JÚLIO CÉSAR FERRAZ JACOB

DINÂMICA OVARIANA E ENDÓCRINA DE ÉGUAS EM DIFERENTES IDADES

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Zootecnia, para obtenção do título de *Doctor Scientiae.*

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BIOGRAFIA

JÚLIO CÉSAR FERRAZ JACOB, filho de Nilda Junqueira Ferraz e José Nilton Negreiros Jacob, nasceu aos 16 de fevereiro de 1961 em São Lourenço, Estado de Minas Gerais.

Em agosto de 1982, ingressou na UFRRJ no curso de Medicina Veterinária, concluindo-o em janeiro de 1987.

Trabalhou como Médico Veterinário autônomo em clínica de sua propriedade (Clínica Veterinária Centauros), em Nova Iguaçu-RJ, juntamente com o sócio e amigo Renato Gáudio. Além disso, trabalhou como consultor de alguns Haras de Manga-Larga Marcador, na Baixada Fluminense.

Em março de 1996, ingressou no curso de mestrado em Medicina Veterinária, área de concentração em Patologia e Reprodução Animal na UFRRJ, concluindo-o em concluindo-o em agosto de 1997.

Nos anos de 1996 e 1997, foi professor substituto do Departamento de Reprodução e Avaliação Animal (DRAA) da UFRRJ.

Em 1998 foi efetivado como professor do DRAA – UFRRJ por meio de Concurso Público Federal.

Em março de 2003, iniciou o curso de doutorado na Universidade Federal de Viçosa, onde permaneceu por dois anos, quando se deslocou para a University of Wisconsin, Madison, WI, EUA, onde permaneceu por quase dois anos. Neste período, realizou os experimentos no laboratório do Prof. Dr. O. J. Ginther, retornando para a UFV em 2007 para a defesa da tese.

Atualmente é professor Assistente IV do DRAA-UFRRJ, ministrando aulas para os cursos de Medicina Veterinária e Zootecnia.

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RESUMO

JACOB, Júlio César Ferraz, D. Sc., Universidade Federal de Viçosa, julho de 2007. **Dinâmica ovariana e endócrina de éguas em diferentes idades**. Orientador: Giovanni Ribeiro de Carvalho. Co-orientadores: Ciro Alexandre Alves Torres e Oliver Joseph Ginter.

O presente estudo foi dividido em três experimentos. No Experimento 1, visou-se caracterizar os hormônios reprodutivos e a população folicular verificados próximos da ovulação em éguas de diferentes idades, a saber: éguas jovens (4 a 6 anos), éguas de meia idade (10 a 14 anos) e éguas velhas (acima de 18 anos). Amostras de sangue foram coletadas diariamente, sendo analisados os hormônios FSH, LH, progesterona e estradiol. Os folículos foram divididos em classes foliculares, de acordo com os seguintes diâmetros: a) 2-5 mm, b) 5,1-10 mm, c) 10,1-15 mm, d) 15,1-20 mm, e) 20,1-25 mm e f) = 25,1 mm. As diferenças encontradas entre os grupos incluem: baixa concentração de LH ao redor da ovulação em éguas pônei com mais de 18 anos e um crescimento folicular mais rápido do folículo dominante em éguas jovens (5-6 anos). No experimento 2, as condições foliculares e hormonais envolvidas na divergência e no desenvolvimento de um folículo (1DF) dominante (= 28 mm) *versus* dois folículos dominantes (2DF) foram estudados em 32 ondas foliculares em éguas. Os folículos foram ordenados a cada dia como F1 (maior) a F3. O início da divergência foi designado como dia 0 e precedido do primeiro aumento da diferença entre os diâmetros dos F1 e F2 para o grupo 1DF e entre a combinação de F1 e F2 *versus* F3 para o grupo 2DF. As éguas apresentaram 1DF e 2DF em 21 (66%) e 11 (34%) das ondas, respectivamente. No Dia 0, as características das ondas com 1DF e 2DF foram, similares apesar da diferença do declíneo do perfil de FSH. Entretanto, o dia específico do declíneo do FSH ou a concentração específica, não foram fatores que iniciaram a divergência. Assim, depreende-se dos resultados obtidos no presente experimento, que as concentrações hormonais não dão sustentação ao desenvolvimento de dois folículos dominantes. No experimento 3, os dados foram coletados diariamente de 23 éguas durante dois interbalos ovulatórios consecutivos (IOIs). Varias e novas significantes ($P < 0{,}05$) observações foram feitas. A concentração de FSH aumentou entre os Dias -1 e 0 (ovulação = Dia 0), com um platô entre os Dias 0 e 1, e houve um rebote entre os dias 1 e 2. A repetibilidade dentro das éguas foi baseada na significante correlação positiva entre os dois IOIs. Os seguintes pontos foram correlacionados significativamente: 1) duração do intervalo entre ovulações ($r = +0.67$) e entre a ovulação e o começo da divergência folicular ($r = +0{,}58$); 2) o diâmetro do folículo pré-ovulatório nos Dias -3 a -1 (média, $r = +0{,}49$); 3) o número de folículos por classes de diâmetro de 2 a 5 mm ($r = +0{,}65$ a média da correlação diária para o IOI; individual correlação significativa diária nos dias 22/23), 5,1 a 10 mm ($r = +0{,}49$; 18/23 dias), 10,1 a 15 mm ($r = +0{,}40$; 12/23 dias), e 15,1 a 20 mm ($r = +0{,}42$; 12/23 dias); e FSH ($r = +0{,}55$; 18/23 dias) e a concentração de LH ($r = +0{,}59$; 22/23 dias). A repetibilidade para o número de folículos foi maior na classe de menor diâmetro (2 a 5 mm). Os resultados demonstraram uma repetibilidade mensurável dentro de cada égua para vários pontos entre consecutivos IOIs. Mais estudos são necessários para se determinar o efeito da idade sobre a atividade reprodutiva de éguas pônei acima de 24 anos de idade.

ABSTRACT

JACOB, Júlio César Ferraz, D. Sc., Universidade Federal de Viçosa, July, 2007. **Ovarian and endocriny dinamyc of mares with different ages.** Adviser: Giovanni Ribeiro de Carvalho. Co-advisers: Ciro Alexandre Alves Torres and Oliver Joseph Ginter.

The present study was divided in tree experiments, led with the intention of characterizing the reproductive hormones and the population follicular close to the ovulation in mares of different ages, young (4 to 6 years), middle (10 to 14 years) and old mares (above 18 years). Samples of blood were collected daily and the analyzed hormones were FSH, LH, progesterone and estradiol. Number of follicles was determined by grouping the follicles diameters into the following categories: a) 2-5 mm, b) 5,1-10 mm, c) 10,1-15 mm, d) 15,1-20 mm, e) 20,1-25 mm, and f) = 25,1 mm. Differences found among age groups include: great concentrations of LH around the ovulation in more 18 years old pony mares and more rapid growth of the largest follicles in the youngest mares (5-6 years). Experiment 2, the follicular and hormonal aspects of diameter deviation and development of one dominant (= 28 mm) follicle (1DF) versus two dominant follicles (2DF) was studied in 32 follicular waves in mares. Follicles were ranked each day as F1 (largest) to F3. The beginning of deviation was designated Day 0 and preceded the first increase in the differences in diameter between F1 and F2 in the 1DF group and between a combination of F1 and F2 versus F3 in the 2DF group, 1DF and 2DF developed in 21 (66%) and 11 (34%) waves, respectively. The similarities on Day 0 in the characteristics of 1DF and 2DF waves despite the differences in the declining portions of the FSH profiles indicated that a specific day of the FSH decline or a specific concentration were not factors in initiating deviation. Results did not indicate a hormonal basis for the development of 2DF. Experiment 3, Repeatability within mares was based on a significant positive correlation between the two IOIs. The following end points were correlated significantly: 1) length of the interval between ovulations (r = +0.67) and between ovulation and the beginning of follicle deviation (r = +0.58); 2) diameter of the preovulatory follicle on Days -3 to -1 (average, r = +0.49); 3) number of follicles in diameter classes of 2 to 5 mm (r = +0.65 average of the daily correlations for the IOI; significant individual daily correlations on 22/23 days), 5.1 to 10 mm (r = +0.49; 18/23 days), 10.1 to 15 mm (r = +0.40; 12/23 days), and 15.1 to 20 mm (r = +0.42; 12/23 days); and FSH (r = +0.55; 18/23 days) and LH concentrations (r = +0.59; 22/23 days). The repeatability for number of follicles was greatest in the smallest diameter class (2 to 5 mm). Results demonstrated measurable repeatability within mares for several end points between consecutive IOIs. More studies are necessary to determine the effect of age in the reproductive activity in mares pony above 24 years of age.

1. INTRODUÇÃO

Fêmeas mamíferas apresentam declínio gradativo da capacidade reprodutiva com o avançar da idade; entretanto, os mecanismos envolvidos na regulação do envelhecimento ovariano em animais senis e na mulher durante o período da menopausa permanecem obscuros (BELLINO, 2000), Ginther (1995) salienta a gerontologia reprodutiva na espécie eqüina como uma das áreas de grande impacto econômico na indústria eqüina. Entretanto, pesquisas objetivando estudar a atividade ovariana e uterina de éguas idosas têm sido surpreendentemente negligenciadas.

A disfunção ovariana tem sido responsabilizada como causa do decréscimo da eficiência reprodutiva em éguas idosas (WESSON e GINTHER, 1981). Neste estudo, observou-se que o número de folículos ovarianos de 2 a 10 mm de diâmetro foi significativamente menor em éguas pôneis idosas (= 15 anos de idade), quando comparado ao de éguas jovens e de média (6 a 15 anos de idade). Além disso, tem sido observado que éguas idosas (acima de 20 anos) apresentaram, além de menor número de folículos, menor taxa de crescimento do folículo ovulatório e intervalo interovulatório (IIO) prolongado (CARNEVALE *et al*., 1993).

O efeito da idade sobre a atividade ovariana tem sido caracterizado também pelo decréscimo do volume ovariano, pela redução do número de folículos antrais e pela menor vascularização do estroma intra-ovariano em mulheres idosas (KUPESIC *et al*., 2003).

Na égua, a idade pode também afetar os níveis dos hormônios reprodutivos (CARNEVALE *et al*., 1993), embora em bovinos os níveis de gonadotropinas não tenham sido afetados pela idade das vacas (MALHI *et al*., 2005). Diante dos trabalhos consultados pretendeu-se com estes experimentos estabelecer o padrão hormonal e a dinâmica folicular durante o ciclo estral de éguas em diferentes idades. Para isto, objetivou-se:

- determinar a dinâmica folicular e a endocrinologia associada à principal onda ovulatória, apresentando uma e duas divergências, e também verificar a associação da presença de duas divergências e de folículo co-dominante;
- comparar o desenvolvimento folicular e endocrinológico de éguas apresentando um ou dois folículos dominantes;
- caracterizar a ocorrência de duas divergências e de folículo co-dominante em éguas de diferentes grupos etários; e
- caracterizar e verificar a repetibilidade da dinâmica dos folículos, gonadotropinas, e estradiol durante dois intervalos interovulatórios consecutivos.

A tese foi dividida em artigos, apresentados de acordo com as normas dos periódicos a serem submetidos. Apenas o primeiro artigo, redigido em português, não teve seu destino ainda definido quanto à publicação. Entretanto, o segundo e terceiro artigos foram encaminhados para a revista *Reproduction in Domestic Animals.*

2. REVISÃO DE LITERATURA

2.1. Hormônios reprodutivos

Concentrações sistêmicas de hormônio folículo estimulante (FSH) e a atividade folicular durante a fase luteal foram maiores em éguas de meia-idade (de 15 a 19 anos), quando comparadas às de éguas idosas (> 20 anos) ou jovens (de 5 a 7 anos) (CARNEVALE *et al*., 1993).

Por outro lado, as concentrações sistêmicas de hormônio luteinizante (LH) não diferiram entre as éguas jovens (de 5 a 7 anos) e com idade intermediária (de 15 a 19 anos), Porém, éguas idosas (= 20 anos) apresentaram menores concentrações de LH durante o segundo período ovulatório, embora esses baixos valores não tenham interferido com o processo de ovulação nesses animais (CARNEVALE *et al*., 1993). Em bovinos, as concentrações de LH não foram diferentes entre vacas e novilhas durante o IIO ou durante a onda de LH no período ovulatório (MALHI *et al*., 2005). Similarmente, em mulheres idosas não têm sido observadas alterações na concentração de LH (KLEIN *et al*., 1996; 2002).

Em éguas, o efeito da idade sobre as concentrações plasmáticas de progesterona não foi observado durante dois períodos ovulatórios (VANDERWALL *et al*., 1993). Estes resultados são similares aos estudos envolvendo bovinos, onde as concentrações plasmáticas de progesterona durante o IIO não deferiram entre vacas idosas e novilhas (MALHI *et al*., 2005). Diferentemente, Carnevale *et al*. (1993) observaram maiores concentrações de progesterona em éguas idosas quando comparadas a éguas com idade intermediária.

Até o presente momento não foi encontrado qualquer estudo evolvendo o efeito da idade sobre as concentrações sistêmicas de estradiol na espécie eqüina. Em bovinos, os níveis plasmáticos de estradiol durante os sete dias antes da ovulação foram maiores (P = 0,01) em vacas idosas do que em novilhas; entretanto, o pico preovulatório de estradiol não diferiu entre os grupos (MALHI *et al*., 2005). Paralelamente, a secreção de estradiol foi significativamente mais elevada durante a fase folicular em mulheres idosas do que em jovens (KLEIN *et al*., 1996; 2002; SANTORO *et al*., 2003).

2.2. Dinâmica folicular

Na égua, o desenvolvimento folicular apresenta um padrão de ondas foliculares (GINTHER, 1992; GINTHER e BERGFELT, 1994), sendo uma onda folicular principal e a outra secundária (GINTHER e BERGFELT, 1992; GINTHER, 1993). Na onda principal, o maior folículo atinge o diâmetro do folículo dominante (= 28 mm). Entretanto, na onda secundária o diâmetro máximo do maior folículo atinge 22 ou 23 mm (GINTHER, 1993; GINTHER *et al*., 2004; 2004d). O futuro folículo dominante e o segundo maior folículo da onda divergem gradualmente de diâmetro, a partir do dia da emergência folicular (GINTHER *et al*., 1989; GINTHER e BERGFELT, 1993). No meio do ciclo estral (aproximadamente no dia 12), sendo o dia zero o dia da ovulação, o folículo dominante atinge seu diâmetro (6 mm) um dia antes do futuro folículo subordinado (GASTAL *et al*., 1997). Quando da divergência para a seleção folicular, o diâmetro folicular é caracterizado por um crescimento continuo do maior folículo, acompanhado pela redução ou cessação da taxa de crescimento ou da regressão dos folículos menores. Quando o maior folículo alcança o diâmetro aproximadamente de 21 a 23 mm, o que ocorre, em média aos seis dias após a emergência, verifica-se o fim da fase comum de crescimento e o início da divergência (GINTHER *et al*., 1989; GASTAL *et al*., 1997; GINTHER, 2000). O mecanismo da divergência deve impedir o crescimento contínuo do futuro folículo subordinado, desde que o mesmo seja capaz de se tornar dominante, como indicado nos estudos envolvendo aspiração do folículo dominante em vacas e éguas (GINTHER, 2000). O início da esperada divergência baseou-se nos diâmetros atingidos pelos folículos, em estudos anteriores, quando a divergência folicular foi utilizada para procedimentos experimentais capazes de interferir ou terminar ao redor da divergência, quando os folículos atingem 22,5 mm de diâmetro em éguas (GINTHER, 2000).

