Reprod Dom Anim 44, 92–99 (2009); doi: 10.1111/j.1439-0531.2007.01003.x
ISSN 0936-6768

Temporal Relationships and Repeatability of Follicle Diameters and Hormone Concentrations within Individuals in Mares

JC Jacob[1,2], EL Gastal[3], MO Gastal[1], GR Carvalho[2], MA Beg[3] and OJ Ginther[1,3]

[1]*Eutheria Foundation, Cross Plains, WI, USA;* [2]*Animal Science Department, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil;* [3]*Pathobiological Sciences, University of Wisconsin, Madison, WI, USA*

Contents

Data were collected daily from 23 mares during two consecutive interovulatory intervals (IOIs). Several significant ($p < 0.05$) new observations on temporal relationships were made. The FSH increase that begins before ovulation temporarily plateaued on the day of discharge of follicular fluid into the peritoneal cavity in association with ovulation. During the declining portion of the pre-ovulatory oestradiol surge, an abrupt reduction in the rate of decrease occurred in synchrony with the peak of the LH surge and is consistent with a negative effect of LH on oestradiol. Repeatability within mares was based on the following positive and significant correlations between the two IOIs: (i) length of the interval between ovulations and between ovulation and the beginning of follicle deviation; (ii) diameter of the pre-ovulatory follicle on days -3 to -1; (iii) number of follicles in diameter classes of 2–5 mm (correlation for 22/23 days of the IOI), 5.1–10 mm (18/23 days), 10.1–15 mm (12/23 days) and 15.1–20 mm (12/23 days) and (iv) concentrations of FSH (18/23 days) and LH (22/23 days). The greatest repeatability for the follicle-diameter classes occurred in the 2–5 mm class, and thereafter the repeatability progressively decreased as the diameters for the classes increased. Results demonstrated measurable repeatability within mares for several end points between consecutive IOIs.

Introduction

Growth of follicles in waves during oestrous or menstrual cycles in monovular species has been characterized in detail by transrectal (cows and mares) and transvaginal (women) ultrasonography (reviewed in Ginther 2000; Ginther et al. 2001, 2003, 2004a; b). After several days of a common-growth phase, the follicles of a wave undergo deviation which is characterized by continued growth of a dominant follicle and reduced growth and atresia of subordinate follicles. Waves that develop a dominant follicle that reaches at least 28 and 13 mm in mares and women, respectively, are termed major waves, and waves without a dominant follicle are termed minor waves. In mares and women, a major anovulatory wave precedes the ovulatory wave in 20–30% of oestrous or menstrual cycles, whereas almost all cycles in heifers and cows have one or two major anovulatory waves preceding the ovulatory wave. Mares are good comparative research models for follicle studies because of many similarities with women in the dynamics of the ovulatory wave. In direct comparative studies (Ginther et al. 2004b, 2005a), the similarities included a constant relative diameter of the largest follicle between the two species at definable events throughout the follicular wave (2.1 or 2.2 times larger in mares).

In mares, regressing follicles from a previous wave overlap with the follicles of the common-growth phase of the ovulatory wave in approximately 25% of ovulatory waves (Ginther et al. 2004a). Therefore, direct information on the day of emergence (e.g. at 6 mm) of the future ovulatory follicle requires monitoring of individually identified follicles from examination to examination. Identification is aided by ablation of follicles to induce a new wave with minimal overlapping of follicles (Gastal et al. 1997). However, the day of emergence of the future dominant follicle can be estimated by using the demonstrations (Gastal et al. 1997; Ginther et al. 2004a) that the growth rate of the ovulatory follicle is similar before and after deviation. Therefore, the post-deviation growth rate can be used for projecting back or retroceding to an estimated day of emergence. Depending on experimental goals, the time-consuming procedure of maintaining follicle identities may not be necessary.

Measurable repeatability within an animal has been shown in association with follicle recruitment and superovulation rates in cattle (Boni et al. 1997; Benyei et al. 2004) and sheep (Bari et al. 2001). The number of 2–6 mm follicles at wave emergence was positively correlated between the first and second waves of an oestrous cycle in cattle (Singh et al. 2004). In humans, the number of antral follicles had a measurable degree of repeatability in individuals between menstrual cycles (Scheffer et al. 1999). Recent studies in cattle have shown considerable repeatability within animals in numbers of follicles during the first post-ovulatory major anovulatory follicular wave and an inverse association of follicle numbers to FSH concentrations (Burns et al. 2005; Ireland et al. 2007). Cows with low (≤15) and high (> 25) follicle numbers were compared for differences in concentrations of FSH aligned to the FSH peak that was associated with the follicular wave. Concentrations of FSH were lower and numbers of follicles ≥3 mm were greater in cows selected for high vs low follicle numbers. Repeatability studies in these species have involved various aspects of follicle numbers, except for the recent inclusion of FSH, and have not considered other aspects of reproduction. In mares, repeatability has not been studied for any characteristic, but it has been noted that immunoreactive (ir)-inhibin concentrations within ovariectomized mares were correlated significantly among days (Ginther et al. 2005b).

The purposes of the present study were to characterize the follicle, FSH, LH and oestradiol dynamics during two consecutive interovulatory intervals (IOIs) with emphasis on the within-mare repeatability between intervals. The temporal associations between follicle diameters and numbers and FSH, LH and oestradiol concentrations were also considered.

