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Relationships between body condition and follicle development in mares[1]

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Abstract

Follicle activity and gonadotropin concentrations were compared between mares with low body condition (n=9) and mares with high body condition (n=8). Examinations began during the anovulatory season (August 14, Southern Hemisphere) and continued until the second ovulation of the year (63 to 141 days). Mares were fed with a complete diet of 1.5–2.0% of body weight in dry matter/day. Body condition increased slightly and similarly for the two groups during the study. Low body condition compared to high body condition was associated significantly with the following: longer interval to first ovulation (77.8 ± 6.9 vs 63.0 ± 3.8 days), smaller maximum diameter of the ovulatory follicle for the first ovulation (45.6 ± 1.4 vs 51.1 ± 1.0 mm) and second ovulation (45.1 ± 1.8 vs 51.4 ± 1.0 mm), fewer medium follicles (11-19 mm) per day preceding the first ovulation (6.0 ± 0.0 vs 9.1 ± 1.5) and fewer large follicles (≥ 20 mm) preceding the second ovulation (1.3 ± 0.2 vs 2.0 ± 0.2). During the last 19 days of the interovulatory interval, each of the four largest follicles was smaller in mares with low body condition than in mares with high body condition. There were no differences between groups in growth rate of the ovulatory follicle or in concentrations of FSH and LH, preceding either the first or second ovulations. Results indicated that low-body condition was associated with reduced follicle development, including diameter of the ovulatory follicle, during the transition between the anovulatory and ovulatory seasons and during the first interovulatory interval of the ovulatory season. These results were not attributable to altered circulating concentrations of FSH and LH.

Keywords: body condition, follicles, mares.

Introduction

Inadequate nutrition or body condition has been associated with delayed onset of the breeding season, decreased pregnancy rate, increased embryo loss, and increased gestation length in mares (Henneke *et al*., 1983, 1984; Hines *et al*., 1987). During the winter, mares with low body condition had fewer medium (11 to 19 mm) and large (≥ 20 mm) follicles than mares with high body condition (Gentry *et al*., 2002). Apparently, the effects of inadequate nutrition or poor body condition on follicle dynamics during the equine ovulatory season are not known. In cattle, chronic or acute dietary restriction resulted in a gradual (Murphy *et al*., 1991; Bossis *et al*., 1999) or rapid (Mackey *et al*., 1999; Comin *et al*., 2002) reduction in growth rate and maximum diameter of the dominant follicle. Low dietary intake tended to increase the occurrence of estrous cycles with three major follicular waves in beef heifers (Murphy *et al*., 1991).

The mechanisms by which feed restriction modifies the reproductive axis are not well understood. Nutrients required for reproduction have not been differentiated from those required for other physiological functions. Initially, glucose, insulin, and fatty acids were considered as potential signals in the regulation of the reproductive axis; however, other studies have concluded that these metabolites do not play a role (Dowining *et al*., 1995; Boukhliq *et al*., 1996; Schreihofer *et al*., 1996; Wade *et al*., 1996).

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The present experiment was designed to study the relationship between body condition and follicle dynamics during the transition between the anovulatory and ovulatory seasons and during the first interovulatory interval in mares.

Materials and Methods

Animals and Groups

Twenty maiden, small draft-type, crossbred Breton mares, 3 to 4 years of age, and weighing 200 to 400 kg were used in the Southern Hemisphere (latitude, 21º). All mares were in the seasonal anovulatory phase characterized by follicles ≤ 20 mm and absence of a corpus luteum as determined by ultrasound examinations (Ginther, 1995). Mares were kept in corrals and fed daily with a diet consisting of forage (green grass, *Pennisetum purpureum schum*) and a mixture of grain, molasses and vitamins (Nutroeste; Nutrição Animal, Goiânia, GO, Brazil) to supply their maintenance requirements. Intake of dry matter per day ranged from 1.5 to 2.0% of body weight. Mares had free access to water and mineralized salt. The experiment was started during the last month of winter (August 14; equivalent to February in the Northern Hemisphere) and was concluded at the second ovulation of the ovulatory season. Mares that did not ovulate by 116 days after the beginning of the experiment were not used because of a requirement for normalizing to ovulation. Body-condition score (1 to 9, lowest to highest; Henneke *et al*., 1983) and body weight were evaluated every 15 days. The score for body condition at each examination was determined by averaging the scores of two operators. At the first ovulation of the ovulatory season, the average of 4 to 6 body-condition scores for each mare was used to assign the mares to two groups: high body condition (score ≥ 5; n=9) and low body condition (score <5; n=11).