Dois folículos dominantes (F1 e F2) são definidos como tal que alcançam > 28 mm de diâmetro em éguas (GINTHER *et al.*, 2004d). Quando dois folículos dominantes estão presentes, o F3 tem sido usado como o maior folículo subordinado para determinar o dia da divergência, que pode ser comparado com o dia da divergência individual, acompanhado por um folículo dominante (GINTHER *et al.*, 2004d). Fisiologicamente, o folículo dominante selecionado cresce, atingindo um maior diâmetro (= 28 mm), embora os outros regridam (onda principal e secundária) ou ovulem (onda ovulatória). O folículo remanescente (folículo subordinado) sofre atresia (GINTHER *et al.*, 2004). A incidência de dois folículos dominantes é maior do que a de dupla ovulação, de forma que um dos folículos pode regredir (GINTHER *et al.*, 2004d).

Comparando-se o maior folículo de uma onda com o maior dos dois folículos dominantes de outra onda, observou-se que a última situação o folículo foi menor que em relação ao folículo dominante da primeira situação, quando os dois folículos estavam no mesmo ovário. Entretanto, esta diferença reduziu para 4 mm quando os dois folículos estavam em ovários opostos (GINTHER, 1995).

O início da divergência ocorre quando há decréscimo de FSH. O decréscimo da concentração de FSH, quando o folículo atinge um estágio apropriado, pode ser anulado pela aplicação de FSH exógeno (ADAMS *et al.*, 1993), pela manutenção da alta concentração endógena de FSH resultante da aplicação de antiinibina (KANEKO *et al.*, 1997) ou progesterona (BERGFELT *et al.*, 2001). Assim, a divergência é experimentalmente adiada ou impedida, o que pode resultar no desenvolvimento de mais de um folículo dominante. Após o início da divergência, a concentração de FSH continua diminuindo por 10 a 20 h em vacas e por vários dias em éguas (GINTHER *et al.*, 2001). Desde o pico da onda de FSH até o começo da divergência, todos os folículos da onda contribuem para o decréscimo do FSH, como demonstrado pela manipulação do número de folículos (DONADEU e GINTHER, 2001).

O declínio da concentração de FSH desde o pico da onda até o início da divergência folicular tem sido descrito à medida que se aumenta a inibina e o número de folículos (DONADEU e GINTHER, 2001). A concentração de inibina total começa a aumentar antes do início do declínio da onda estimulatória de FSH (BERGFELT *et al.*, 1991; IRVINE *et al.*, 2000; BERGFELT *et al.*, 2001; DONADEU e GINTHER, 2001).

A elevação passageira de LH ocorre durante a divergência como parte da onda ovulatória de LH (GASTAL *et al*., 1997; 1999a; 2000; BERGFELT *et al*., 2001). Em éguas, a onda de LH ocorre em torno do 6º ou 7º dia antes da ovulação, alcançando o máximo um dia após a ovulação (WHITMORE *et al*., 1973).

O estradiol ou qualquer fração protéica do fluido folicular suprime a concentração de FSH circulante (MILLER *et al*., 1979; 1981; BERGEFELT e GINTHER, 1986). Ao redor de um dia antes da divergência o estradiol circulante começa a aumentar (GASTAL *et al*., 1999a; 1999c; BERGFELT *et al*., 2001). Desse modo, o aumento do estradiol aparentemente não contribui para o declínio do FSH até um dia antes da divergência (GINTHER *et al*., 2001).

Estudos recentes em vacas têm demonstrado uma repetibilidade considerável, dentro de animais, quanto ao número de folículos durante a onda folicular e uma associação inversa entre o número de folículos e a concentração de FSH (BURNS *et al.*, 2005; IRELAND *et al.*, 2007). Vacas com um pequeno (= 15) ou elevado (> 25) número de folículos foram comparadas para diferentes concentrações de FSH, alinhadas ao pico de FSH e associadas com a primeira onda anovulatória, pós-ovulação**.** A concentração de FSH foi baixa e o número de folículos = 3 mm foi maior em vacas selecionadas para elevado número de folículos *versus* baixo número de folículos. Estudos similares de repetibilidade não têm sido citados para éguas e mulheres; porém, tem sido notado em recente estudo que a concentração de inibina em éguas ovariectomizadas teve correlação significativa entre dias (GINTHER *et al*., 2005b).

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EFEITO DA IDADE SOBRE A DINÂMICA OVARIANA E ENDÓCRINA DE ÉGUAS

Resumo: O presente estudo foi conduzido visando caracterizar os hormônios reprodutivos e a população folicular verificados próximos da ovulação em éguas de diferentes idades, a saber: éguas jovens (de 4 a 6 anos), éguas de meia-idade (de 10 a 14 anos) e éguas velhas (acima de 18 anos). Amostras de sangue foram coletadas diariamente, sendo analisados os hormônios FSH, LH, a progesterona e o estradiol. Folículos = 15 mm foram mensurados (média da largura x altura), bem como se estimou o diâmetro de todos os outros folículos (= 2,0 mm a < 15,0 mm). Os diâmetros dos seis maiores folículos foram anotados, sem que se considerasse a sua identidade dia-a-dia. Todos os folículos, mensurados e estimados, foram utilizados para se determinar o número de folículos a cada dia. Os folículos foram divididos em classes foliculares, de acordo com os seguintes diâmetros: a) 2 a 5 mm; b) 5,1 a 10 mm; c) 10,1 a 15 mm; d) 15,1 a 20 mm; e e) 20,1 a 25 mm; e f) = 25,1 mm. As diferenças encontradas entre os grupos incluem: baixa concentração de LH ao redor da ovulação em éguas pônei com mais de 18 anos e um crescimento folicular mais rápido do folículo dominante em éguas jovens (de 5 a 6 anos). Ao redor da ovulação, de quatro dias antes a quatro dias após a sua ocorrência, o número de folículos para as classes foliculares de 5,1 a 0,10, 1 a 15, 15,1 a 20 mm foi menor nas éguas velhas. Embora tenham sido encontradas algumas diferenças entre os grupos etários, este estudo demonstrou serem os hormônios reprodutivos e a atividade folicular mantidos em éguas velhas. Mais estudos são necessários para se determinar o efeito da idade sobre a atividade reprodutiva de éguas pônei acima de 24 anos de idade.

EFFECT OF AGE ON OVARIAN AND ENDOCRINY DINAMYC IN MARES

Abstract: The present study was led with the intention of characterizing the reproductive hormones and the population follicular close to the ovulation in mares of different ages, young (4 to 6 years), middle (10 to 14 years) and old mares (above 18 years). Samples of blood were collected daily and the analyzed hormones were FSH, LH, progesterone and estradiol. Diameters of follicles = 15 mm were measured (average of height and width) and the diameters of all other follicles (= 2 mm and < 15 mm) were estimated. The diameters of the six largest follicles on each day were recorded without regard to day-to-day identity. The measured and estimated diameters of all follicles were used to determine the number of follicles on each day. Number of follicles was determined by grouping the follicles diameters into the following categories: a) 2-5 mm; b) 5.1-10 mm; c) 10.1-15 mm; d) 15.1-20 mm; e) 20.1-25 mm; and f) = 25.1 mm. Differences found among age groups include: great concentrations of LH around the ovulation in more 18 years old pony mares and more rapid growth of the largest follicles in the youngest mares (5-6 years), Around ovulation, mean 4 days before to 4 days after ovulation, the number of the follicles of the follicular classes 5.1-10, 10.1-15, 15.1-20 mm was smallest in old mares. Although found some differences among groups of age, that study demonstrated that reproductive hormones and activity follicular are maintained in considered old mares. More studies are necessary to determine the effect of age in the reproductive activity in mares pony above 24 years of age.

1. Introdução

Fêmeas mamíferas apresentam declínio gradativo da capacidade reprodutiva com o avançar da idade. Entretanto, os mecanismos envolvidos na regulação do envelhecimento ovariano em animais senis e na mulher durante o período da menopausa permanecem obscuros (BELLINO, 2000). Ginther (1995) salienta a gerontologia reprodutiva na espécie eqüina como uma das áreas de grande impacto econômico na indústria eqüina. Entretanto, pesquisas objetivando estudar a atividade ovariana e uterina de éguas idosas têm sido surpreendentemente negligenciadas.

A disfunção ovariana tem sido responsabilizada como causa do decréscimo da eficiência reprodutiva em éguas idosas (WESSON e GINTHER, 1981). Neste estudo, observou-se que o número de folículos ovarianos de 2 a 10 mm de diâmetro foi significativamente menor em éguas pôneis idosas (= 15 anos de idade), quando comparado ao de éguas jovens e de meia-idade (6 a 15 anos de idade). Além disso, cinco éguas com idade entre 17 e 25 anos apresentaram atrofia ovariana caracterizada pela ausência de atividade folicular e, ou, corpo lúteo (WESSON e GINTHER, 1981). Além disso, tem sido observado que éguas idosas (acima de 20 anos) apresentaram, além de um menor número de folículos, menor taxa de crescimento do folículo ovulatório e intervalo interovulatório (IIO) prolongado (CARNEVALE *et al*., 1993). A duração do intervalo interovulatório não variou com a idade, quando foram comparadas vacas (13 a 14 anos de idade) com suas filhas (1 a 4 anos de idade) (MALHI *et al*., 2005). Em mulheres idosas durante a fase de transição que precede a menopausa as ovulações ocorrerem regularmente, embora os ciclos menstruais comecem a reduzir gradativa-mente em decorrência da redução da fase folicular (KLEIN *et al*., 2002). O efeito da idade sobre a atividade ovariana tem sido caracterizado também pelo decréscimo do volume ovariano, pela redução do número de folículos antrais e pela menor vascularização do estroma intra-ovariano (KUPESIC *et al*., 2003),

Originalmente, éguas têm 40.000 folículos primordiais e ciclam continuamente por 25 anos; assim, usando em média 100 folículos/ciclo poderá esgotar toda a sua reserva de folículos primordiais (100 folículos x 16 ciclos/ano x 25 anos = 40.000 folículos primordial) neste período. Presumivelmente, o pool de folículos primordiais é finito, principalmente por não ter sido citado, até o presente momento, a ocorrência de mitoses em oogônias de potras ou éguas (GINTHER, 1993).

Concentrações sistêmicas de hormônio folículo estimulante (FSH) associadas à atividade folicular durante a fase luteal foram maiores em éguas de meia-idade (de 15 – 19 anos), quando comparadas às de éguas idosas (> 20 anos) ou jovens (5 a 7 anos; CARNEVALE *et al.*, 1993). Esses resultados corroboram achados em bovinos, quando foram observadas que durante o IIO as concentrações plasmáticas de FSH foram mais elevadas em vacas idosas do que em novilhas (MALHI *et al.*, 2005). Em mulheres idosas, o aumento da concentração sistêmica de FSH tem sido atribuído à redução da secreção de inhibina B (KLEIN *et al.*, 2002; SANTORO *et al.*, 2003).

Em éguas, as concentrações sistêmicas de hormônio luteinizante (LH) não diferiram entre os grupos etários, envolvendo éguas jovens (de 5 a 7 anos) e de idade intermediária (de 15 a 19 anos); porém, éguas idosas (= 20 anos) apresentaram menores concentrações de LH durante o segundo período ovulatório. Entretanto, esses baixos valores não interferiram com o processo de ovulação nesses animais (CARNEVALE *et al.*, 1993). Em bovinos, as concentrações de LH não foram diferentes entre vacas e novilhas durante o IIO ou durante a onda de LH no período ovulatório (MALHI *et al.*, 2005). Similarmente, em mulheres idosas não têm sido observadas alterações quanto a concentração de LH (KLEIN *et al.*, 1996; 2002).

Em éguas, o efeito da idade sobre as concentrações plasmáticas de progesterona não foi observado durante dois períodos ovulatórios (VANDERWALL *et al.*, 1993). Esses resultados são similares aos encontrados em bovinos, onde as concentrações plasmáticas de progesterona durante o IIO não deferiram entre vacas idosas e novilhas (MALHI *et al.*, 2005). Diferentemente, Carnevale *et al.* (1993) observaram maiores concentrações de progesterona em éguas idosas quando comparadas às de éguas com idade intermediária.

Até o presente momento, não tem sido estudado o efeito da idade sobre as concentrações sistêmicas de estradiol na espécie eqüina. Em bovinos, os níveis plasmáticos de estradiol durante os sete dias antes da ovulação foram maiores ($P = 0,01$) em vacas idosas do que em novilhas; entretanto, o pico preovulatório de estradiol não diferiu entre os grupos (MALHI *et al.*, 2005). Paralelamente, durante a fase folicular inicial a secreção de estradiol foi significativamente mais elevada em mulheres idosas do que em jovens (KLEIN *et al.*, 1996, 2002; SANTORO *et al.*, 2003).

Outros estudos relacionando o efeito da idade sobre a capacidade reprodutiva de fêmeas têm focalizado anormalidades morfológicas do trato genital. Dentro deste contexto, tem-se concluído que anormalidades histopatológicas são mais comuns em animais idosos.

O objetivo desse trabalho foi estudar a dinâmica folicular e o padrão hormonal de éguas, de diferentes idades, durante o ciclo estral.

2. Material e Métodos

2.1. Animais e delineamento experimental

As éguas foram manipuladas de acordo com o Guia de Cuidados utilizado pelo Ministério da Agricultura e Animais de Pesquisa e Ensino (EUA). Éguas mestiças de pôneis, não-lactantes (n = 24), em boa condição corporal e pesando entre 250 a 400 kg foram usadas durante o período de transição anovulatório para o período ovulatório e durante a estação ovulatória de 2005 (abril a agosto, Hemisfério Norte). Os animais foram mantidos sob luz natural em um abrigo aberto e piquetes ao ar livre, às quais se forneceu feno de alfafa e grama, com acesso a água e sal mineralizado. Éguas cíclicas foram selecionadas pelo temperamento dócil e pela ausência de anormalidades aparentes do trato reprodutivo, determinado, pelo exame ultra-sonográfico (Ginther 1995). Características dentárias (AMERICAN ASSOCIATION OF EQUINE PRACTITIONERS, 2002) foram utilizadas para estimar a idade das éguas, distribuídas em três grupos experimentais com quatro anos de diferença entre os grupos, a saber: a) jovens (5 a 6 anos, n = 8), b) éguas com idade intermediária (10 a 14 anos, n = 8) e c) velhas (= 18 anos, n = 8).