Materials and Methods

Animals and ultrasound end points

Mares were handled according to the United States Department of Agriculture Guide for Care and Use of Agricultural Animals in Agricultural Research and Teaching. The mares were mixed breeds of large ponies and apparent pony-horses crosses weighing 250–400 kg and aged 5 to ≥18 years. A total of 24 mares with a docile temperament and no apparent abnormalities of the reproductive tract as determined by ultrasound examinations (Ginther 1995) were used in two consecutive IOIs during April–August (Northern Hemisphere). A lighting program (Ginther 1992) was used during the preceding December–February, so that the ovulatory season began on average in March. The mares were kept under natural light in an open shelter and outdoor paddock and were maintained on alfalfa/grass hay with access to water and trace-mineralized salt. All mares remained healthy and in good body condition throughout the study.

Transrectal B-mode ultrasonographic examinations were carried out daily starting 15 days after an ovulation and encompassed three subsequent ovulations or two IOIs. The experimental period extended from 4 days before the first ovulation to 4 days after the third ovulation (ovulation = day 0). A real-time ultrasound scanner with a linear-array 7.5 MHz transducer was used for examination of the ovaries. Diameters of follicles ≥ 15 mm were measured (average of height and width) with the electronic calipers, and diameters of all other follicles (2 to < 15 mm) were estimated from a calibrated grid that was placed on the ultrasound screen. Numbers of follicles were grouped into diameter classes as 2–5, 5.1–10, 10.1–15, 15.1–20, 20.1–25 and > 25 mm. Number of dominant follicles (≥ 28 mm) for the ovulatory wave was also considered. The diameters of the six largest follicles were recorded without regard to the day-to-day identity and were defined as F1 (largest) to F6.

Normalization of the interovulatory interval

Data were normalized to the mean length of the IOIs rounded to 23 days for both the first (23.0 ± 0.5 days) and second (22.9 ± 0.4) IOIs (Fig. 1). Days -4 to 4 for each of the three periovulatory periods remained intact. Days preceding the end of each IOI were designated days -18 to -1. If the IOI was longer than 23 days, the data were discontinued at day -18; if the IOI was shorter, missing data were used to complete the interval. Thus, a break in continuity occurred between day 4 after the first ovulation and day -18 before the second ovulation. The day of the break was selected on the basis of minimal likelihood that critical events would occur during that time (e.g. follicle emergence and deviation, onset and continuation to maximum of the ovulatory LH surge). For study and analyses, the two IOIs were partitioned as shown in Fig. 1. Data for days -

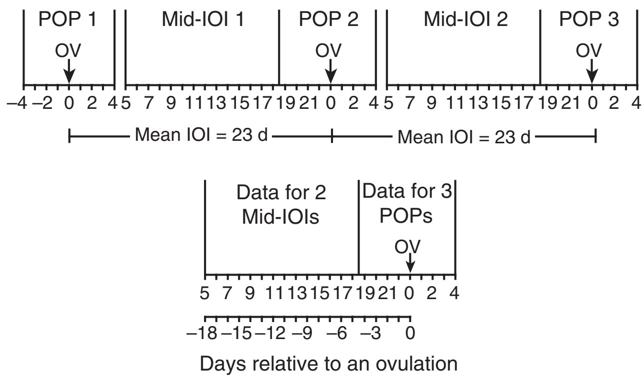

Fig. 1. Scheme for normalization of the interovulatory intervals (IOIs) to the mean length of 23 days. A break in continuity was made between days 4 and 5 by designating day 5 as day -18 before the ovulation at the end of an IOI. The middle of the two IOIs was designated Mid-IOI 1 and 2, and the three periovulatory periods (POPs) (days -4 to 4) were designated POP 1, 2 and 3. For some analyses, data were combined for the two Mid-IOIs and the three POPs

4 to 4 for each of the three periovulatory periods were designated POP 1, POP 2 and POP 3. Data for days 5 to 18 after an ovulation (corresponds to days -18 to -5 before an ovulation) for the middle of the two IOIs were designated Mid-IOI 1 and Mid-IOI 2. Data for the three periovulatory periods and data for the two middle IOIs were combined, as shown in Fig. 1, when indicated by the absence of a significant day-by-group interaction.

The day of emergence at 6 mm of the future ovulatory follicle and the beginning of deviation were estimated for each mare and each IOI by using the mean growth rate of the dominant follicle (F1) during 4 days (3 day-to-day growth rates) after the apparent beginning of deviation. This approach was based on the following previous studies: (i) diameter growth rate of the future dominant follicle beginning at 6 mm and until several days after deviation was the same before and after deviation (Gastal et al. 1997; Ginther et al. 2004b) and (ii) the diameter of the future dominant follicle at the beginning of deviation was 22.5 mm averaged over many reports (Ginther et al. 2004a). The mean growth rate for the 4-day post-deviation was used for retroceding to the day with the diameter closest to 22.5 and 6.0 mm to estimate the days of the beginning of deviation and emergence of the future dominant follicle, respectively. The calculation of F1 growth rates began at the first consistent ≥5 mm separation between F1 and F2 that was followed by four visually selected days that appeared to have the most consistent growth rate among days. However, in two IOIs, only three suitable days were available because of a short interval between deviation and ovulation.

Repeatability and correlations

The repeatability of an end point within each mare was examined by correlation between the first and second IOIs over all mares. Sequential end points were correlated for each day and were diameter of F1, number of follicles in each of the five diameter classes, and concentrations of FSH, LH and oestradiol. Correlations between IOIs for single-point data were diameter of F1 on day -1; length of the IOI; days of estimated emergence and deviation of the future ovulatory follicle; intervals from emergence to deviation and from deviation to ovulation; growth rate of the ovulatory follicle on 4 days post-deviation, on days -3 to -2, and on days -2 to -1; and number of dominant follicles in the ovulatory wave.

