FSH pulses was consistent from mare to mare. Pulsatility is a general operating characteristic of many endocrine systems and it has been proposed that target organs respond to changes in hormone concentrations and not to stable concentrations, whether high or low (Yates, 1981). For example, gonadotrophs of many species respond better when GnRH is delivered in pulses rather than as a continuous infusion (Knobil, 1980; McIntosh and McIntosh, 1985), which can reduce responsiveness to the point that gonadal activity ceases (Knobil, 1980). However, in mares, folliculogenesis and ovulation can be induced by constant delivery of GnRH (for review, see Irvine, 1995), although the limited data available on gonadotrophin secretion patterns during such treatment suggest that they remain pulsatile (Hyland *et al.* 1987). The optimum pattern of FSH stimulation for recruiting equine follicles remains to be determined experimentally; however, if it is assumed that hormonal patterns become fixed in a breed because they maximize reproductive efficiency, then infrequent, high amplitude FSH pulses may be the best means to convey the signal for folliculogenesis in mares. Equine follicles may respond better to repeated changes in FSH concentration and folliculogenesis may be controlled more precisely and efficiently by pulses rather than by other patterns of stimulation.

Unlike FSH, LH profiles were similar whether based on daily means or simulated once-a-day sampling. Similarly, previous reports have generally agreed on the pattern of LH concen-

trations during the cycle of mares (for review, see Ginther, 1992). One explanation for the stability in LH profiles compared with FSH profiles is that LH concentrations peak during the periovulatory surge when pulses occur so rapidly ($1\text{--}2\text{ h}^{-1}$ as measured in pituitary blood; Alexander and Irvine, 1987; Irvine and Alexander, 1994) that they merge in jugular blood and are not detected readily even with sampling every 5 min (Fitzgerald *et al.*, 1985). Therefore, once-a-day sampling is adequate to determine mean LH concentrations during the surge. During dioestrus, LH pulse amplitude, unlike that of FSH, declined steadily and mean LH concentrations reached their nadir. Thus, sampling at the peak of a dioestrous pulse would distort the cyclic profile less for LH than for FSH.

In the present study, the FSH and LH pulses revealed by sampling at 4 h intervals were synchronous, confirming earlier studies (Alexander and Irvine, 1987; Thomson *et al.*, 1987; Irvine and Alexander, 1993, 1994, 1997; Irvine and Alexander, 1994; Irvine and Alexander, 1997). However, FSH and LH pulse amplitude varied independently during dioestrus (present study) and oestrus (Irvine and Alexander, 1994). This disparity between FSH and LH pulse amplitude was reflected in the significant negative cross-correlation between daily mean FSH and LH concentrations and in the 15-fold increase in the FSH:LH ratio from oestrus to dioestrus. The apparent contradiction of concordant timing but discordant changes in amplitude of LH and FSH pulses arises because, although GnRH initiates LH and FSH secretory pulses, pituitary responsiveness to GnRH determines pulse amplitude (Alexander and Irvine, 1993; Irvine and Alexander, 1994). Responsiveness is regulated differentially for FSH and LH and depends both on the pattern of GnRH stimulation and on ovarian hormone feedback. Rapid GnRH pulses favour LH synthesis and secretion, while slow GnRH pulses favour FSH synthesis and secretion (Knobil, 1980; Turner and Irvine, 1991; Haisenleder *et al.*, 1991; Albanese *et al.*, 1996). Gonadal feedback also modulates pituitary responsiveness differentially, with inhibin preferentially decreasing FSH synthesis and secretion (Bergfelt *et al.*, 1991; Clayton, 1993; Albanese *et al.*, 1996; Mather *et al.*, 1997), and oestrogens increasing LH responsiveness, but inhibiting FSH (Marshall and Griffin, 1993). Progesterone decreases GnRH pulse frequency (Irvine and Alexander, 1997), thereby modulating pituitary responsiveness indirectly, as discussed above.

Between day 4 and day 10 after ovulation, neither the frequency nor the amplitude of FSH pulses varied significantly. In no mare did there appear to be a discrete 'surge' of FSH secretion, as has been described in studies using less frequent sampling (Evans and Irvine, 1975; Bergfelt and Ginther, 1993). The follicle destined next to ovulate can first be detected 6 (Ginther and Bergfelt, 1993), 7.4 (Ginther, 1993) or 12 (Bergfelt and Ginther, 1993) days after the preceding ovulation, or approximately 10 days before its subsequent ovulation using ultrasonography of the ovaries of horse mares once per day. The emergence of this dominant follicle has been linked to the occurrence in the previous 8 days of one or more FSH surges spanning an average of 4.5 days (Bergfelt and Ginther, 1993). The present data suggest that the dominant follicle appears after exposure to 2–8 days of rhythmic, high amplitude FSH pulses. In women in whom plasma FSH concentrations are not clearly pulsatile (Filicori *et al.*, 1986), the recruitment of the ovulatory follicle is thought to depend on post-menstruation

plasma FSH concentrations exceeding a certain threshold value for several days (Fauser and van Heusden, 1997). In mares, it appears that high FSH concentrations need only to be attained intermittently to select the ovulatory follicle.