2.2. Ultra-sonografia e outras considerações

Exame ultra-sonográfico B-mode em tempo real foi realizado diariamente do dia 15 até o quarto dia após a segunda ovulação, utilizando-se uma probe linear com transdutor de 5 MHz (Aloka 900). Folículos com diâmetros = 15 mm foram mensurados (média da altura e largura) estimando-se também o diâmetro de todos os outros folículos (= 2,0 mm e < 15,0 mm), por meio da utilização de uma transparência quadriculada (5 x 5 mm) por cima da tela do ultra-som. Durante a avaliação ultra-sonográfica todas as informações foram gravadas fonograficamente e mais tarde registradas por escrito em um caderno de anotações. Os diâmetros dos seis maiores folículos em cada dia foram anotados sem que se considerasse a sua identidade dia-a-dia. Os diâmetros estimados e mensurados de todos os folículos foram usados para determinar a classe à qual pertenciam, a saber: a) 2 a 5 mm; b) 5,1 a 10 mm; c) 10,1 a 15 mm; d) 15,1 a 20 mm; e) 20,1 a 25 mm; e f) = 25,1 mm. O diâmetro (média da altura e largura) do corpo lúteo foi anotado do dia zero até a estrutura não ter sido mais detectada. A ecotextura uterina foi determinada usando-se o sistema de escore de 1 a 4 (mínimo a máximo, incluindo frações como 1,5, 2,5 e 3,5), baseando-se na extensão do edema (áreas anecóicas vista ao US) e pregas uterinas (GINTHER, 1995). Antes do exame ultra-sonográfico, o tônus uterino foi determinado por palpação transrectal, como descrito previamente por Ginther (1993). O corno uterino foi colocado entre os dedos e comprimido gentilmente para determinar a sua consistência, variando o escore de 1 (mínimo ou flácido), a 4 (máximo ou túrgido). A taxa de crescimento (mudanças nos diâmetros entre exames) do folículo dominante foi anotada do dia -4 a -1 (dia 0 = ovulação). O período interovulatório (PIO) para cada égua foi normalizado para uma duração média do período interovulatório das éguas (23 dias; variação de 19 a 27 dias; BERGFELT, 1993). O procedimento para normalização foi feito da seguinte forma: a) para éguas com intervalo (PIO) mais longo do que a média, os dias excedentes foram sorteados e deletados; b) para éguas com intervalos mais curtos que a média, os dias que faltavam para a média foram sorteados e incluídos; c) os valores dos dias inseridos foram considerados como dados perdidos; e d) dias deletados ou inseridos não foram utilizados nem os primeiros quatro dias do ciclo nem os quatro dias que precederam a ovulação, assim como para dias consecutivos para determinada égua. Assim, utilizou-se do 5º ao 18º dia após a ovulação. Conseqüentemente, o perfil diário de todas as avaliações (concentrações de FSH e LH, diâmetro dos seis maiores folículos e classes foliculares) para cada égua e o perfil significa a média normalizada da duração do período interovulatório (PIO).

2.3. Amostras de sangue e ensaio hormonal

Amostras de sangue foram diariamente coletadas da jugular em tubos heparinizados do 15º dia do ciclo ou do quarto dia antes da primeira ovulação até o quarto dia após a terceira ovulação. As amostras de sangue foram centrifugadas (1.500 x*g* por 10 min), decantadas e o plasma foi estocado (-20 °C) até a análise. As concentrações de FSH e LH foram determinadas por radioimunoensaio e de estradiol e progesterona por kits comercial, como validado e descrito por vários pesquisadores (LH e FSH, DONADEU e GINTHER, 2002; progesterona, GINTHER *et al.*, 2005a; estradiol, GINTHER *et al.,* 2005b). O coeficiente de variação intra e interanálise e a sensitividade da análise foram, respectivamente, 9,2%, 18,4% e 1,1 ng/ml para FSH; 7,8%, 8,3%, e 0,2 ng/ml para LH; 10,0%, 4,9%, 0,1 pg/ml para estradiol e 5,6% (intra-análise CV) e 0,04 ng/ml para progesterona. As concentrações de Estradiol e Progesterona foram dosados dos dias -4 a +4 (D -4 a D +4) após a ovulação, sendo considerado como o dia zero, o dia da ovulação.

2.4. Análise estatística

Os dados foliculares e hormonais foram normalizados para ovulação durante os períodos interovulatorio e ovulatório. Dados hormonais foram testados para valores extremos pelo teste de Dixon. Todos os dados hormonais e foliculares foram testados para normalidade, por meio do teste de Kolmogorov-Smirnov. Quando da ausência de normalidade, os dados hormonais e foliculares foram excluídos. Dados seqüenciais dos diâmetros dos seis maiores folículos e das concentrações plasmáticas de gonadotropinas foram analisados para determinação dos efeitos de ciclo, dia e interação ciclo-por-dia dentro de cada grupo, por meio do modelo linear, considerando-se efeitos fixos e ocasionais (efeitos mesclados) e com expressão repetitiva para confirmação da auto-correlação entre dados seqüenciais (Proc Mixed; 8,2 Version; SAS, Institute Inc., Cary, NC). O teste-t para dados não-pareados foi utilizado visando estabelecer diferenças entre as médias quando o efeito ciclo, dia ou interação foi obtido. Quando da ausência de efeito significativo para o ciclo ou da interação ciclo por dia, os dados por ciclo foram combinados para se determinar o efeito grupo e a interação grupo por dia, desconsiderando-se o ciclo. Dados não seqüenciais foram analisados por ANOVA.

3. Resultados

Foi excluída do projeto uma égua de 6 anos de idade, do Grupo 1, por ter tido uma prolongada fase luteal (29 dias) com persistência do CL. Das 23 éguas que ovularam, duas éguas velhas tiveram dupla ovulação, sendo uma sincrônica e a outra ocorrendo dois dias após a primeira ovulação. A duração do PIO para éguas jovens, com idade intermediária e velhas foi de 22,6 ± 0,48, 22,4 ± 0,38, e 23,9 ± 0,55 dias, respectivamente (P = 0,06). As características das concentrações plasmáticas de FSH, LH, estradiol e progesterona foram centralizados para o dia da ovulação, estando apresentadas de forma a ilustrar a relação temporária entre esses pontos (Figura 1). O efeito de grupo foi significativo (P < 0,0001) para o LH, no intervalo de -4 a +4 dias da ovulação. Assim, observa-se que a concentração de LH começou a aumentar nas éguas jovens quatro dias antes da ovulação e alcançou o pico um dia após a sua ocorrência, decrescendo e permanecendo mais elevada que nas éguas velhas, embora não houvesse diferença do perfil hormonal apresentado, neste período, para as éguas com idade intermediária. Quanto às concentrações de FSH, estradiol, progesterona e a dinâmica dos seis maiores folículos não houve diferença (P > 0,05) entre as éguas jovens, com idades intermediárias e velhas.

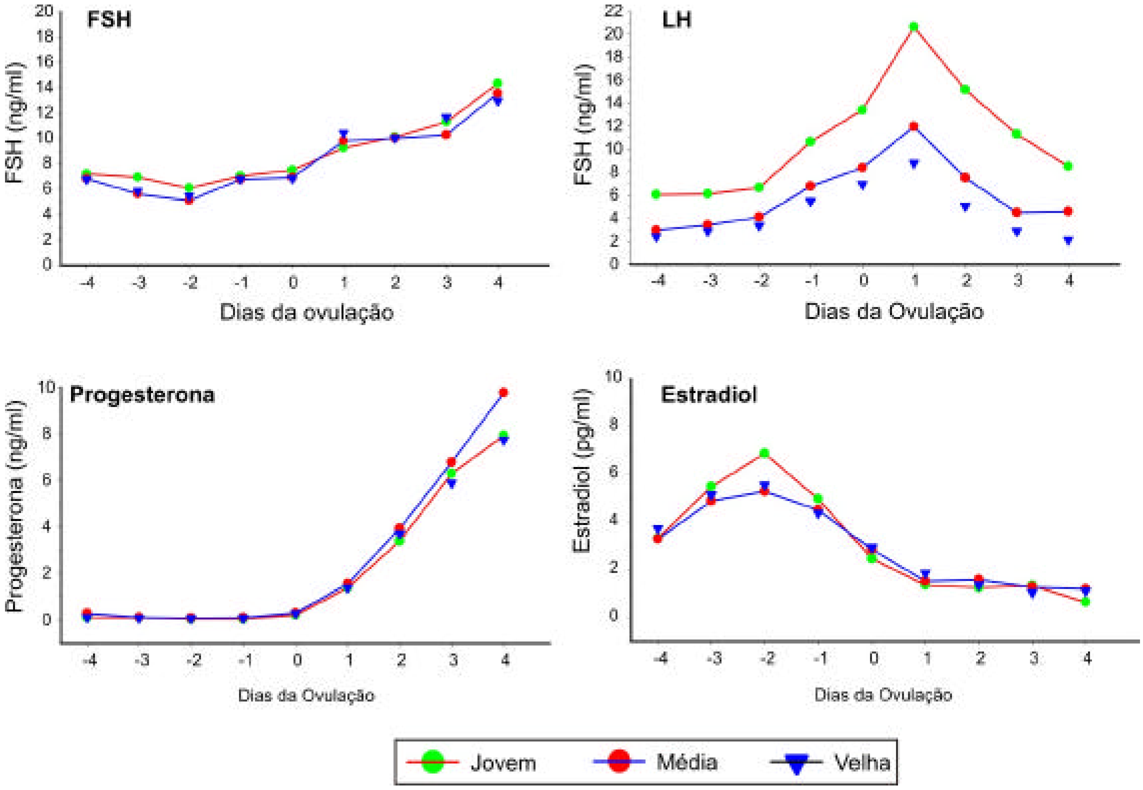

Figura 1 – FSH, LH, progesterona e estradiol centralizado para ovulação.

3.1. Diâmetro dos seis maiores folículos

Observa-se na Tabela 1 que não houve efeito de grupo (P > 0,05) sobre o diâmetro do folículo dominante, um dia antes da ovulação e no dia de sua ocorrência. A taxa de crescimento do maior folículo nos quatro dias antes da ovulação foi maior nas

Tabela 1 – Duração do ciclo e dinâmica folicular de éguas de diferentes idades no intervalo de -4 a +4 dias da ovulação

Diâmetro dos Maiores Folículos	**Jovem (n = 14)**	**Meia-idade (n = 16)**	**Velhas (n = 16)**	**P-Valor**
Duração do ciclo	22,6 ± 0,48	22,4 ± 0,38	23,9 ± 0,55	0,0567
Um dia antes da ovulação				
Primeiro	41,7 ± 0,9	42,3 ± 1,1	39,1 ± 1,3	0,0973
Segundo	23,5 ± 1,7	21,8 ± 1,3	18,9 ± 1,8	NS
Terceiro	19,0 ± 1,1^{a}	18,2 ± 0,8^{a}	14,3 ± 1,3^{b}	0,0075
Quarto	15,6 ± 1,0^{a}	16,0 ± 0,7^{a}	11,9 ± 1,1^{b}	0,0058
Quinto	13,9 ± 1,0^{ab}	14,8 ± 0,8^{a}	10,8 ± 1,0^{b}	0,0100
Sexto	12,6 ± 0,1^{ab}	13,3 ± 0,7^{a}	9,8 ± 0,9^{b}	0,0119
No dia da ovulação				
Primeiro	22,3 ± 1,8	21,1 ± 1,3	17,1 ± 1,7	0,0663
Segundo	17,1 ± 1,2^{a}	17,6 ± 0,8^{a}	13,1 ± 1,2^{b}	0,0079
Terceiro	14,5 ± 1,1^{a}	15,0 ± 0,7^{a}	10,7 ± 0,9^{b}	0,0019
Quarto	12,8 ± 1,0^{a}	13,6 ± 0,8^{a}	9,8 ± 0,8^{b}	0,0065
Quinto	11,1 ± 0,8^{ab}	12,7 ± 0,8^{a}	9,3 ± 0,8^{b}	0,0118
Sexto	9,9 ± 0,7^{ab}	11,9 ± 0,8^{a}	11,9 ± 0,8^{b}	0,0236
Taxa de crescimento (mm/dia) do maior folículo nos quatro dias antes da ovulação	3,0 ± 0,18^{a}	2,5 ± 0,13^{ab}	2,1 ± 0,32^{b}	0,0407

Valores com letras sobrescritas diferentes há diferença (P < 0,05) entre os grupos pelo teste de Kolmogorov-Smirnov.

éguas jovens do que nas éguas velhas, embora não houvesse diferença entre as jovens e as com idade intermediária, e nem entre essas e as éguas velhas. Não houve diferença (P > 0,05) quanto ao segundo maior folículo, um dia antes da ovulação, entre os grupos; entretanto, no dia da ovulação o segundo maior folículo foi menor nas éguas velhas em relação aos das éguas jovens e com idade intermediária (P < 0,008). Além disso, comparando-se do terceiro ao sexto maior folículo entre os grupos, observa-se diferença entre os mesmos, como demonstrado na Tabela 1.

3.2. Classes foliculares

O número de folículos dentro de cada classe folicular um dia antes da ovulação e no dia de sua ocorrência diferiu (P < 0,05) entre os grupos etários nas classes de 15,1 a 20 mm, para ambos os dias. As éguas velhas apresentaram menos folículos do que as de meia-idade, embora não houvesse diferenças entre elas e as éguas jovens (Tabela 2).

Tabela 2 – Número de folículos por classe e faixa etária em éguas no intervalo de -4 a +4 dias da ovulação

Diâmetro dos Maiores Folículos	**Jovem (n = 14)**	**Meia-idade (n = 16)**	**Velhas (n = 16)**	**P-Valor**
Um dia antes da ovulação				
2 a 5 mm	21,2 ± 2,7	19,9 ± 2,3	22,8 ± 2,7	NS
5,1 a 10 mm	11,8 ± 1,8	9,6 ± 0,9	9,8 ± 1,3	NS
10,1 a 15 mm	3,6 ± 0,7	5,1 ± 0,7	3,4 ± 0,7	NS
15,1 a 20 mm	2,1 ± 0,4^{ab}	2,4 ± 0,4^{a}	1,1 ± 0,2^{b}	0,0224
20,1 a 25 mm	0,6 ± 0,2	0,5 ± 0,2	0,4 ± 0,2	NS
> 25 mm	1,5 ± 0,2	1,3 ± 01	1,2 ± 0,1	NS
No dia da ovulação				
2 a 5 mm	27,1 ± 2,7	23,4 ± 2,3	25,3 ± 2,8	NS
5,1 a10 mm	13,6 ± 2,1	11,3 ± 0,9	12,2 ± 1,7	NS
10,1 a 15 mm	2,9 ± 0,7	4,3 ± 0,6	3,0 ± 0,7	NS
15,1 a 20 mm	1,4 ± 0,3^{ab}	2,1 ± 0,4^{a}	0,8 ± 0,3^{b}	0,0185
20,1 a 25 mm	0,5 ± 0,2	0,6 ± 0,2	0,2 ± 0,1	NS
> 25 mm	0,4 ± 0,2	0,2 ± 0,1	0,1 ± 0,1NS	NS

Valores com letras sobrescritas diferentes há diferença (P < 0,05) entre os grupos pelo teste de Kolmogorov-Smirnov.