To consider whether the repeatability results were confounded by age effects, the mares were grouped into younger mares (5–14 years; n = 15) and older mares (≥ 18 years; n = 8). Three end points were evaluated that had high positive correlations between IOIs as determined over all mares in the present study and have been shown previously to be affected by age (Carnevale et al. 1993). The reported results were higher LH on day 1, more follicles 10–16 mm on day 13 and a shorter IOI in younger mares (5–19 years) than in older mares (≥ 20 years). The correlations between IOIs within mares in the present study were determined for each of these end points within each age range.

In addition to the correlation approach for repeatability between IOIs within mares, correlations were performed between end points of interest. The mean of each mare averaged over the two IOIs was used in determining the correlations. The following combinations of end points were analyzed: (i) number of follicles in a diameter class with each other diameter class, using the four diameter classes with the greatest expected turnover (2–5, 5.1–10, 10.1–15 and 15.1–20 mm); (ii) FSH with each of the four diameter classes; (iii) FSH with LH and (iv) LH with diameter of F1 on day -1.

Blood samples and hormone assays

Daily jugular blood samples were collected into heparinized tubes. Blood samples were centrifuged (1500 ***g*** for 10 min) and decanted, and the plasma was stored at −20°C until assayed. Plasma gonadotropin concentrations were assayed for all samples from 4 days before the first ovulation to 4 days after the third ovulation. Oestradiol was assayed from 4 days before to 4 days after deviation. In addition, oestradiol and progesterone were assayed for days -4 to 4 by using pooled samples from the three periovulatory periods. Samples were assayed for FSH and LH by radioimmunoassay (Donadeu and Ginther 2002) and for oestradiol (Ginther et al. 2005b) and progesterone (Ginther et al. 2005a) by commercial kits, as validated and described for mare plasma in our laboratory. The intra- and interassay coefficients of variation (CV) and mean sensitivity, respectively, were 9.2%, 18.4% and 1.1 ng/ml for FSH; 7.8%, 8.3% and 0.2 ng/ml for LH; 10.0%, 4.9%, 0.1 pg/ml for oestradiol and 5.6% (intra-assay CV) and 0.04 ng/ml for progesterone.

Statistical analyses

End points that were not normally distributed, according to Kolmogorov-Smirnov tests, were transformed to natural logarithms. Sequential number of follicles in the diameter classes, diameter of F1 and F2, and concentrations of FSH and LH were analyzed for the two mid-IOIs (group effect) and the 14 days between days 5 and 18 (day effect; 2×14 factorial). Oestradiol for the 9 days encompassing the estimated beginning of deviation was analyzed in a 2 (group) × 9 (day) factorial. Diameter of F1 on days -4 to -1 (4 days) and concentrations of FSH and LH for days -4 to 4 (9 days) were analyzed for the three periovulatory periods in 3×4 or 3×9 factorials. Oestradiol and progesterone data of the ovulatory periods were examined for a day effect only, given that the plasma samples for the three periovulatory periods were combined for each day before assayed. The data were analyzed by SAS MIXED procedure with a REPEATED statement to account for autocorrelation between sequential measurements (8.2 Version; SAS, Institute Inc., Cary, NC, USA). If a significant effect of day was detected, without a significant effect of group (two mid-IOIs or three periovulatory periods) or an interaction, paired Student's *t*-tests were used to locate selected differences between days using data combined for the two mid-IOIs or the three periovulatory periods. Significant interactions were further examined by paired Student's *t*-tests within days. Correlations between the first and second IOIs as an indication of repeatability for a specific day and end point were carried out by the Spearman test. The Spearman correlation test was selected because it uses ranked data and is less affected by extreme values (Conover 1999). Single-point data were analyzed by one-way ANOVA, and frequency data were analyzed by chi-squared test. A probability of $p \leq 0.05$ indicated that a difference was significant, and probabilities between $p > 0.05$ and $p \leq 0.1$ indicated that a difference approached significance. Data are given as the mean ± SEM, unless otherwise stated.

Results

One mare was removed from the experiment, owing to the development of a haemorrhagic anovulatory follicle (Ginther et al. 2006a) during the second expected ovulatory period. Two complete IOIs with three periovulatory periods were obtained for each of the remaining 23 mares. For the mid-IOIs (days 5–18), significant ($p < 0.0001$) day effects, without an effect of group (first vs second mid-IOI) or an interaction, were obtained for diameters of F1 and F2; number of follicles 2–5, 5.1–10, 15.1–20, 20.1–25 and > 25 mm; and concentrations of FSH and LH. For these end points, except diameters of F1 and F2, the first significant ($p < 0.05$) and consistent increase or decrease combined for the two mid-IOIs are shown (Fig. 2). For number of follicles 10.1–15 mm, the main effect of group (greater number in the second IOI), as well as day, was significant, but the interaction was not. Oestradiol, centralized to the estimated beginning of deviation, showed only a day effect ($p < 0.0001$); the initial increase ($p < 0.05$) between days is shown (Fig. 2).