Follicle Evaluations

An ultrasound scanner (Aloka SSD-500V; Aloka, Wallingford, CT) equipped with a 5 MHz linear-array transducer was used for transrectal ovarian examinations. Ovarian activity was evaluated over a span of 63 to 141 days, encompassing the first and second ovulations of the year; examinations were discontinued at the second ovulation in individual mares. Examinations were done every three days until a 25-mm follicle developed. After the largest follicle was ≥ 25 mm, scanning was done daily until ovulation, using day-to-day identity of the largest follicle (Ginther, 1995).

Number of follicles per day was grouped into three categories: small (5-10 mm), medium (11-19 mm), and large (≥ 20 mm). Number of follicles was assessed for 55 days before the first ovulation and 19 days before the second ovulation, using the mean value per mare over the indicated days in the comparison between groups. The number of major anovulatory waves per mare was taken from the beginning of the experiment to the first ovulation and between the first and second ovulations. A major anovulatory wave was identified by the presence of a nonovulatory follicle that reached ≥ 30 mm (Ginther, 1993). Other follicle and gonadotropin end points were assessed for 31 days before the first ovulation and 19 days before the second ovulation. End points for the ovulatory follicles were diameter at maximum and at Day -1 (Day 0 = ovulation) and growth rate during the 7 days before ovulation. The four largest follicles were ranked as F1, F2, F3, and F4, according to descending diameter on each day of examination. The lengths of intervals from the beginning of the experiment to a ≥ 25-mm ovulatory follicle, from the beginning to the first ovulation, and from the first to the second ovulations (interovulatory interval) were also recorded.

Blood Samples and Hormone Assays

Jugular blood samples were collected every 3 days from each mare into heparinized tubes and centrifuged (500 xg for 10 min), decanted, and stored (–20 °C) until assay. Plasma samples were assayed for FSH and LH by radioimmunoassay as validated (Whitmore *et al*., 1973; Freedman *et al*., 1979, respectively) and modified (Donadeu & Ginther, 2002) in our laboratory. The intra- and interassay coefficients of variation and mean sensitivity were 12.7%, 11.7%, and 1.1 ng/mL for FSH and 8.4%, 5.0%, and 0.4 ng/mL for LH, respectively.

Statistical Analyses

Follicle data and plasma concentrations of gonadotropins were analyzed to determine effects of group, day and the interaction, using a mixed linear model with a repeated statement to account for the autocorrelation between sequential measurements (SAS, Institute Inc., Cary, NC). If a significant effect of group or group-by-day interaction was detected, unpaired t-tests were used to locate the mean differences between groups within a day, and paired t-tests were used between days within a group. The difference between groups in frequency of the number of mares with a major anovulatory wave was analyzed by chi-square. Pearson correlations between scores for body condition and maximum diameter of the ovulatory follicle were performed. A probability of P≤ 0.05 indicated that a difference was significant. All data are expressed as the mean ± SEM.

Results

Two mares from the low body-condition group did not ovulate by 116 days, resulting in nine mares in the group. A mare was removed from the high body-condition group because of an injury, resulting in eight mares in the group. Body-condition scores from the day at the beginning and the day at end of the experiment showed a main effect (P<0.0001) of group (high, 6.2 ± 0.3; low, 4.2 ± 0.1) and an effect (P<0.0006) of day (beginning, 4.6 ± 0.2; end, 5.5 ± 0.3), but the interaction was not significant. For body weight, there was an effect (P<0.0001) of group (high, 370 ± 12; low, 297 ± 12 kg) with no other significant differences.