Para o mesmo período, não foram observadas diferenças entre as outras classes foliculares. Ao redor da ovulação, ou seja, quatro dias antes e quatro dias depois de sua ocorrência, as éguas jovens e de meia-idade apresentaram mais folículos que as velhas para as classes foliculares de 5,1 a 10 mm (P = 0,04) e 15,1 a 20 mm (P = 0,0005). Para a classe folicular de 10,1 a 15 mm, as éguas velhas tiveram menos folículos que as de meia-idade (P = 0,002), embora não diferissem das éguas jovens. Para as outras classes foliculares não foram encontradas diferenças (P < 0,05) entre os grupos de idade (Tabela 2).

4. Discussão

Observa-se na Tabela 1 que a duração do ciclo estral não foi influenciada (P > 0,05) pela idade das éguas. Resultados similares foram obtidos por Vanderwall *et al.* (1993) que também não encontraram diferenças (P > 0,10) na duração (dias) do ciclo estral entre éguas velhas (25,5 ± 1,5) e jovens (23,2 ± 0,7), da primeira para a segunda ovulação. Diferentemente, Carnevalle (1993) encontrou intervalos de 23,9 ± 0,6, 23,0 ±

0,8 e 26,5 ± 0,7 dias para éguas jovens, com idade intermediária e velhas, respectivamente. Esse prolongado período interovulatório deveu-se ao prolongamento da fase folicular e, conseqüentemente ao prolongado intervalo da indução da luteólise até a ovulação. Um prolongado período interovulatório foi citado Vanderwall *et al.* (1993) em éguas velhas (16 a 20 anos), quando comparado ao das fêmeas entre 10 e 11 anos de idade. Entretanto, como todas as éguas foram cobertas, os autores sugeriram que o prolongado intervalo entre ovulações pode ter se devido à não-detecção de perda embrionária precoce nos animais velhos. Em vacas velhas, a duração do período interovulatório não mudou com a idade quando foram comparadas vacas velhas (13 a 14 anos) com as suas filhas (1 a 4 anos) (MALHI *et al.*, 2005). Em mulheres, observa-se que nos anos que precedem o período de transição para a menopausa, ocorrem ovulações regulares, enquanto o ciclo menstrual é progressivamente encurtado. Tal fenômeno se deve ao encurtamento da fase folicular em mulheres velhas (KLEIN *et al.*, 2002), No presente estudo, observou-se um efeito significativo de grupo sobre o LH ao redor da ovulação e no período interovulatório. Assim, a concentração de LH em éguas jovens ao redor da ovulação foi mais elevada do que em éguas de idade intermediária e nas velhas. Resultados similares em éguas foram observados por Carnevalle *et al.* (1993), quando a concentração de LH não diferiu entre as fêmeas do Grupo 1 (5 a 7 anos) e Grupo 2 (15 a 19 anos), embora as do Grupo 3 (20 anos ou mais) apresentassem uma baixa concentração de LH durante um segundo período ovulatório; porém, a concentração de LH foi suficiente para induzir a ovulação. A redução das concentrações de LH relatada em éguas velhas pode ter sido devido ao prolongamento da fase folicular (Carnevalle *et al.*, 1993), Contudo, no presente estudo não foi encontrada uma fase folicular prolongada, nem houve diferenças entre os grupos quanto à fase luteal.

Verificou-se, ainda, na Tabela 1, um efeito de idade sobre a taxa de crescimento folicular envolvendo o folículo dominante. Em éguas velhas, a taxa de crescimento folicular não diferiu das éguas de meia-idade, porém foi menor ($P < 0,05$) que nas éguas jovens, mesmo não havendo diferenças entre ciclos nem no diâmetro do maior folículo. Observou-se também que nas éguas velhas os folículos subordinados ao redor da ovulação foram menores ($P < 0,05$) do que as de éguas de meia-idade e jovens. Quanto à classe folicular, observa-se na Tabela 2 que um dia antes da ovulação e no dia de sua ocorrência, éguas velhas diferiram das de meia-idade, mas não das éguas jovens, no que se refere ao menor número de folículos de 15,1 a 20 mm de diâmetro. Wesson e Ginther (1981), examinando éguas pônei em matadouro, observaram que o número de folículos de 2 a 10 mm e de 11 a 20 mm de diâmetro foi significativamente menor em éguas velhas mais de 15 anos comparadas ao de éguas de 6 a 15 anos de idade. Observaram, também, que muitas éguas velhas (> 20 anos) eram reprodutivamente ativas. Porém, os ovários de poucas éguas, de 17 a 25 anos de idade, poderiam se classificados, aparentemente, como senescentes. Ainda sobre a classe folicular, Carnevalle *et al.* (1993) encontraram efeito de idade, incluindo efeito significativo de grupo sobre o número de folículos de 16 a 20 mm de diâmetro, bem como interação significativa dia *versus* grupo, para o diâmetro do maior folículo e número de folículos de 11 a 15 mm. Os autores sugeriram que a maior atividade folicular ocorreu no final da fase lútea para o Grupo 2 (15 a 19 anos) em relação aos Grupos 1 (5 a 7 anos) e 3 (20 anos ou mais), associada aos altos níveis de FSH durante o meio da fase luteal. Apesar de estes achados terem sugerido baixa atividade reprodutiva do grupo de éguas velhas, a inspeção dos ovários e do trato reprodutivo demonstrou que muitas éguas velhas (> 20 anos) apresentavam atividade reprodutiva (WESSON e GINTHER, 1981). Em outro estudo, não foram observadas diferenças ($P < 0,10$) do número de folículos ovarianos de 11 a 20 mm de diâmetro, quando foram comparadas éguas velhas com éguas jovens, durante o período periovulatório na primeira e segunda ovulação (VANDERWALL *et al.*, 1993). No grupo de éguas jovens, verificou-se também que teve um rápido aumento do número de folículos de 6 a 10 mm. Até o presente momento não foram determinadas as razões para o rápido crescimento folicular em éguas jovens, embora os folículos das mesmas possam ser mais responsivos ao estímulo hormonal, uma vez não terem sido detectadas diferenças entre os grupos às concentrações hormonais.

Vanderwall *et al.* (1993) não encontraram diferenças significativas nas concentrações plasmáticas de progesterona em duas ovulações entre éguas velhas e jovens. Porém, Carnevalle *et al.* (1993) demonstraram o efeito de idade, sendo que o Grupo 3 (éguas velhas) apresentou concentração de progesterona mais elevada, após a segunda ovulação, do que o Grupo 2 (éguas de meia-idade), embora o mesmo não tenha sido observado na primeira ovulação. A literatura tem registrado falha de ovulação associadas à idade. Em dois experimentos foram documentadas que quatro éguas velhas não ovularam durante um período de 60 e 90 dias na estação fisiológica (VANDERWALL *et al.*, 1993). Entretanto, essas éguas tinham 29, 30, 30 e 33 anos de idade enquanto tais falhas não foram observadas em éguas jovens.

No presente estudo não houve efeito de idade sobre as concentrações de FSH, progesterona e estradiol, demonstrando-se que éguas pônei com meia-idade ou velhas, apresentam, ainda, atividade reprodutiva normal.

5. Conclusão

As diferenças encontradas entre os grupos incluem: 1) baixa concentração de LH ao redor da ovulação em éguas pônei com mais de 18 anos; 2) crescimento folicular mais rápido do folículo dominante em éguas jovens (5 a 6 anos); 3) os diâmetros dos folículos subordinados foram menores em éguas velhas do que em éguas jovens e de idade intermediária (10 a 14 anos); 4) um dia antes e no dia da ovulação o número de folículos para a classe folicular de 15,1 a 20 mm foi maior nas éguas de meia-idade do que nas éguas velhas; e 5) ao redor da ovulação, de quatro dias antes a quatro dias após a sua ocorrência, o número de folículos para as classes foliculares de 5,1 a 10, 10,1 a 15,15,1 a 20 mm foi menor nas éguas velhas. Embora tenham sido observadas algumas diferenças entre os grupos etários nesse estudo, demonstrou-se que os hormônios reprodutivos e a atividade folicular são mantidos em éguas velhas. Mais estudos são necessários para determinar-se o efeito da idade sobre a atividade reprodutiva de éguas pônei acima de 24 anos de idade.

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FOLLICLE DEVIATION IN OVULATORY FOLLICULAR 1 WAVES WITH ONE OR TWO DOMINANT FOLLICLES IN MARES

Abstract: The follicular and hormonal aspects of diameter deviation and development of one dominant (= 28 mm) follicle (1DF) versus two dominant follicles (2DF) was studied in 32 follicular waves in mares. Follicles were ranked each day as F1 (largest) to F3. The beginning of deviation was designated Day 0 and preceded the first increase in the differences in diameter between F1 and F2 in the 1DF group and between a combination of F1 and F2 versus F3 in the 2DF group, 1DF and 2DF developed in 21 (66%) and 11 (34%) waves, respectively. Double ovulations occurred in only one of the waves with 2DF. In 8/11 waves with 2DF, a second deviation occurred between F1 and F2 on 2.5 ± 0.4 d after the first deviation. The first deviation in waves with 1DF and 2DF occurred on a similar day of the interovulatory interval and were similar on Day 0 in number of follicles, difference in diameter between F1 and F2, and plasma concentrations of LH, estradiol, and immunoreactive (ir)-inhibin. The interval from maximum FSH concentration to Day 0 was longer ($P < 0.05$) and FSH concentration was lower ($P < 0.05$) on Days -1 to 4 in the 2DF group. The similarities on Day 0 in the characteristics of 1DF and 2DF waves despite the differences in the declining portions of the FSH profiles indicated that a specific day of the FSH decline or a specific concentration were not factors in initiating deviation. Results did not indicate a hormonal basis for the development of 2DF.

DIVERGÊNCIA FOLICULAR NA ONDA OVULATÓRIA COM UM OU DOIS FOLÍCULOS DOMINANTES EM ÉGUAS

Resumo: Condições foliculares e hormonais envolvidas na divergência e no desenvolvimento de um folículo (1DF) dominante (= 28 mm) *versus* dois folículos dominantes (2DF) foram estudadas em 32 ondas foliculares em éguas. Os folículos foram ordenados a cada dia, como F1 (maior) a F3. O início da divergência foi designado como dia 0 e precedido do primeiro aumento da diferença entre os diâmetros dos F1 e F2 para o grupo 1DF e entre a combinação de F1 e F2 *versus* F3 para o grupo 2DF. As éguas apresentaram 1DF e 2DF em 21 (66%) e 11 (34%) das ondas, respectivamente. Ocorreu dupla ovulação somente em uma onda com 2DF. Em 8/11 ondas com 2DF, a segunda divergência ocorreu entre F1 e F2 aos 2,5 ± 0,4 dias após a primeira divergência. A primeira divergência nas ondas com 1DF e 2DF ocorreu no mesmo dia do intervalo interovulatório e foram semelhantes no Dia 0, quanto ao número de folículos, diferença no diâmetro entre F1 e F2, e na concentração plasmática de LH, estradiol, e (ir)-inibina imunoreativa. O intervalo da concentração máxima de FSH até o Dia 0 foi maior ($P < 0,05$) e a concentração de FSH menor ($P < 0,05$) nos dias -1 até +4 para o grupo 2DF. No Dia 0, as características das ondas com 1DF e 2DF foram similares, apesar da diferença do declínio do perfil de FSH. Entretanto, o dia específico do declínio do FSH ou a concentração específica não foram fatores que iniciaram a divergência. Assim, depreende-se dos resultados obtidos no presente experimento, que as concentrações hormonais não dão sustentação ao desenvolvimento de dois folículos dominantes.

1. Introduction

Follicle selection in monovular animals (e.g., mares, cows, women) is the process wherein usually only one follicle develops from a wave of growing follicles and continues to grow and ovulates. The eminent selection event during a follicular wave is diameter deviation and is retrospectively identifiable by continued growth of the developing dominant follicle and reduced growth and regression of the remaining follicles (subordinates; Ginther et al., 1997). The characteristics and the intrafollicular and systemic hormonal events associated with the beginning of deviation have been reviewed for mares (Ginther, 2000; Ginther et al., 2004a) and mares and heifers (Ginther et al., 2001, 2003, Beg and Ginther, 2006) and have been compared between mares and women (Ginther et al 2004b, 2005a). Briefly, dramatic changes in the insulin-like growth factor (IGF)1 system lead to increased free IGF1 in the most developed follicle before the beginning of diameter deviation and play a crucial role in the events that lead to deviation in both horses and cattle. Estradiol and LH receptors also play a role, at least in cattle. The intrafollicular events prepare the selected follicle for the decreasing availability of FSH from the wave-stimulating FSH surge and increasing availability of LH, Other follicles of the wave have a capability for future dominance similar to that of the largest follicle but do not have adequate time to attain the required preparatory stage. In this regard, the essence of deviation in heifers is a close two-way functional coupling between FSH and products of the follicles (inhibin, estradiol), so that the establishment of deviation or the destiny of the future dominant and subordinate follicles occurs in < 8 h.

In a direct comparative study of the ovulatory follicular waves between mares and women, deviation began when the largest follicle and second-largest follicle were 22.7 and 20.3 mm in mares and 10.3 and 9.0 mm in women (Ginther et al., 2004b). The relative diameter of the largest follicle between the two species was similar at discernible events throughout the follicular wave. Thus, the follicle was 2.1 or 2.2 times larger in mares than in women at the peak of the wave-stimulating FSH surge (13.0/6.0 mm), at the beginning of deviation (22.7/10.3 mm), and at maximum diameter of the preovulatory follicle (44.8/21.8 mm). Other similarities in the ovulatory wave between mares and women include 1) emergence of the future dominant follicle before the future largest subordinate follicle, 2) length of intervals between sequential emergence of follicles of the wave, 3) percentage increases in follicle diameter during the common growth phase preceding deviation, 4) incidence of major anovulatory waves during the interovulatory interval, 5) incidence of predeviation follicles that reach maximum diameter an average of 1 d before the true deviation, and 6) occurrence of deviation during the FSH decline about 3 d after the peak of the FSH surge (Ginther et al., 2004b; 2005a), The large size of equine follicles and accessibility by the transrectal and transvaginal ultrasonographic approaches provide superb intrafollicular experimental access. These considerations and the remarkable similarities between mares and women throughout the ovulatory follicular wave encourage the use of mares as a comparative research model for study of the mechanisms of follicle deviation.