For the periovulatory periods, the day effect was significant ($p < 0.0001$) for diameters of F1 and F2 (days -4 to -1) and for concentrations of FSH and LH (days -4 to 4), without an effect of group (first, second and third periods) or an interaction. The day effect for LH involved an increase over days -4 to 0, followed by a decrease. The rate of increase in LH concentrations on

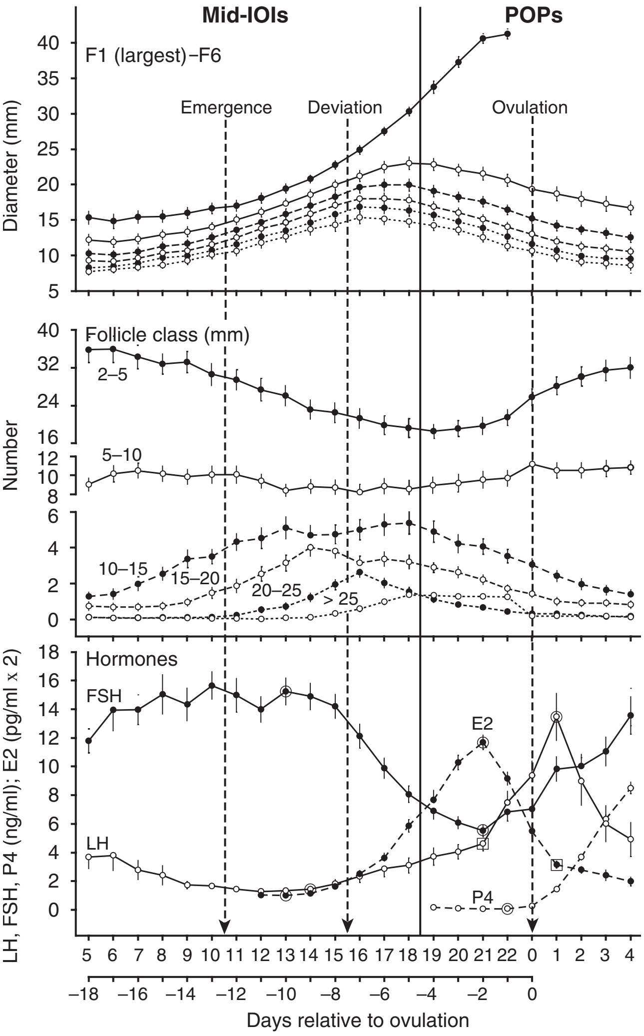

Fig. 2. Mean (±SEM) diameter of the six largest follicles, number of follicles in a diameter class, and concentrations of hormones combined for two Mid-IOIs and three periovulatory periods (POPs) in 23 mares. All diameters, follicle numbers and hormone concentrations showed a significant day effect. A circle around a hormone mean indicates the first day of a progressive increase or decrease ($p < 0.05$). A box indicates an abrupt change ($p < 0.05$) in the rate of increase or decrease

days -4 to -2 (0.5 ± 0.1 ng/ml/days) was less ($p < 0.0001$) than for days -2 to 0 (2.9 ± 0.3 ng/ml/days). Oestradiol and progesterone (samples pooled for each day of the three periovulatory periods) each showed a day effect ($p < 0.0001$). The first significant ($p < 0.05$) increase and decrease are shown (Fig. 2). The significant effect of day for oestradiol involved an increase between days -4 and -2, followed by a decrease. The rate of decrease in oestradiol concentrations on days -2 to 1 (1.5 ± 0.1 pg/ml/days) was greater ($p < 0.0001$) than for days 1–4 (0.2 ± 0.1 pg/ml/days). Progesterone concentrations combined for the three periods began to increase slightly but significantly ($p < 0.05$) between days -1 and 0; in all of the 23 individual mares, except one, the concentration on day 0 was higher than on day -1.

The intervals from ovulation to estimated emergence of the ovulatory follicle at 6 mm, emergence to the estimated beginning of deviation, ovulation to deviation and ovulation to ovulation were not significantly different between the two IOIs, but the deviation to ovulation interval approached being shorter ($p < 0.08$) for the second IOI (Table 1). For the growth rate of F1 during the three sets of days (4 days post-deviation, days -3 to -2, and days -2 to -1), there was a main effect of sets of days ($p < 0.0001$), without a difference between IOIs or an interaction. The F1 growth rate for days -2 to -1 was less ($p < 0.05$) than for each of the other two growth rates.

The number of days during the 23-day IOI with a positive and significant correlation between IOIs and the mean of the daily correlations are shown (Table 2). To illustrate the extent of the differences in follicle and hormone values among IOIs, the lowest and highest mean/IOI for each end point are also given. The mean for the 23 daily correlations within mares between the two IOIs for diameter of the largest follicle reflected positive and significant correlations only for days 15 ($r = +0.49$), 16 (+0.48), 20 (+0.56), 21 (+0.47) and 22 (+0.44); day 22 corresponds to day -1. The number of days with significant correlations and the mean of the daily correlations for five follicle classes are shown. The greatest number of days with positive correlations and greatest mean correlation occurred in the 2–5 mm class and the lowest in the 20.1–25 mm class. The number of follicles in the 2–5 mm class was significantly correlated on each of the 23 days, except that the correlation approached significance ($p < 0.08$) on day 8. In the 5.1–10 mm class, the correlations were significant

Table 1. Mean (±SEM) lengths of intervals between events and growth rates of the ovulatory follicle (F1) for the first and second interovulatory interval (IOI) and correlations between IOIs within mares (n = 23 mares)