Mean plasma FSH concentrations did not start to increase from the oestrous nadir until after ovulation and reached an early dioestrous peak on days 4 and 5. However, a previous study found that FSH increased gradually throughout oestrus to peak with LH just after ovulation (Evans and Irvine, 1975). This pattern might be expected to occur in mares, since concurrent periovulatory FSH and LH surges are observed in other species, for example, women (Guillemin, 1972), rats (Butcher *et al.*, 1974) and ewes (Findlay and Clarke, 1987). It seems unlikely that the difference between the present study and our previous studies on the timing of the increase in FSH after ovulation was due to the sampling regimen used, since few FSH pulses were detected during oestrus when samples were taken at 4 h intervals. FSH pulses occur rapidly at oestrus (Irvine and Alexander, 1994) and merge in jugular blood, as do LH pulses, so that once-a-day sampling is adequate to determine mean concentrations. Another explanation for the discrepancy between our present and previous results may be that the radioimmunoassay used to measure FSH in our laboratory has changed. A different antibody against human FSH is now used, and equine, not human, FSH is iodinated as tracer (Evans and Irvine, 1975; Irvine and Alexander, 1993). This is relevant because equine FSH is polymorphic (Irvine, 1979) and different antibodies can vary in their affinity for each isoform, leading to discrepant potency estimates (Alexander *et al.*, 1987). Moreover, the range of FSH isoforms in blood changes during the ovulatory cycle of mares, shifting from predominantly neutral-basic forms on the day of ovulation to acidic forms on day 9 (Bleichwitz, 1996). An identical switch in the nature of circulating FSH occurs during the human menstrual cycle (for review, see Ulloa-Aguirre *et al.*, 1995). Therefore, it is possible that our original radioimmunoassay had a higher affinity for basic FSH isoforms than the assay used in the present study, with the result that the original assay detected a periovulatory FSH increase.

Although not significant, the amplitude of FSH pulses tended to decrease around day 6. It may be that this was an artifact and due to the fact that pulse maxima cannot always be sampled with blood collection at 4 h intervals. Nevertheless, four of six mares showed a decrease in amplitude at this time, suggesting that it was unlikely to be a fluke of inadequate sampling frequency. Consequently, daily mean FSH concentrations decreased on day 6, separating two periods of significantly increased FSH in the group mean profile. Alternate increasing and decreasing dioestrous FSH concentrations with a periodicity of approximately 5–7 days has been reported in cows (Adams *et al.*, 1992; Evans *et al.*, 1997) and ewes (McNeilly *et al.*, 1992) on the basis of sampling once or twice per day. The FSH increases are associated with new waves of follicular growth, and the FSH troughs have been attributed to increased oestradiol negative feedback (McNeilly *et al.*, 1992). In the mares used in the present study, ovarian activity was not monitored with sufficient frequency or accuracy to detect when follicular waves first appeared; however, others have observed waves to emerge during the first 5 days after ovulation (Bergfelt and Ginther, 1993). Moreover, plasma oestrogen

conjugate concentrations increase slightly in mares between day 4 and day 6 after ovulation (Daels *et al.*, 1991). It is possible that, in the mares used in the present study, FSH pulse amplitude was transiently decreased by an increase in oestrogen feedback.

In summary, plasma FSH concentrations during most of dioestrus were characterized by fairly rhythmic, low frequency, high amplitude pulses; a pattern that was consistent from mare to mare. This marked pulsatility provided a major source of variation in profiles derived from once-a-day sampling. No discrete FSH 'surges' were observed during dioestrus as originally described by Evans and Irvine (1975) on the basis of blood collection three times per week. However, in the present study, there were periods during dioestrus in which mean FSH concentrations were high owing to the fact that FSH pulse amplitude tended to increase and decrease, albeit not significantly. Ovulation followed a period of higher amplitude FSH pulses by 10.2 ± 0.7 days. This temporal relationship is compatible with recruitment of the ovulatory follicle at the time of the large pulses. Therefore, it is possible that, in mares, the signal for follicular recruitment is intermittent excursions of plasma FSH concentrations above a given threshold value.

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