The occurrence of multiple (usually two) dominant follicles per wave reflects concentrations of circulating gonadotropins that differ from the changing concentrations for a single dominant follicle, centralized to the beginning of deviation in heifers (Kulick et al., 2001; Beg et al., 2003; Acosta et al., 2005) and cows (Lopez et al., 2005). Therefore, a wave with two dominant follicles is a natural model for studying the mechanisms of deviation. A dominant follicle has been defined as one that attained at least 28, 10, 10, and 13 mm in mares (Ginther, 1993), heifers (Kulick et al., 2001), cows (Lopez et al., 2005), and women (Ginther et al., 2004b), respectively. The 28 and 13 mm definitions of a dominant follicle in mares and women, respectively, are in a similar ratio between species (28/13 = 2,2 mm) as for the other events during the ovulatory wave, listed above. Two dominant follicles in heifers may involve a single deviation or two separate deviations (Kulick et al., 2001; Beg et al., 2003; Acosta et al., 2005). Two deviations are characterized by a first deviation involving the two largest follicles (future codominant follicles) versus the third largest follicle, followed an average of 2 d later by a second deviation between the two largest follicles. The phenomenon of two deviations has been reported only for heifers and apparently has not been studied in mares, cows, and women. The ovulatory outcome of two dominant follicles in cattle was not determined; all studies were done during the first anovulatory wave. In mares, two dominant follicles during the ovulatory wave may result in double ovulations, but usually only one follicle ovulates, and the other follicle regresses after attaining the diameter of a dominant follicle (Ginther et al., 2004b).

The purpose of the present experiment was to determine the incidence of two dominant follicles with one or two deviations during the ovulatory follicular wave in mares. Changes in follicle diameters, and systemic concentrations of FSH, LH, estradiol, and ir-inhibin were compared among waves with one versus two dominant follicles and results were interpreted in terms of the hormonal aspects of deviation.

2. Materials and Methods

2.1. Animals

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-horse crosses weighing 250 to 400 kg and aged 5 to 20 yr. A total of 24 mares with a docile temperament and no apparent abnormalities of the reproductive tract as determined by ultrasound examinations (Ginther, 1995) was used in two consecutive estrous cycles during April–August (Northern Hemisphere). 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.

2.2. Ultrasonography and end points

B-mode ultrasonographic examinations were done daily starting 15 days after an ovulation and encompassed three subsequent ovulations or two interovulatory intervals. The experimental period extended from 4 d before the first ovulation to 4 d after the third ovulation, but only the data temporally involving deviations were used in this study. A real-time ultrasound scanner with a linear-array 7.5 MHz transducer was used for transrectal 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 (= 5 mm to < 15 mm) were estimated. The diameters of the three largest follicles on each day were recorded without regard to day-to-day identity and were defined as F1 (largest) to F3. A dominant follicle was defined as one that attained = 28 mm (Ginther, 1993).

Observed diameter deviation in each follicular wave was based on retrospective examination of the diameter-data profiles of individual follicles. Waves were removed from the analyses when the day of deviation was not apparent, owing to only one follicle per wave or obscurement by either intermingling follicles of a previous wave (Ginther et al., 2004a) or predeviation follicles within the ovulatory wave (Ginther et al., 2004b). In the remaining waves, the beginning of deviation was identified by the day preceding the first apparent change in the differences in diameter between follicles (Ginther et al., 1997). Days of deviation were established before other follicular and hormonal data were inspected or analyzed. For waves with one dominant follicle, deviation was detected by comparing the diameter growth profiles for F1 with the profile for F2. For waves with two dominant follicles, deviation was detected by comparing the combined diameter profiles of F1 and F2 with the profile for F3. For two dominant follicles, the subsequent profiles of F1 and F2 were examined for a second deviation. The single deviation for waves with one dominant follicle and a single or first deviation for waves with two dominant follicles were both defined as Deviation 1, and the day of occurrence was designated Day 0, For waves with two dominant follicles and two deviations, the second deviation was defined as Deviation 2, and its position in the wave was based on the number of days after Deviation 1.

The ovulatory waves were divided into a group with one dominant follicle (1DF) and a group with two dominant follicles (2DF). Sequential end points for comparisons among the two groups were diameter of F1 to F3 on Days -1 to 4; number of follicles 5.1 to 10.0 mm, 10.1 to 15.0 mm, 15.1 to 20.0 mm, and 20.1 to 25.0 mm on Days -1 and 0; concentrations of FSH, LH, and estradiol on Days -4 to 4; and concentrations of ir-inhibin on Days -2, 0, and 2, Postdeviation (Days 0 to 4) hormonal data for LH were also considered separately. Concentrations of ir-inhibin were assessed only on days -2, 0, and 2, owing to a limited availability of antigen. Single-point characteristics for the day of Deviation 1 were number of days from the preceding ovulation and to the next ovulation; diameter of F1, F2, and F3; concentration of FSH, LH, estradiol, and ir-inhibin; subsequent growth rate of F1 (Days 0–4); day and concentration for maximum FSH between ovulation and Deviation 1; and number of days between maximum FSH and Deviation 1, Follicular waves with two dominant follicles were categorized into those with one or two deviations for characterization purposes.

2.3. Blood samples and hormone assays

Daily jugular blood samples were collected into heparinized tubes, Blood samples were centrifuged (1,500 xg for 10 min) and decanted, and the plasma was stored at -20ºC until assay. Plasma samples were assayed for FSH and LH by radioimmunoassay and estradiol and ir-inhibin by commercial kits, as validated and described for mare plasma in our laboratory (LH and FSH, Donadeu & Ginther, 2002; estradiol, Ginther et al., 2005b; ir-inhibin, Donadeu & Ginther, 2001). The ir-inhibin antibody recognizes dimeric forms of inhibin, as well as free alpha subunits (Roser et al., 1994). The intra- and interassay coefficients of variation 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%, and 0.1 pg/ml for estradiol, For ir-inhibin, the intraassay CV was 12.1% and sensitivity was 2.8 ng/ml.

2.4. Statistical analyses

Data for follicular and hormonal end points that were not normally distributed, according to a Kolmogorov-Smirnov test, were transformed to natural logarithms. Sequential diameters of follicles and plasma concentrations of hormones were normalized to Day 0 (day of Deviation 1) and were analyzed to determine the main effects of group (1DF vs 2DF) and day and for their interaction. The SAS MIXED procedure with a REPEATED statement was used to account for the autocorrelation between sequential measurements (8.2 Version; SAS Institute Inc., Cary, NC). If a significant effect of group or group-by-day interaction was detected, unpaired Student's *t*-tests were used to locate differences in means between groups. If a significant day effect was obtained, differences between means within a group were examined by paired Student's *t*-tests, Single point data were analyzed by one-way ANOVA, and frequency data were analyzed by Chi-square. Data are given as the mean ± SEM, unless otherwise stated. A probability of $P = 0.05$ indicated that a difference was significant, and probabilities between $P > 0.05$ and $= 0.1$ indicated that a difference approached significance.

3. Results

Of the 48 ovulatory waves in the 24 mares, 32 waves were used in the analyses. Waves were omitted for the following reasons: absence of ovulation (n = 3); no detectable deviation, owing to inadequate number of follicles (n = 5); and obscured deviation (n = 8). The number of waves in the group with one dominant follicle (1DF) was 21 (66%) and in the group with two dominant follicles (2DF) was 11 (34%). The number of waves with one and two deviations in the 2DF group was three and eight, respectively. Two ovulations occurred in 1/32 waves (3%) in a wave with two dominant follicles and one deviation.

Single-point data for Day 0 (day of Deviation 1) for the 1DF and 2DF groups are shown (Table 1). Length of intervals from ovulation to Deviation 1 and from Deviation 1 to ovulation; diameter of F3; growth rate of F1 between Days 0 to 4; concentrations of LH, estradiol, and ir-inhibin; and day and concentration for maximal FSH did not differ between groups. The number of follicles 5.1 to 10.0 mm, 10.1 to 15.0 mm, 15.1 to 20.0 mm, and 20.1 to 25.0 mm was not different between the 1DF and 2DF groups on Day -1 (data not shown) or on Day 0 (Table 1). The diameter of F1 on Day 0 was greater, diameter of F2 approached being greater, concentration of FSH was less, and number of days from maximum FSH to deviation was greater in the 2DF group than in the 1DF group.

The main effect of day was significant for diameter of F1, F2, and F3 on Days -1 to 4 (Fig. 1). The group effect was significant for F1, F2, and F3, owing to greater diameters in the 2DF group. The interaction of group and day was significant for F2 and F3, owing to increasing diameter in the 2DF group and decreasing diameter in the 1DF group, For FSH, the main effects of group and day were significant (Fig. 2); concentrations were less on Days -2 to 4 in the 2DF group. Concentrations of LH showed only a significant day effect. When the analysis was limited to Days 0 to 4, the interaction was significant ($P < 0.03$), owing to greater concentrations in the 2DF group on Day 3 ($P < 0.07$) and Day 4 ($P < 0.05$). Averaged over the two groups, LH concentrations first increased ($P < 0.03$) between Days -2 (1.5 ± 0.1) and -1 (1.7 ± 0.2). There were no significant effects for estradiol, except for the main effect of day. Combined over the two groups, estradiol concentrations first increased ($P < 0.001$) between Days -4 (0.4 ± 0.05) and -3 (0.5 ± 0.06); concentrations increased between Days -4 and -2 in 94% of the waves and in all waves by Day 0, For concentrations of ir-inhibin on Days -2, 0, and 2, only the effect of day was significant (data not shown). Concentrations of ir-inhibin increased between Days -2 (18.4 ± 0.4 ng/ml) and 0 (21.0 ± 0.3 ng/ml) in the 1DF group ($P < 0.002$) and between Days -2 (15.5 ± 0.5 ng/ml) and 0 (19.7 ± 0.6 ng/ml) in the 2DF group ($P < 0.01$), An increase between Days 0 and 2 was not significant for either group.

Table 1 - Mean ± SEM for characteristics at the beginning of the first follicle deviation in ovulatory follicular waves with one or two dominant follicles

Characteristics involving the first deviation	**Dominant follicles (= 28 mm)**		
	One (1DF)[a]	**Two (2DF)**	***p* value**
No. ovulatory waves	21	11	-
Day of occurrence (No. days)			
After ovulation	15.3 ± 0,5	16.0 ± 0.6	NS
To next ovulation	7.3 ± 0.3	7.4 ± 0.4	NS
Diameter (mm) of:			
F1 (largest follicle)	22.7 ± 0.4	23.9 ± 0.4	$P < 0.02$
F2	21.7 ± 0.5	22.7 ± 0.3	$P < 0.08$
F3	20.3 ± 0.5	20.8 ± 0.5	NS
F1 minus F2	1.0 ± 0.3	1.1 ± 0.5	NS
Growth rate (mm/day) of F1 on Days 0 to 4	3.1 ± 0.19	3.4 ± 0.02	NS
No. follicles	8.9 ± 1.0	8.5 ± 1.4	NS
5.1–10.0 mm	5.8 ± 0.7	5.0 ± 1.1	NS
10.1–15.0 mm	3.4 ± 0.5	3.8 ± 0.9	NS
15.1–20.0 mm	3.4 ± 0.5	3.8 ± 0.9	NS
20.1–25.0 mm	2.0 ± 0.3	2.5 ± 0.7	NS
Concentrations of:			
FSH (ng/ml)	12.5 ± 0.8	9.1 ± 0.7	$P < 0.005$
LH (ng/ml)	1.9 ± 0.2	1.8 ± 0.3	NS
Estradiol (pg/ml)	0.9 ± 0.1	0.9 ± 0.1	NS
Ir-inhibin (ng/ml)	21.0 ± 0.3	19.7 ± 0.6	NS
Maximum FSH[b]			
No. days after ovulation	9.8 ± 0.7	8.7 ± 0.9	NS
No. days to deviation	5.5 ± 0.6	7.3 ± 0.9	$P < 0.05$
Concentration (ng/ml)	25.7 ± 2.5	26.5 ± 3.3	NS

[a] 1DF and 2DF are the abbreviations used in text.

[b] Maximum concentration between ovulation and deviation.

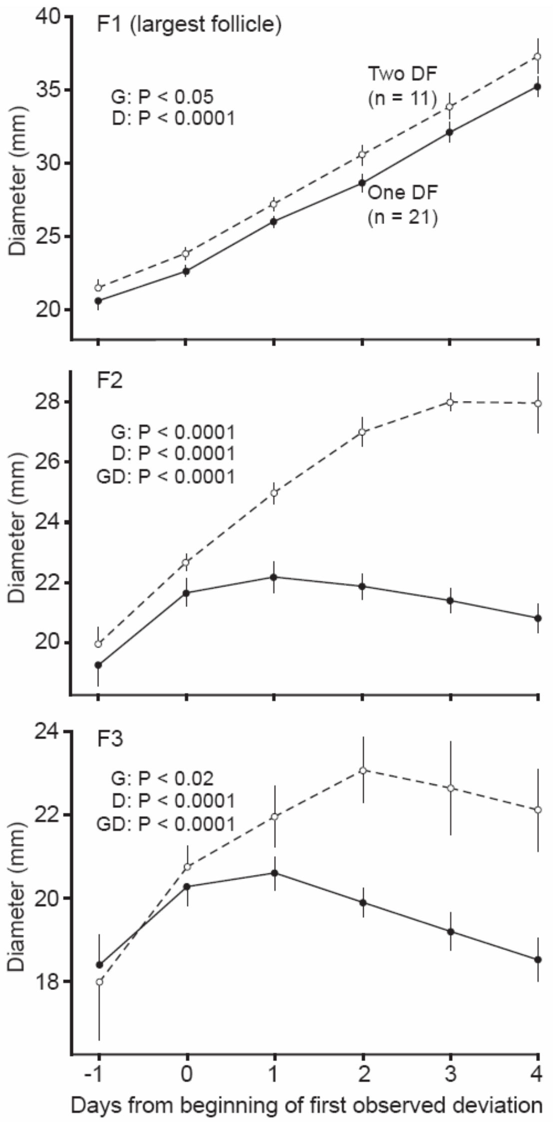

Figure 1 – Mean (± SEM) diameters of the three largest follicles (F1, F2, F3) in ovulatory follicular waves with one versus two dominant follicles (DF). Probabilities for a main effect (G = group; D = day) and an interaction (GD) are shown.