	Interovulatory interval		Correlation		
End points	First	Second	Combined[a]	*r*	Probability
Intervals (days)					
Ovulation to emergence	10.3 ± 0.5	10.8 ± 0.5	10.5 ± 0.4	–	ns
Emergence to deviation	5.1 ± 0.3	5.0 ± 0.2	5.1 ± 0.2	–	ns
Ovulation to deviation	15.4 ± 0.4	15.8 ± 0.5	15.6 ± 0.4	+0.58	$p < 0.004$
Deviation to ovulation	7.5 ± 0.2	7.1 ± 0.2	7.3 ± 0.2	–	ns
Ovulation to ovulation	23.0 ± 0.5	22.9 ± 0.4	22.9 ± 0.4	+0.67	$p < 0.0005$
F1 growth rate (mm/day)					
Four-day post-deviation	3.4 ± 0.2	3.4 ± 0.1	3.4 ± 0.1[x]	–	ns
Days -3 to -2	3.2 ± 0.4	3.0 ± 0.3	3.1 ± 0.3[x]	–	ns
Days -2 to -1	1.2 ± 0.5	0.1 ± 0.3	0.6 ± 0.3[y]	–	ns

[a]No significant differences ($p < 0.05$) between the two IOIs.
[x,y]Means with a different superscript alphabets are different ($p < 0.05$).
ns, not significant.

Table 2. Mean (±SEM) of the daily correlations between interovulatory intervals (IOI), number of days with a significant correlation and the IOI with the lowest and highest mean

End points	No. of days with a significant correlation	Mean of daily correlations	IOI with	
			Lowest mean	Highest mean
Diameter largest follicle (mm)	5/23	+0.29 ± 0.03	16.5 ± 1.2	27.0 ± 1.3
Follicles/mare/day (no.)				
2–5 mm	22/23[a]	+0.65 ± 0.02[a]	13.9 ± 0.5	48.3 ± 2.2
5.1–10 mm	18/23[ab]	+0.49 ± 0.20[b]	1.7 ± 0.2	14.8 ± 0.7
10.1–15 mm	12/23[b]	+0.40 ± 0.03[c]	0.3 ± 0.1	5.9 ± 0.7
15.1–20 mm	12/23[b]	+0.42 ± 0.04[c]	0.2 ± 0.1	4.1 ± 0.3
20.1–25 mm	2/23[c]	+0.11 ± 0.06[d]	0.1 ± 0.0	1.7 ± 0.2
Hormones				
FSH (ng/ml)	18/23[a]	+0.55 ± 0.03[a]	6.5 ± 0.4	21.2 ± 1.6
LH (ng/ml)	22/23[a]	+0.59 ± 0.03[a]	1.4 ± 0.1	12.9 ± 1.9
Oestradiol (pg/ml)	0/9[b]	+0.1 ± 0.1[b]	0.4 ± 0.1	1.8 ± 0.3

[a,b,c,d]For each column, values within the five follicle diameter classifications and within the three hormones without a common superscript are different ($p < 0.05$).

on 18 of the 23 days, but approached significance ($p < 0.09$) on the remaining days. In the 10.1–15 mm class, the correlations were significant consistently for days 13–22. In the 15.1–20 mm class, the days of consistency were days 10–19. The number of days with correlations did not differ significantly between the 5.1–10 mm class and the next two classes but significance was approached ($p < 0.06$). The correlation for the number of follicles in the 20.1–25 mm class was significant on only day 5 ($r = +0.45$; $p < 0.03$) and day 11 ($r = +0.99$; $p < 0.0001$); during the days of the greatest numbers (approximately days 12–21), the correlations were not significant. The number of dominant follicles in the ovulatory wave (1.4 ± 0.1) was not correlated between IOIs within mares. For FSH and LH, the correlations within mares between the two IOIs were positive for each day. The two gonadotropins did not differ in the mean of the correlations; however, significance was approached (higher for LH) for the mean of the correlations ($p < 0.1$) and for the number of days with a significant correlation ($p < 0.08$). There was no indication of a significant correlation in oestradiol concentrations for any of the 9 days centred on the estimated beginning of deviation. The growth rates of the pre-ovulatory follicle were not correlated between IOIs within mares.

The difference between younger vs older mares was significant for LH concentration on day 1 (15.5 ± 1.8 vs 8.1 ± 1.0 ng/ml; $p < 0.003$), number of follicles 10.1–15 mm on day 13 (6.0 ± 0.7 vs 3.5 ± 0.6; $p < 0.02$), and length of the IOI (22.5 ± 0.3 vs 23.8 ± 0.5 days; $p < 0.02$). Correlations were positive ($r = +0.53$ to $+0.92$) and significant ($p < 0.04$) for each end point between IOIs within each age range.

The correlations between end points averaged over the two IOIs within mares were positive and significant between number of follicles in a diameter class and the number in the next class as follows: (i) 2–5 mm and 5.1–10 mm ($r = +0.43$, $p < 0.04$), (ii) 5.1–10 mm and 10.1–15 mm ($r = +0.70$, $p < 0.0002$) and (iii) 10.1–15 mm and 15.1–20 mm ($r = +0.80$, $p < 0.0001$). There were no other correlations for any of the combinations of the four diameter classes, for FSH and each of the diameter classes, for FSH and LH, or for LH and diameter of F1 on day -1.