Diameters of F1, F2, and F3 are shown for the 1DF group and for the waves in the 2DF group with two deviations (Fig. 3). For the 1DF group, the difference in diameter between F1 and F2 was less ($P < 0.0001$) for Day 0 (1.0 ± 0.3 mm) than for Day 1 (3.9 ± 0.5 mm). Differences in diameter of F2 and F3 were not significant between Days 0 and 1. For the waves with two-dominant follicles and two deviations (n = 8), the interval between Deviations 1 and 2 was 2 d for five waves and 3 or 4 d for the remaining waves (mean, 2.5 ± 0.4 d). The waves with 2 d between deviations were used to depict the characteristics of two deviations and two dominant follicles (Fig. 3). The differences for F1 and F2 were not significant between Days 0 (1.3 ± 0.2 mm) and 1

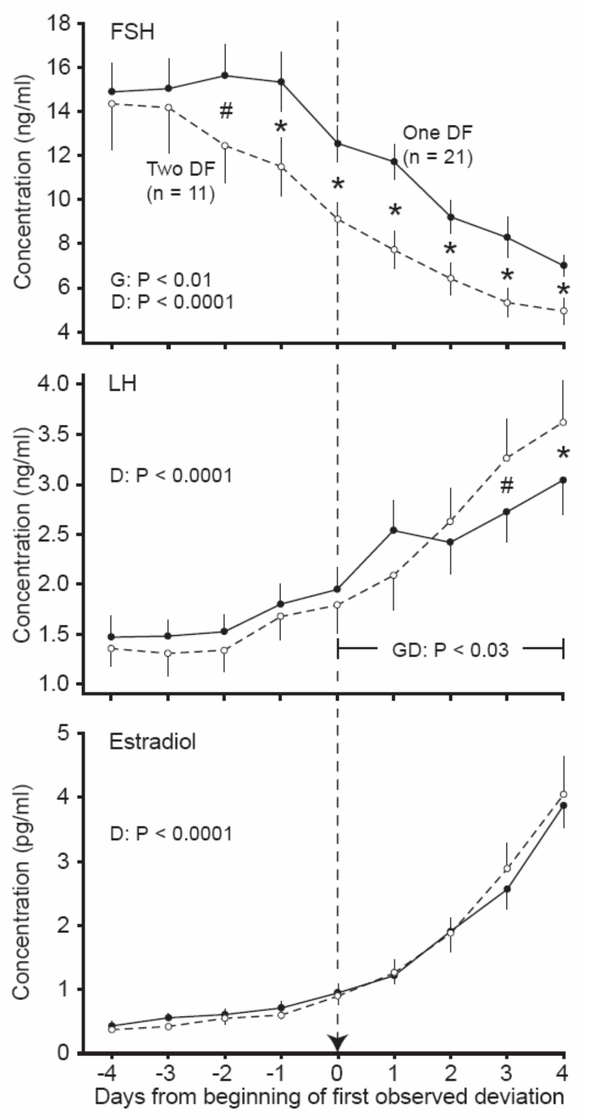

Figure 2 – Mean (± SEM) concentrations of plasma hormones for waves with one and two dominant follicles (DF). Probabilities for a main effect (G = group; D = day) and an interaction (GD) are shown. For LH, data were also analyzed separately for Days 0 to 4. An asterisk indicates days of a significant difference ($P < 0.05$) between groups, and a pound mark (#) indicates a difference that approached significance ($P < 0.07$).

(1.6 ± 0.6 mm). The difference between F1 and F3 was less ($P < 0.002$) for Day 0 (2.9 ± 1.4 mm) than for Day 1 (5.3 ± 1.5 mm). For the second deviation, the difference in diameter between F1 and F2 was less ($P < 0.003$) for Day 2 (1.0 ± 0.5 mm) than for Day 3 (6.3 ± 1.3 mm). The difference in diameter between F1 and F2 showed a group-by-day interaction ($P < 0.006$) in the comparison of the 1DF group (one deviation) and the waves in the 2DF group that had two deviations (Fig. 3); the interaction represented significant differences that began on Day 1 ($P < 0.02$) and continued thereafter.

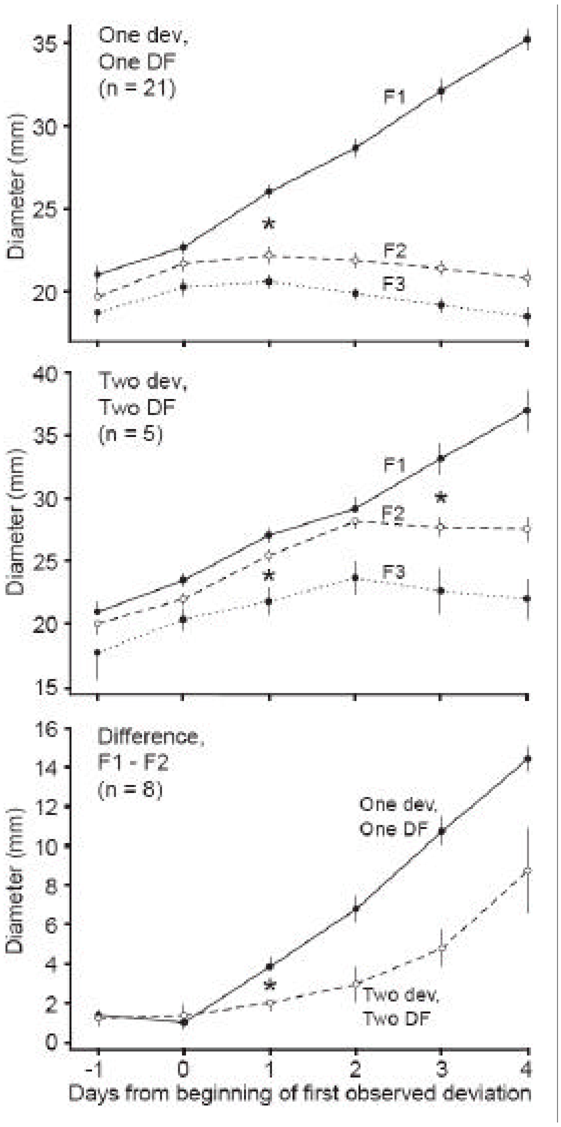

Figure 3 – Mean (± SEM) diameters of F1 (largest follicle), F2, and F3 for waves with one dominant follicle (DF) and one deviation (dev) and for waves with two dominant follicles and two deviations with the second deviation occurring on Day 2. A comparison of the difference between the two groups in diameter of F1 and F2 for all mares in the two groups is shown in the lower panel. In the upper two panels, an asterisk indicates the day after the beginning of a deviation or the first day when the difference in diameter between the two indicated follicles is greater ($P < 0.05$) than for the previous day. In the lower panel, the asterisk indicates the first day of a significant difference ($P < 0.05$) between groups.

Maximum diameter of the follicle that reached dominant status (based on diameter) and then regressed in the 2DF group was 30.8 ± 0.7 mm. Maximum diameter was attained 2.8 ± 0.3 d after Deviation 1 or 19 d after ovulation and 4 d before the next ovulation. The diameter of the preovulatory follicle on the day before ovulation in waves with only one ovulation approached significance ($P < 0.08$) between waves with one dominant follicle (40.3 ± 0.9 mm) and waves with two dominant follicles (42.7 ± 1.4 mm).

4. Discussion

This is the first study of follicle deviation that used waves with two dominant follicles as a separate experimental group in mares. The results are especially relevant on a species comparison basis, owing to the striking similarities between mares and women in relative diameter of the follicles among events throughout the ovulatory follicular wave and in the temporal relationships between deviation and the profile of the wave-stimulating FSH surge (*see Introduction*). The experiment was dependent on the reliability of assigning the day of the beginning of deviation by inspection of graphs that showed the changes in diameters of individual follicles. The process of deviation as observed during inspection of data has been substantiated mathematically by agreement between inspection and the results of a segmented linear regression approach in heifers (Bergfelt et al., 2003) and on a limited basis in mares (Ginther et al., 2003). In the present study, the follicles were ranked by diameter each day without regard to day-to-day identity. This approach was used to minimize the length of the transrectal examinations and has been used previously to estimate the day of the beginning of deviation in mares (Ginther et al., 2007). Ovulatory waves were not used when the day of the beginning of deviation was obscured by overlapping regressing follicles. For the same reason, follicles preceding the beginning of deviation (common growth phase) were not studied before Day -1.

The occurrence of two deviations during the ovulatory wave in most mares with two dominant follicles (incidence 8/11) has not been reported previously. A similar phenomenon has been reported for heifers during the first anovulatory wave (Beg et al., 2003; Acosta et al., 2005). The interval of 2 d between the first and second deviation in five of the eight waves was useful in illustrating the mean changes in diameters for F1, F2, and F3 in association with each deviation (Fig, 3). Between Deviations 1 and 2, F1 and F2 increased in diameter in parallel as indicated by similar diameter differences between F1 and F2; diameter of F3 began to decrease at Deviation 1. As expected, the first significant increase in the mean difference between follicles in the eight mares with two deviations and two dominant follicles was on the day after the beginning of observed deviation for both Deviations 1 and 2.

The day of the first observed deviation (Deviation 1; Day 0) did not differ between mares with one or two dominant follicles, as previously reported for heifers (Acosta et al., 2005) and cows (Lopez et al., 2005). The species differed in that F1 was a mean of 1.2 mm larger on the day of Deviation 1 in waves with two dominant follicles than in waves with one dominant follicle in mares, but there were no differences in heifers for either F1, F2, or F3. The difference in diameter between F1 and F2 on Day 0 was similar between groups within each species and cannot be considered a factor in the development of one versus two dominant follicles. In summary, the only characteristics of the follicle population detected on the day before and on the day of the beginning of deviation that were associated with development of the two dominant follicles during equine ovulatory waves was a slightly greater diameter of F1 and a tendency (approached significance) for a greater diameter of F2. In contrast, the number of follicles = 4 mm but not the diameters of F1 and F2 was greater in heifers that developed two dominant follicles in the first anovulatory follicular wave (Kulick et al., 2001; Acosta et al., 2005).

Concentrations of FSH began to decrease before the beginning of deviation in the groups with one and two dominant follicles, but the decrease began 2 d earlier in the group with two dominant follicles. The earlier beginning of the decrease led to the longer interval from maximal FSH to deviation and apparently to the lower concentrations on Days -1 to 4 in the group with two dominant follicles. The earlier decrease in FSH relative to deviation is not attributable to the occurrence of deviation earlier in the estrous cycle; the intervals from ovulation to Deviation 1 and from Deviation 1 to the next ovulation were not different between the two groups. The lower FSH concentrations before the beginning of deviation in waves that later developed two dominant follicles in mares contrasts with greater FSH concentrations 1 d before deviation in waves that developed two dominant follicles in heifers (Kulick et al., 2001; Acosta et al., 2005) and cows (Lopez et al., 2005). Thus, higher FSH concentrations, as well as more follicles before deviation, is compatible with the production of more waves with two dominant follicles in cattle, but neither characteristic accounted for waves with two dominant follicles in mares.

The dependence of deviation upon decreasing FSH has been well demonstrated by many temporal and FSH-manipulative studies in the three monovular species (*see reviews cited in the Introduction*). The differences in the predeviation FSH profile between the present two groups are compatible with the concept that the FSH role in the initiation of deviation is a decrease in concentrations without dependence on the length of time from the beginning of the decline or the actual FSH concentration at the time of initiation. Apparently, therefore, other factors, as well as the FSH decline, contribute to the initiation of deviation (potential examples: stage of follicle development, increasing LH concentrations).

The lower FSH concentration after deviation in waves with two dominant follicles in the present study in mares, has been reported for heifers (Kulick et al., 2001; Beg et al., 2003; Acosta et al., 2005) and a similar tendency has been reported for cows (Lopez et al., 2005). The reduced postdeviation concentrations in FSH in waves with two dominant follicles may account for the occurrence of a second deviation in 8 of 11 waves with two dominant follicles, as suggested for heifers (Kulick et al., 2001). However, this hypothesis has not been tested in either species.

Ir-inhibin increased during the FSH decrease associated with deviation, as previously reported (Bergfelt et al., 2001; Ginther et al., 2005b). However, the increase was similar in the groups with one and two dominant follicles, despite the lower levels of FSH in the group with two dominant follicles. Also, estradiol concentrations were not different between the two groups during the days FSH was lower in the group with two dominant follicles. Thus, the two hormones associated with FSH depression in mares (ir-inhibin and estradiol; Ginther et al., 2004a) did not account for the lower concentrations of FSH in the waves with two dominant follicles. The present study involved total inhibin, and the role of inhibins in deviation will not likely be clarified until assays are available that distinguish between immunoreactive and bioactive forms. It is noteworthy, however, that the assay system that did not implicate ir-inhibin in the low FSH concentrations in mares with two dominant follicles is the same system that has indicated consistently a negative relationship between the two hormones in many previous studies in mares (Bergfelt et al., 1991; 2001; Donadeu and Ginther, 2001; 2003; 2004; Ginther et al., 2005b). Furthermore, during the last half of the anovulatory season and early ovulatory season the profiles of ir-inhibin (Donadeu and Ginther, 2003) were similar to those for Inhibin-A (Watson et al., 2002).

The predeviation increase in both plasma estradiol and LH in mares confirms previous findings (Ginther et al., 2004a). Concentrations of LH were not different between groups with one and two dominant follicles before deviation and at the beginning of deviation. This contrasts with findings in cattle of higher LH concentrations the day before the beginning of deviation in anovulatory waves with two dominant follicles in heifers (Kulick et al., 2001; Acosta et al., 2005) and cows (Lopez et al., 2005). There were no differences between the groups with one and two dominant follicles in postdeviation LH concentrations in the equine follicular waves during the first 2 d after Deviation 1. Similarly, LH concentrations did not differ in cattle during the first 2 d after the beginning of deviation (Kulick et al., 2001; Acosta et al., 2005; Lopez et al., 2005). Concentrations of LH did increase 3 or 4 d after the beginning of Deviation 1 in the group with two dominant follicles. However, estradiol did not differ between the two groups and did not account for the differences in LH, Estradiol, as well as LH, did increase postdeviation in cows with two dominant follicles (Lopez et al., 2005).

In follicular waves with two dominant follicles, double ovulations would originate from waves that have only one deviation, considering that a second deviation would involve regression of one of the two follicles. In the present study, only one wave produced double ovulations and was one of the three waves with two dominant follicles and one deviation. One double ovulation in 32 waves (3%) is consistent with reported low double ovulation rates (2 to 3%) in ponies (Ginther, 1992). Equine breeds and types vary widely in double ovulation rates (example: Thoroughbreds, 25%; Standardbreds, 15%) and is influenced by other factors (example: nonlactating and lactating Quarter Horses, 7 and 14%, respectively; Ginther, 1992). It is expected, therefore, that the incidence of two dominant follicles with one deviation (double ovulations) would be much higher in other breeds and types.

Diameter of follicles that produce double ovulations are smaller on the day before ovulation than for single ovulations (Ginther, 1992). In the present series, preovulatory diameters of the one set of double-ovulating follicles were smaller (33.5 and 33.9 mm) than for all except one of the other 31 ovulations. The tendency (approached significance) for a larger preovulatory follicle on the day before ovulation in waves with two dominant follicles, but only one ovulation, than in waves with one dominant follicle was unexpected and will require confirmation. However, the greater concentration of LH 4 d after Deviation 1 may have been involved in the greater diameter on the day before ovulation. Apparently, the presence of a second nonovulatory dominant follicle that reached a maximum mean diameter of 31 mm an average of 4 d before ovulation did not diminish the diameter of the other follicle the day before it ovulated. This observation indicates that the reduced diameter of a preovulatory follicle in waves with double ovulations occurred after the time the nonovulating dominant follicle in other waves reached maximum diameter.

5. Conclusion

In conclusion, the study produced the first information for the ovulatory follicular waves in mares on the incidence of two dominant follicles (= 28 mm; 11 of 31 waves) and two deviations (8 of 11 of the waves with two dominant follicles). The first deviation occurred on a similar day as the deviation in waves with one dominant follicle and was between a combination of F1 and F2 versus F3. The second deviation occurred a mean of 2 d later and was between F1 and F2. The only follicle difference preceding or at the beginning of deviation between the groups with one and two dominant follicles was a slightly larger F1 and a tendency for a larger F2 in the group that developed two dominant follicles. No differences were found between waves that developed one versus two dominant follicles in LH, estradiol, and ir-inhibin concentrations during 2 d before to 2 d after the beginning of deviation. Concentrations of FSH were lower in waves with two dominant follicles before, during, and after the beginning of deviation, and the FSH decline began earlier. An increase in FSH or LH before deviation did not account for the development of two dominant follicles in mares. Previous studies have documented the necessity of decreasing FSH concentrations for the initiation of deviation. The earlier decline in FSH and the lower concentrations at the beginning of deviation in the group with two dominant follicles suggests that the length of time that FSH has been declining and the attainment of a specific concentration are not requisite components of the FSH decline for the initiation of deviation.

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REPEATABILITY OF FOLLICLE DIAMETERS AND GONADOTROPIN AND ESTRADIOL CONCENTRATIONS BETWEEN CONSECUTIVE INTEROVULATORY INTERVALS WITHIN INDIVIDUAL MARES[1]

Abstract: Data were collected daily from 23 mares during two consecutive interovulatory intervals (IOIs). Several significant ($P < 0.05$) new observations were made. Concentrations of FSH increased between Days -1 and 0 (ovulation = Day 0), plateaued between Days 0 and 1, and rebounded on Days 1 and 2. During the declining portion of the preovulatory estradiol surge, the rate of decrease was greater for Days -2 to 1 than for Days 1 to 4; the abrupt change in estradiol output on Day 1 was synchronous with the peak of the LH surge. Repeatability within mares was based on a significant positive correlation between the two IOIs. The following end points were correlated significantly: 1) length of the interval between ovulations ($r = +0.67$) and between ovulation and the beginning of follicle deviation ($r = +0.58$); 2) diameter of the preovulatory follicle on Days -3 to -1 (average, $r = +0.49$); 3) number of follicles in diameter classes of 2 to 5 mm ($r = +0.65$ average of the daily correlations for the IOI; significant individual daily correlations on 22/23 days), 5.1 to 10 mm ($r = +0.49$; 18/23 days), 10.1 to 15 mm ($r = +0.40$; 12/23 days), and 15.1 to 20 mm ($r = +0.42$; 12/23 days); and FSH ($r = +0.55$; 18/23 days) and LH concentrations ($r = +0.59$; 22/23 days). The repeatability for number of follicles was greatest in the smallest diameter class (2 to 5 mm). Results demonstrated measurable repeatability within mares for several end points between consecutive IOIs.

REPETIBILIDADE DO DIÂMETRO FOLICULAR, CONCENTRAÇÕES DE GONADOTROFINAS E ESTRADIOL ENTRE CONSECUTIVOS INTERVALOS INTEROVULATÓRIOS EM ÉGUAS

Resumo: Os dados foram coletados diariamente de 23 éguas durante dois intervalos ovulatórios consecutivos (IOIs). Varias e novas significantes ($P < 0.05$) observações foram feitas. A concentração de FSH aumentou entre os Dias -1 e 0 (ovulação = Dia 0), com um platô entre os Dias 0 e 1, e houve um rebote entre os dias 1 e 2. Durante o declínio da onda de estradiol no período pré-ovulatório, a taxa de decréscimo foi maior para os Dias -2 a 1 do que para os Dias 1 a 4; a abrupta mudança na produção de estradiol no Dia 1 foi sincrônica com o pico da onda de LH. A repetibilidade dentro das éguas foi baseada na significante correlação positiva entre os dois IOIs. Os seguintes pontos foram correlacionados significativamente: 1) duração do intervalo entre ovulações (r = +0,67) e entre a ovulação e o começo da divergência folicular (r = +0,58); 2) o diâmetro do folículo pré-ovulatório nos Dias -3 a -1 (média, r = +0,49); 3) o número de folículos por classes de diâmetro de 2 a 5 mm (r = +0,65 a média da correlação diária para o IOI; individual correlação significativa diária nos dias 22/23), 5,1 a 10 mm (r = +0,49; 18/23 dias), 10,1 a 15 mm (r = +0,40; 12/23 dias), e 15,1 a 20 mm (r = +0,42; 12/23 dias); e FSH (r = +0,55; 18/23 dias) e a concentração de LH (r = +0,59; 22/23 dias). A repetibilidade para o número de folículos foi maior na classe de menor diâmetro (2 a 5 mm). Os resultados demonstraram uma repetibilidade mensurável dentro de cada égua para vários pontos entre consecutivos IOIs.

1. Introduction

Growth of follicles in waves during estrous or menstrual cycles in monovular species has been characterized in detail by transrectal (cows, mares) and transvaginal (women) ultrasonography (reviewed in Ginther 2000; Ginther et al., 2001; 2003; 2004a; 2004b). After several days of a common-growth phase, the follicles of a wave may dissociate or deviate. Deviation is characterized by continued growth of a dominant follicle and reduced growth and atresia of subordinate follicles. Waves that develop a dominant follicle or one that reaches 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 to 30% of estrous or menstrual cycles, whereas almost all cycles in heifers and cows have one or two major anovulatory waves preceding the ovulatory wave.

In recent years, it has become apparent that mares are good experimental animals 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), similarities between mares and women were shown for the following characteristics: 1) relative diameter of the largest follicle at discernible events throughout the follicular wave (2.1 or 2.2 times larger in mares), 2) emergence of the future dominant follicle before the future largest subordinate follicle, 3) length of intervals between sequential emergence of follicles of the wave, 4) percentage increases in follicle diameter during the common growth phase preceding deviation, 5) incidence of major anovulatory waves during the interovulatory interval, and 6) occurrence of deviation during the decline in the wave-stimulating FSH surge about 3 d after the peak of the surge. The experimental accessibility of the large follicles of mares and the remarkable similarities between mares and women throughout the ovulatory follicular wave encourage the use of mares as a comparative research model for study of the ovulatory follicular wave.

In mares, the intermingling of follicles from a previous wave with the follicles of the common-growth phase of the ovulatory wave occurs in about 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 diameter-based growth rate of the ovulatory follicle is similar before and after deviation. Therefore, the postdeviation 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.

Recent studies in cattle have shown considerable repeatability within animals in numbers of follicles during a 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 associated with the first postovulatory major anovulatory wave. Concentrations of FSH were lower and numbers of follicles = 3 mm were greater in cows selected for high versus low follicle numbers. Similar repeatability studies apparently have not been reported for mares and women. However, it has been noted that 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, gonadotropin, and estradiol dynamics during two consecutive interovulatory intervals with emphasis on the within-mare repeatability between intervals. The temporal associations between follicle diameters and numbers and gonadotropins and estradiol concentrations were also considered.

2. Materials and Methods

2.1. Animals and ultrasound and 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 to 400 kg and aged 5 to = 18 yr. A total of 24 mares with a docile temperament and no apparent abnormalities of the reproductive tract as determined by ultrasound examinations (Ginther, 1995) was used in two consecutive interovulatory intervals during April to August (Northern Hemisphere). 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 done daily starting 15 days after an ovulation and encompassed three subsequent ovulations or two interovulatory intervals. The experimental period extended from 4 d before the first ovulation to 4 d 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 to 5 mm, 5.1 to 10 mm, 10.1 to 15 mm, 15.1 to 20 mm, 20.1 to 25 mm, 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.

2.2. Normalization of the interovulatory interval (IOI)

Data were normalized to the mean length of the IOIs rounded to 23 d for both the first (23 ± 0.5 d) and second (22.9 ± 0.4) IOIs (Fig. 1), Days -4 to 4 for each of the three periovulatory periods were not adjusted. Days preceding the end of each IOI were designated Days -18 to -1. If the IOI was longer than 23 d the data were discontinued at Day -18; if the IOI was shorter, missing data were used to complete the interval. Thus, an average break in continuity occurred between Day 4 and Day -18. 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 (Fig. 1). Data for Days -4 to 4 for each of the three periovulatory periods were designated POP 1, POP 2, and POP 3, Data for Day 5 to 18 after an ovulation (corresponds to Days -18 to -5 before ovulation) for the middle of the two IOIs were designated Mid-IOI 1 and Mid-IOI 2.

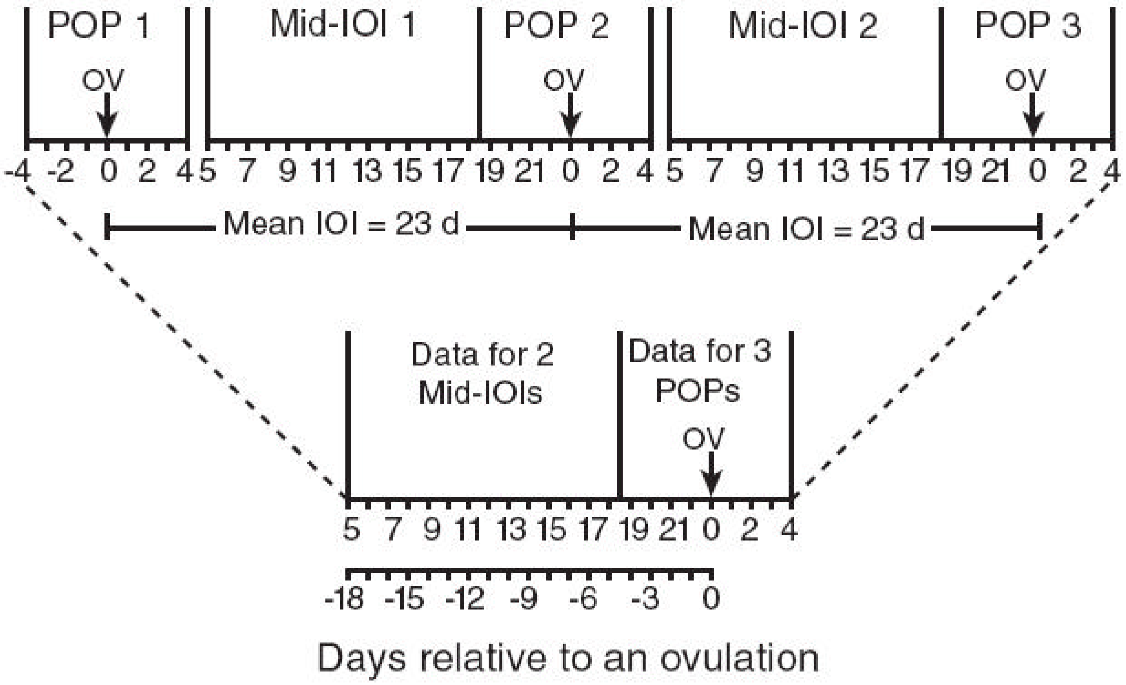

Figure 1 – Scheme for normalization of the interovulatory intervals (IOIs) to the mean length of 23 d. A break in continuity was made between Days 4 and 5 by designating Day 5 as Day -18 before ovulation at the end of an IOI. The middle of the two IOIs were designated Mid–IOI 1 and 2, and the three periovulatory periods (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.

Data for the three periovulatory periods and data for the two middle IOIs were combined, as shown (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 diameter growth rate of the dominant follicle (F1) during 4 d (three day-to-day growth rates) after the apparent beginning of deviation. This approach was based on the following previous studies: 1) 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 2) 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 four days postdeviation was used for retroceding to the day with the diameter closest to 22.5 mm 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.

2.3. Repeatability and correlations

The repeatability of an end point was examined by correlation between the first and second IOI for each mare. Sequential end points were correlated for each day and were diameter of F1, number of follicles in each of the five diameter classes, number of dominant follicles in the ovulatory wave, and concentrations of LH and FSH for 23 d and estradiol for 9 d. Correlations between IOIs for single–point data were diameter of F1 on Day -1; length of the IOI; days of estimated deviation and emergence of the future ovulatory follicle; intervals from emergence to deviation and from deviation to ovulation; and growth rate of the ovulatory follicle per day on four days postdeviation, on Days -3 to -2, and on Days -2 to -1.

To consider whether the repeatability results were confounded by age effects, the mares were grouped into younger mares (5 to 14 yr; n =15) and older mares (= 18; 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 follicles11 to 15 mm on Day 13, and shorter IOIs in younger mares (5 to 19 yr) than in older mares (= 20 yr). The correlations between IOIs within mares in the present study were determined for each of these end points within each age group.

In addition to the correlation approach for repeatability between IOIs within mares, correlations were done 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 correlated: 1) number of follicles in a diameter class with each other diameter class, using the four diameter classes with the greatest expected turnover (2 to 5 mm, 5.1 to 10 mm, 10.1 to 15 mm, and 15.1 to 20 mm); 2) FSH with each of the four diameter classes; 3) FSH with LH; and 4) LH with diameter of F1 on Day -1.

2.4. Blood samples and hormone assays

Daily jugular blood samples were collected into heparinized tubes. Blood samples were centrifuged (1500 x g for 10 min) and decanted. and the plasma was stored at -20^{o}C until assayed. Plasma gonadotropin concentrations were assayed for all samples from 4 d before the first ovulation to 4 d after the third ovulation. Estradiol was assayed from 4 d before to 4 d after deviation. In addition, estradiol 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 & Ginther. 2002) and for estradiol (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 estradiol; and 5.6% (intraassay CV) and 0.04 ng/ml for progesterone.

2.5. 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 and the 14 days between Days 5 to 18 (2 x 14 factorial). Estradiol for the 9 d encompassing the estimated beginning of deviation was analyzed in a 2 x 9 factorial. Diameter of F1 on Days -4 to -1 (4 d) and concentrations of FSH and LH for Days -4 to 4 (9 d) were analyzed for the three periovulatory periods in 3 x 4 or 3 x 9 factorials. Estradiol 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 SAS MIXED procedure with a REPEATED statement was used to account for the autocorrelation between sequential measurements (8.2 Version; SAS. Institute Inc., Cary. NC). 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 interovulatory intervals as an indication of repeatability for a specific day and end point were done 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-square. Data are given as the mean ± SEM, unless otherwise stated. A probability of $P = 0.05$ indicated that a difference was significant, and probabilities between $P > 0.05$ and $P = 0.1$ indicated that a difference approached significance.

3. Results

One mare was removed from the experiment, owing to the development of a hemorrhagic anovulatory follicle (Ginther et al., 2006a) during the second expected ovulatory period. Two complete interovulatory intervals (IOIs) with three periovulatory periods were obtained for each of the remaining 23 mares. For the mid-IOIs (Days 5 to 18), significant ($P < 0.0001$) day effects, without an effect of mid-IOI (first vs second) or an interaction, were obtained for diameters of F1 and F2; number of follicles 2 to 5 mm, 5.1 to 10 mm, 15.1 to 20 mm, 20.1 to 25 mm, 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 to 15 mm, the main effect of mid-IOI (greater number in the second IOI), as well as day, was significant, but the interaction was not. Estradiol, 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.