Discussion

The use of a large data mass with centralization to the day of ovulation resulted in detection of several temporal relationships between hormones that have not been reported previously. Mean concentrations of FSH began to increase and oestradiol began to decrease in synchrony on day -2. These two changes are likely related, given that exogenous oestradiol has a negative effect on FSH in mares (Donadeu and Ginther 2003). The FSH increase between days -2 and -1 was followed by a transient suspension in the increase or a plateau in FSH between days -1 and 0. In this regard, ovulation (day 0) was indicated by a collapsed follicle, and therefore actual collapse occurred sometime between day -1 and day 0. A spike in ir–inhibin concentrations occurs on day 0 (Bergfelt et al. 1991), owing to discharge of follicular fluid into the abdomen with absorption of the inhibin component into the circulation (Nambo et al. 2002). A similar indication that oestradiol of the discharged follicular fluid altered the profile of plasma oestradiol was not apparent. Thus, the absorption of inhibin from the follicular fluid in the peritoneal cavity accounts for the transient suspension in the FSH increase between days -1 and 0. An increase, apparently a rebound, in FSH occurred between days 0 and 1. This FSH response likely reflected the release of FSH from the suppressing effects of inhibin during the absorption of inhibin from the peritoneal cavity. In this regard, a rebound in FSH began 24 h after administration of a proteinaceous fraction of follicular fluid in ovariectomized mares (Miller et al. 1979) and ovarian-intact mares (Bergfelt and Ginther 1985). The rapid decrease in oestradiol concentrations on days -2 to 1 and a slower decrease on days 1 to 4 resulted in an abrupt change in the rate of decrease on day 1 in synchrony with the mean day of the LH peak. The rapid decrease in oestradiol is attributable to a negative effect of the rapidly increasing LH on oestradiol (Gastal et al. 2006; Ginther et al. 2007b), and the slower decrease is attributable to a diminishing negative effect of the decreasing LH.

Other characteristics and temporal interrelationships of changes in follicle diameters, follicle numbers, and hormone concentrations confirmed those of previous studies. The confirmations included: (i) changes in diameter of the six largest follicles measured without regard to day-to-day follicle identity (Ginther et al. 2007a); (ii) day of the beginning of follicle deviation as determined by retroceding the post-deviation growth rate in this study (day 15.6) and by observation of the growth profiles of follicles in the individual waves (day 16.0; Ginther et al. 2007a); (iii) changes in numbers of follicles in several diameter classes (Pierson and Ginther 1987), consistent with progressive increases in follicle diameter during the common-growth phase preceding deviation; (iv) a reduction or plateau in growth rate of the pre-ovulatory follicle on the day before ovulation (Gastal et al. 2006); (v) profile for LH concentration, including basal levels on day 12 followed by slight but significant increases 1 and 2 days before the mean beginning of deviation, as previously reported for mares (Ginther et al. 2006b) and women (Ginther et al. 2005a) and a subsequent slow increase ending and a rapid increase beginning on the day of the oestradiol peak (Ginther et al. 2006b); (vi) increase in oestradiol beginning 1 or 2 days before the beginning of deviation in both mares (Gastal et al. 1999; Ginther et al. 2007b) and women (Ginther et al. 2005a); (vii) oestradiol and LH surges reaching peak concentrations 2 days before and 1 days after ovulation, respectively (Ginther et al. 2006b); (viii) beginning of deviation during decreasing FSH, as previously reported for both mares and women (Ginther et al. 2005a) and (ix) beginning of a progesterone increase in the plasma between days -1 and 0 (Townson et al. 1989).

A positive and significant correlation for an end point between IOIs within mares was taken as an indicator of repeatability within individual mares and that the magnitude of the correlation was an indicator of the extent of repeatability. The repeatability involved two consecutive IOIs for each mare, and therefore the extent of repeatability throughout the ovulatory season was not examined. However, the experimental period extended from April to August and encompassed 5 months of a 9-month ovulatory season (March to November) in this herd under a lighting program. Inspection of data did not suggest that repeatability was affected by month during the 5 months.

The finding that the pre-ovulatory follicle tends to reach a diameter that is characteristic of the mare may be useful knowledge in equine breeding programs. The significant positive correlations for diameter of the pre-ovulatory follicle occurred on the 3 days before ovulation. These days encompassed the day that the follicle first reached ≥35 mm in approximately 70% of the IOIs; ≥35 mm is a common diameter for administration of an ovulation-inducing dose of hCG (Ginther 1992). These observations suggest that knowledge of the mare's history on the diameter preceding ovulation may be useful for estimating the optimal follicle diameter for a given mare for ovulation induction, as well as for the optimal time for breeding before spontaneous ovulation. Considering the close similarity between mares and women in relative diameter of the follicles during the ovulatory wave (see Introduction), a history of the diameter of the pre-ovulatory follicle in individual women may be an aid in assisted reproduction programs. The reliability and practicality of this approach would require specific study in each species.

The correlations between IOIs in the interval from ovulation to deviation and in F1 diameter at approximately the beginning of deviation indicated measurable repeatability in individual mares in the time of initiation of deviation. The length of the IOI was also correlated significantly between the IOIs, but the intervals from ovulation to emergence, emergence to deviation, and deviation to ovulation were not. Therefore, the only detected interval that accounted for repeatability in the length of the IOI was the interval from ovulation to deviation.

The repeatability for number of follicles in each of the five diameter classes indicated that the highest average positive correlation and the most number of days⁄IOI with a significant correlation occurred in the 2- to 5-mm class. When the number of follicles 10.1–20 mm was considered as a single class, the number of days⁄IOI with a significant correlation decreased and the magnitude of the positive correlations decreased progressively as the diameters for the classes increased (i.e. 2–5, 5.1–10, 10.1–20 and 20.1–25 mm). At a diameter of 20.1–25 mm, the correlations were significant on only 2 of the 23 days. In summary, the repeatability involved primarily the smallest follicles detected by the ultrasound procedure, and thereafter the repeatability progressively decreased. Our interpretation is that the number of small follicles (≤5 mm) is a characteristic of each mare and therefore the most repeatable between IOIs. The extent of repeatability diminishes as the small follicles enter the larger classes in the dynamics of the turnover involved in follicle growth and atresia.

Repeatability within mares in concentrations of FSH and LH was found for 78% and 96%, respectively, of the 23 days of the IOI, as indicated by positive and significant correlations between IOIs. No significant correlations were found for oestradiol during the days encompassing the beginning of deviation. Thus, mares with higher concentrations of FSH and LH during an IOI are likely to be the mares with the higher concentrations during the next IOI. This finding is especially important in statistical analyses of sequential data between experimental groups. Clinically, predicting the time of occurrence of an event (e.g. ovulation) on the basis of another (e.g. attainment of a given LH concentration) is not likely to be successful without knowledge of the history for each individual.

The relationship between pairs of selected end points was examined by correlation between average values over the two IOIs for the two end points for each mare. The turnover among the diameter classes was represented by positive and significant correlations within mares between each set of sequential classes. For example, the number of follicles in the 2–5 mm class was correlated with the number in the 5.1–10.1 class but not with the number in other classes. Similarly, the number in the 5.1–10 mm class was correlated with the number in the 10.1–15 mm class, and the number in the 10.1–15 mm class was correlated with the number in the 15.1–20 mm class. These correlations are taken as a reflection, at least in part, of the movement of follicles to the next class during the common-growth phase of a follicular wave. The FSH concentration averaged over all days within mares was not correlated significantly with any of the follicle end points. This appears to further support the interpretation that the number of small follicles is inherent in each mare, independently of average FSH output/mare. However, the repeatability of the follicle/FSH relationship was not studied with consideration of day-to-day change and will require a specific approach. The other examinations of the overall correlations between end points did not indicate significance between any of the other comparisons (FSH and LH concentrations, LH concentration and F1 diameter on day -1).

Although a detailed study of age effects was not a goal, the effect of age on the repeatability demonstrations was considered. This was carried out by examining three end points and days previously shown (Carnevale et al. 1993) to differ between younger and older mares. Comparisons among end points for the age ranges confirmed the reported findings (higher LH on day 1, more follicles 10–16 mm on day 13, and a shorter IOI in younger mares). Although the study was limited by the comparisons between only two age ranges, the repeatability (high positive correlations) between IOIs within each age range for each of the three end points did not suggest that the overall repeatability demonstrated for the 23 mares was confounded by age; repeatability was a characteristic within each age range. Further study will be needed to confirm these findings on age and to determine the factors contributing to repeatability.

In conclusion, several new aspects of hormonal temporal interrelationships during the periovulatory period were detected. Mean concentrations of FSH began to increase and oestradiol began to decrease on day -2, consistent with a negative effect of oestradiol on FSH. The FSH between days -2 and -1 was followed by a transient suspension in the increase between days -1 and 0, consistent with a discharge of hormonal-laden follicular fluid into the peritoneal cavity during ovulation. A change from a rapid to slow decrease in oestradiol concentrations occurred on day 1 in synchrony with the peak concentration of LH, consistent with a negative effect of LH on oestradiol. A positive and significant correlation between IOIs within mares, indicating a measurable degree of repeatability was found for the following: (a) diameter of the preovulatory follicle during the 3 days before ovulation; (b) length of intervals from ovulation to deviation and from ovulation to ovulation; (c) numbers of follicles in the 2–5, 5.1–10, 10.1–15, 15.1–20 mm classes, with the greatest repeatability (number of days/IOI with a significant correlation and mean correlation for the IOI) in the 2–5 mm class and (d) concentrations of FSH and LH.

Acknowledgements

Supported by the Eutheria Foundation. Project P1-JC-05. J.C. Jacob was on leave from the Department of Reproduction and Animal Evaluation, Federal Rural University of Rio de Janeiro, Rio de Janeiro, Brazil. The authors thank Dee Cooper for assistance with the figures and statistical analyses and A.F. Parlow of the National Pituitary and Peptide Program (NPPP) for eFSH and eLH.

References

Bari F, Khalid M, Wolf B, Haresign W, Murray A, Merrell B, 2001: The repeatability of superovulatory response and embryo recovery in sheep. Theriogenology **56,** 147–155.

Benyei B, Gaspardy A, Komlosi I, Pecsi A, 2004: Repeatability and heritability of ovulation number and embryos in dam-daughters pairs in superovulated Holstein-Friesian cows. Reprod Domest Anim **39,** 99–102.

Bergfelt DR, Ginther OJ, 1985: Delayed follicular development and ovulation following inhibition of FSH with equine follicular fluid in the mare. Theriogenology **24,** 99–108.

Bergfelt DR, Mann BG, Schwartz NB, Ginther OJ, 1991: Circulating concentrations of immunoreactive inhibin and FSH during the estrous cycle of mares. J Equine Vet Sci **11,** 319–322.

Boni R, Roelofsen MWM, Pieterse MC, Kogut J, Kruip TA, 1997: Follicular dynamics, repeatability and predictability of follicular recruitment in cows undergoing repeated follicular puncture. Theriogenology **48,** 277–289.

Burns DS, Jimenez-Krassel F, Ireland JLH, Knight PG, Ireland JJ, 2005: Numbers of antral follicles during follicular waves in cattle: evidence for high variation among animals, very high repeatability in individuals, and an inverse association with serum follicle-stimulant hormone concentrations. Biol Reprod **73,** 54–62.

Carnevale EM, Bergfelt DR, Ginther OJ, 1993: Aging effects on follicular activity and concentrations of FSH, LH, and progesterone in mares. Anim Reprod Sci **31,** 287–299.