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 periods (first, second, and third) 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 Days -4 to -2 (0.5 ± 0.1 ng/ml/d) was less ($P < 0.0001$) than for Days -2 to 0 (2.9 ± 0.3 ng/ml/d). Estradiol 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 estradiol involved an increase between Days -4 to -2, followed by a decrease. The rate of decrease in estradiol concentrations on Days -2 to 1 (1.5 ± 0.1 pg/ml/d) was greater ($P < 0.0001$) than for Days 1 to 4 (0.2 ± 0.1 pg/ml/d).

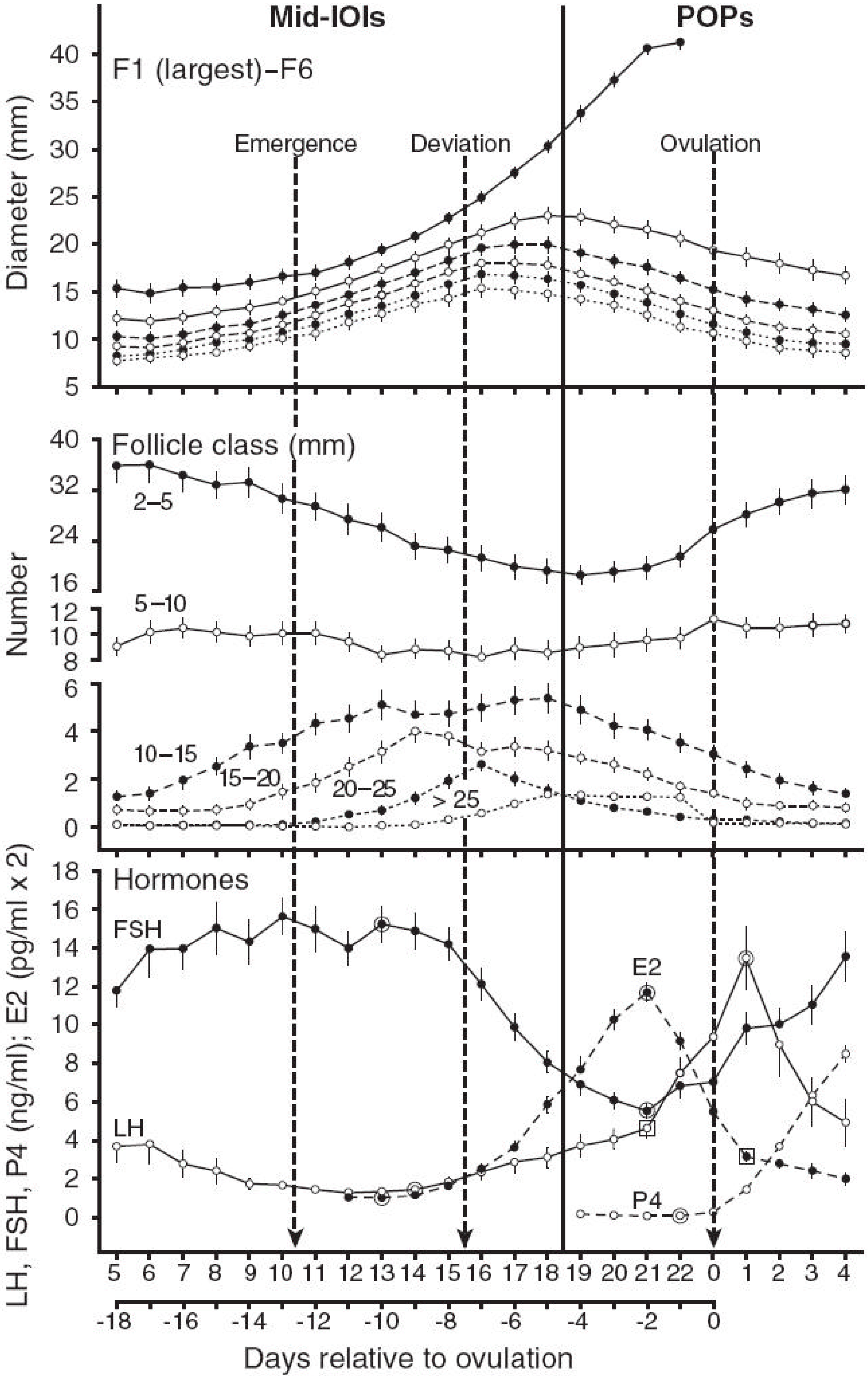

Figure 2 – Mean (± SEM) diameter of the six largest follicles. Number of follicles in a diameter class, and concentrations of hormones combined for two Mid–IOIs and three periovulatory periods (POP) in 23 mares. All diameters, follicle numbers, and hormone concentrations showed a significant day effect. 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.

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 four days postdeviation, on Days -3 to -2, and on Days -2 to -1, there was a main effect of the day groups ($P < 0.0001$), 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.

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 within mares between IOIs (n = 23 mares)

End points	**Interovulatory interval**			**Correlation**	
	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/d)					
Four days postdeviation	3.4 ± 0.2	3.4 ± 0.1	3.4 ± 0.1	-	NS
Days -3 to -2	3.2 ± 0.4	3.0 ± 0.3	3.1 ± 0.3	-	NS
Days -2 to -1	1.2 ± 0.5	0.1 ± 0.3	0.6 ± 0.3	-	NS

[a] No significant differences between the two IOIs.
NS = not significant.

The number of days during the 23–d IOI with a positive and significant correlation and the mean of the daily correlations are shown (Table 2). To illustrate the extent of the differences in follicle and hormone values among mares, the lowest and highest mean/mare for each end point are also given. The mean for the 23 daily correlations within mares between the two IOIs for diameter of F1 reflected positive and significant correlations only for Days 15 (+0.49), 16 (+0.48), 20 (+0.56), 21 (+0.47), and 22 (+0.44); Day 22 corresponds to Day -1.

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	**Average of daily correlations**	**No. days with a significant correlation**	**IOI with**	
			Lowest mean	**Highest mean**
Diameter largest follicle (mm)	+0.29 ± 0.03	5/23	16.5 ± 1.2	27.0 ± 1.3
Follicles/mare/day (No.)	+0.65 ± 0.02^{a}	22/23^{a}	13.9 ± 0.5	48.3 ± 2.2
2 to 5 mm	+0.49 ± 0.2^{b}	18/23^{b}	1.7 ± 0.2	14.8 ± 0.7
5.1 to 10 mm	+0.49 ± 0.2^{b}	18/23^{b}	1.7 ± 0.2	14.8 ± 0.7
10.1 to 15 mm	+0.40 ± 0.03^{c}	12/23^{c}	0.3 ± 0.08	5.9 ± 0.7
15.1 to 20 mm	+0.42 ± 0.04^{c}	12/23^{c}	0.2 ± 0.07	4.1 ± 0.3
20.1 to 25 mm	+0.11 ± 0.06^{d}	2/23^{d}	0.1 ± 0.04	1.7 ± 0.2
Hormones	+0.55 ± 0.03^{a}	18/23^{a}	6.5 ± 0.4	21.2 ± 1.6
FSH (ng/ml)	+0.59 ± 0.03^{b}	22/23^{b}	1.4 ± 0.1	12.9 ± 1.9
LH (ng/ml)	+0.1 ± 0.1^{c}	0/9^{c}	0.4 ± 0.1	1.8 ± 0.3
Estradiol (pg/ml)				

^{Abcd} For each column, values within the five follicle–diameter classifications and within the three hormones with different superscript are significantly different ($P < 0.05$) or approach significance ($P < 0.1$).

The mean of the daily correlations and number of days with significant correlations for five follicle classes are shown. The greatest mean correlation and number of days with positive correlations occurred in the 2 to 5 mm class and the lowest in the 20.1 to 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 to 10 mm class, the correlations were significant on 18 days, but approached significance ($P < 0.09$) on the remaining days. In the 10.1 to 15 mm class, the correlations were significant consistently for Days 13 to 22. In the 15.1 to 20 mm class, the days of consistency were Days 10 to 19.

The correlation for the number of follicles in the 20.1 to 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 to 21) the correlations were not significant. The number of dominant follicles in the ovulatory wave (1.4 ± 0.1) was not correlated 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 only correlations; however, significance was approached 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 estradiol concentrations for any of the nine days centered on the estimated beginning of deviation. The growth rates of the preovulatory follicle were not correlated between IOIs within mares.

The difference between younger and older groups was significant ($P < 0.02$)for LH concentration on Day 1 (7.9 ± 1.6 vs 2.5 ± 0.4 ng/ml), number of follicles 10.1 to 15 mm on Day 13 (6.0 ± 0.7 vs 3.5 ± 0.6), and length of the IOI (22.5 ± 0.3 vs 23.8 ± 0.5 d). Correlations were positive (+0.53 to +0.78) and significant ($P < 0.04$) for each end point within each age group.

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 class with the next largest diameter as follows: 1) 2 to 5 mm and 5.1 to 10 mm (+0.43, $P < 0.04$), 2) 5.1 to 10 mm, and 10.1 to 15 mm (+0.70, $P < 0.0002$), and 3) 10.1 to 15 mm, and 15.1 to 20 mm (+0.80, $P < 0.0001$). There were no other correlations for any of the combinations of the four diameter classes or for FSH and each of the diameter classes, for FSH and LH, or for LH and diameter of F1 on Day -1.

4. Discussion

The characteristics and temporal interrelationships of changes in follicle diameters, follicle numbers, and hormone concentrations were similar to those of previous studies, but some new observations were made. The similarities included: 1) changes in diameter of the six largest follicles measured without regard to day-to-day follicle identity (Ginther et al., 2007a); 2) day of the beginning of deviation as determined by retroceding the postdeviation 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); 3) changes in numbers of follicles in several diameter classes (Pierson and Ginther, 1987). consistent with progressive increases in diameter during the common-growth phase preceding deviation; 4) a reduction or plateau in growth rate of the preovulatory follicle on the day before ovulation (Gastal et al., 2006); 5) 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 estradiol peak (Ginther et al., 2006b); 6) increase in estradiol beginning 1 or 2 d before the beginning of deviation in both mares (Gastal et al., 1999, Ginther et al., 2007b) and women (Ginther et al., 2005a); 7) estradiol and LH surges reaching peak concentrations 2 d before and 1 d after ovulation, respectively (Ginther et al., 2006b); 8) beginning of deviation during decreasing FSH, as previously reported for both mares and women (Ginther et al., 2005a); and 9) beginning of a progesterone increase in the plasma between Day -1 and 0 (Townson et al., 1989).

Characteristics that have not been reported previously likely were detected in this study because of the large data mass centralized to the day of ovulation. Mean concentrations of FSH began to increase and estradiol began to decrease in synchrony on Day -2. These two changes are likely related, given that exogenous estradiol has a negative effect on FSH in mares (Donadeu & Ginther, 2003). The FSH increase between Day -2 and -1 was followed by a transient suspension in the increase or a plateau in FSH between Days -1 and 0. This phenomenon occurred in the means of each of the three periovulatory periods. In this regard, ovulation (Day 0), as indicated by a collapsed follicle, ovulation occurred sometime between Day -1 and Day 0. A spike in immunoreactive (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). 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 apparent rebound in FSH occurred between Days 0 and 1. This FSH response likely reflected the release of FSH from the inhibiting 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 estradiol concentrations on Days -2 to 1 and a slower decrease on Days 1 to 4 resulted in an abrupt change in the rate of output on Day 1 in synchrony with the mean day of the LH peak. The rapid decrease in estradiol is attributable to a negative effect of the rapidly increasing LH (Ginther et al., 2007b), and therefore the slower decrease is attributable to the gradual loss of LH.

A positive and significant correlation for an end point between IOIs within mares was taken as an indicator that the magnitude of the end point was toward repeatability within individual mares and that the magnitude of the correlation was an indicator of the extent of repeatability. On this basis, for example, the finding that the preovulatory 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 preovulatory follicle occurred on the three 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 mares history of 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 preovulatory 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.

In addition to the three days before ovulation. F1 diameter showed a positive correlation on Days 15 and 16 or approximately at the beginning of deviation. The interval from ovulation to deviation was also correlated. These results suggested measurable repeatability in individual mares in the time of initiation of deviation. The length of the interovulatory interval was also correlated significantly, but the intervals from ovulation to emergence, emergence to deviation, and deviation to ovulation were not. Therefore, the only interval that apparently 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 involved the highest average positive correlation and the most number of days with a significant correlation in the 2 to 5 mm class. During the next diameter classes, the magnitude of the positive correlations decreased and the number of days with a significant correlation decreased progressively as the diameter that defined the classes increased. At a diameter of 20 to 25 mm. the correlations were significant on only 1 or 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 interovulatory interval, as indicated by positive and significant correlations between IOIs. No significant correlations were found for estradiol 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. The standard error of the mean for a day of interest in each group would be affected by repeatability within mares, resulting in a decrease in the ability to detect real differences between groups.

The relationship between pairs of selected end points was examined by correlation between average values for each mare. The turnover among the diameter classes was represented by positive and significant correlations within mares between sequential classes. For example, the number of follicles in the 2 to 5 mm class was correlated with the number in the 5.1 to 10.1 class but not with the number in other classes. Similarly, the number in the 5.1 to 10 mm class was correlated with the number in the 10.1 to 15 mm class, and the number in the 10.1 to 15 mm class was correlated with the number in the 15.1 to 20 mm class. These correlations are taken as a reflection 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 further appears to 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, LH 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 done by examining three end points and days previously shown (Carnevale et al., 1993) to differ for older mares. Comparisons among end points for the age groups confirmed the reported findings (lower LH on Day 1. fewer follicles 10 to 16 mm on Day 13. and a longer IOI in older mares). Although the study was limited by the comparisons between only two age groups, the repeatability (high positive correlations) between IOIs within each age group 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 group. Further study will be needed to confirm these findings on age and determine the factors contributing to repeatability.

5. Conclusion

In conclusion, several new aspects of the hormonal interrelationships during the periovulatory period were detected. Mean concentrations of FSH began to increase and estradiol began to decrease on Day -2. The FSH between Days -2 and -1 was followed by a transient suspension in the increase between Days -1 and 0, followed by an increase, apparently a rebound, on Days 0 to 1. A change from a rapid to slow decrease in estradiol concentrations occurred on Day 1 in synchrony with peak concentration of LH. 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 on Days -3. -2, and -1; b) length of intervals from ovulation to deviation and from ovulation to ovulation; c) numbers of follicles in the 2 to 5. 5.1 to 10. 10.1 to 15, 15.1 to 20 mm classes, with the greatest repeatability (mean correlation) and number of days with a significant correlation in the 2 to 5 mm class; and d) concentrations of FSH and LH. Repeatability characteristics known to be affected by age occurred in both younger and older mares.

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