Conover WJ, 1999: Practical Nonparametric Statistics, 3rd edn. John Wiley & Sons Inc, New York, pp. 269–427.

Donadeu FX, Ginther OJ, 2002: Follicular waves and circulating concentrations of gonadotrophins, inhibin, and oestradiol during the anovulatory season in mares. Reproduction **124,** 875–885.

Donadeu FX, Ginther OJ, 2003: Suppression of circulating concentrations of FSH and LH by inhibin and estradiol during the initiation of follicle deviation in mares. Theriogenology **60,** 1423–1434.

Gastal EL, Gastal MO, Bergfelt DR, Ginther OJ, 1997: Role of diameter differences among follicles in selection of a future dominant follicle in mares. Biol Reprod **57,** 1320–1327.

Gastal EL, Gastal MO, Wiltbank MC, Ginther OJ, 1999: Follicle deviation and intrafollicular and systemic estradiol concentrations in mares. Biol Reprod **61,** 31–39.

Gastal EL, Gastal MO, Ginther OJ, 2006: Relationships of changes in B-mode echotexture and colour-Doppler signals in the wall of the preovulatory follicle to changes in systemic oestradiol concentrations and the effects of human chorionic gonadotrophin in mares. Reproduction **131,** 699–709.

Ginther OJ1992: Reproductive Biology of the Mare: Basic and Applied Aspects, 2nd edn. Equiservices Publishing Cross Plains, WI.

Ginther OJ, 1995: Ultrasonic Imaging and Animal Reproduction: Book 2, Horses. Equiservices Publishing Cross Plains, WI.

Ginther OJ, 2000: Selection of the dominant follicle in cattle and horses. Anim Reprod Sci **60–61,** 61–79.

Ginther OJ, Beg MA, Bergfelt DR, Donadeu FX, Kot K, 2001: Follicle selection in monovular species. Biol Reprod **65,** 638–647.

Ginther OJ, Beg MA, Donadeu FX, Bergfelt DR, 2003: Mechanism of follicle deviation in monovular farm species. Anim Reprod Sci **78,** 239–257.

Ginther OJ, Beg MA, Gastal MO, Gastal EL, 2004a: Follicle dynamics and selection in mares. Anim Reprod **1,** 45–63.

Ginther OJ, Gastal EL, Gastal MO, Bergfelt DR, Baerwald AR, Pierson RA, 2004b: Comparative study of the dynamics of follicular waves in mares and women. Biol Reprod **71,** 1195–1201.

Ginther OJ, Beg MA, Gastal EL, Gastal MO, Baerwald AR, Pierson RA, 2005a: Systemic concentrations of hormones during the development of follicular waves in mares and women: a comparative study. Reproduction **130,** 379–388.

Ginther OJ, Gastal EL, Gastal MO, Beg MA, 2005b: Regulation of circulating gonadotropins by the negative effects of ovarian hormones in mares. Biol Reprod **73,** 315–323.

Ginther OJ, Gastal EL, Gastal MO, Beg MA, 2006a: Conversion of a viable preovulatory follicle into a hemorrhagic anovulatory follicle in mares. Anim Reprod **3,** 29–40.

Ginther OJ, Utt MD, Bergfelt DR, Beg MA, 2006b: Controlling interrelationships of progesterone/LH and estradiol/LH in mares. Anim Reprod Sci **95,** 144–150.

Ginther OJ, Utt MD, Beg MA, 2007a: Follicle deviation and diurnal variation in circulating hormone concentrations in mares. Anim Reprod Sci **100,** 197–203.

Ginther OJ, Utt MD, Beg MA, Gastal EL, Gastal MO2007b: Negative effect of estradiol on LH throughout the ovulatory LH surge in mares. Biol Reprod doi:10.1095/biolreprod.107.061705.

Ireland JJ, Ward F, Jimenez-Krassel F, Ireland JLH, Smith GW, Lonergan P, Evans ACO2007: Follicle numbers are highly repeatable within individual animals but are inversely correlated with FSH concentrations and the proportion of good-quality embryos after ovarian stimulation in cattle. Hum Reprod doi:10.1093/humrep/demo071.

Miller KF, Wesson JA, Ginther OJ, 1979: Changes in concentrations of circulating gonadotropins following administration of equine follicular fluid to ovariectomized mares. Biol Reprod **21,** 867–872.

Nambo Y, Hasegawa T, Sato F, Oki H, Kusunose R, Nakai R, Nagata S, Watanabe G, Taya K, 2002: The release of follicular fluid into the peritoneal cavity during ovulation in mares. Theriogenology **58,** 545–548.

Pierson RA, Ginther OJ, 1987: Follicular population dynamics during the estrous cycle of the mare. Anim Reprod Sci **14,** 219–231.

Scheffer GJ, Broekmans FJ, Dorland M, Habbema JD, Looman CW, te Velde ER, 1999: Antral follicle counts by transvaginal ultrasonography are related to age in women with proven natural fertility. Fertil Steril **72,** 845–851.

Singh J, Dominguez M, Jaiswal R, Adams GP, 2004: A simple ultrasound test to predict the superstimulatory response in cattle. Theriogenology **62,** 227–243.

Townson DH, Pierson RA, Ginther OJ, 1989: Characterization of plasma progesterone concentrations for two distinct luteal morphologies in mares. Theriogenology **32,** 197–204.

Submitted: 21 Aug 2007

Author's address (for correspondence): O.J. Ginther, Pathobiological Sciences, 1656 Linden Drive, University of Wisconsin, Madison, Wisconsin 53706, USA. E-mail: ginther@svm.vetmed.wisc.edu