Data associated with each of the two ovulations and the results of the statistical analyses are shown (Table 1). The mares with low versus high body condition had a longer interval from beginning of the

Table 1. Mean ± SEM follicle characteristics for mares with high and low body-condition scores.

	Body condition[a]				
End points	High		Low		Probability
Intervals (days)					
Beginning of the experiment					
To ≥ 25 mm ovulatory follicle	50.4	± 3.6	68.8	± 6.8	P<0.02
To the first ovulation	63.0	± 3.8	77.8	± 6.9	P<0.04
25-mm ovulatory follicle to ovulation					
First ovulation	12.6	± 2.1	8.8	± 0.8	P<0.04
Second ovulation	10.3	± 0.8	9.9	± 0.9	NS[b]
First to second ovulation	22.8	± 1.1	24.0	± 2.6	NS
Ovulatory follicle					
Maximum diameter (mm)					
First ovulation	51.1	± 1.0	45.6	± 1.4	P<0.004
Second ovulation	51.4	± 1.0	45.1	± 1.8	P<0.003
Day –1[c] diameter (mm)					
First ovulation	49.4	± 1.5	45.2	± 1.4	P<0.03
Second ovulation	51.1	± 1.0	44.3	± 1.4	P<0.001
Growth rate (mm/day)[d]					
First ovulation	1.3	± 0.4	1.8	± 0.3	NS
Second ovulation	2.5	± 0.4	1.9	± 0.6	NS
Number of follicles/day					
First ovulation					
5-10-mm	6.6	± 1.3	4.6	± 0.9	P<0.1
11-19-mm	9.1	± 1.5	6.0	± 0.0	P<0.05
≥ 20-mm	2.0	± 0.2	2.0	± 0.2	NS
Total (≥ 5 mm)	17.7	± 2.7	12.5	± 1.9	P<0.06
Second ovulation					
5-10-mm	5.9	± 0.7	5.2	± 0.9	NS
11-19-mm	5.8	± 0.8	4.0	± 1.0	P<0.09
≥ 20-mm	2.0	± 0.2	1.3	± 0.2	P<0.03
Total (≥ 5 mm)	14.0	± 1.5	10.0	± 1.7	P<0.05
Number of major anovulatory waves/mare[e]					
First ovulation	0.8	± 0.2	1.3	± 0.4	P<0.1
Second ovulation	0.4	± 0.2	0.4	± 0.2	NS

[a]Mares were grouped into those with high (≥ 5; n=8) versus low (<5; n=9) scores.
[b]NS = not significant.
[c]Day 0 = ovulation.
[d]Growth rate between Day -7 and -1.
[e]Largest follicle ≥ 30 mm.

experiment to a ≥ 25-mm ovulatory follicle, longer interval to the first ovulation, and a shorter interval from a ≥ 25-mm ovulatory follicle to ovulation. Diameter of the ovulatory follicle was smaller at the maximum diameter and at the day before the first and second ovulations in the low body-condition group. Diameter of the ovulatory follicle averaged over Days -7 to -1 before the first and second ovulations was larger (P<0.01) for the high-condition group (46.6 ± 0.7 and 44.6 ± 0.8 mm, respectively) than for the low-condition group (41.0 ± 0.7 and 39.5 ± 1.0 mm). There were fewer small and medium follicles per day before the first ovulation and fewer medium and large follicles before the second ovulation in the low- than in the high-condition group (Table 1). The total number of follicles (≥5 mm) per day was greater for mares with high body condition than for mares with low body condition before the first and second ovulations. A greater mean number of major anovulatory waves preceding the first ovulation in the low than in the high body-condition group approached significance. A greater number of mares with multiple major anovulatory waves in the low group (3/7) than in the high group (0/6) approached significance (P<0.07). For the mares that had one or more major anovulatory waves (7/9 and 6/8 in the low and high groups, respectively), there were more (P<0.04) waves per mare in the low group (1.4 ± 0.2) than in the high group (1.0 ± 0.0). The body-condition score was positively correlated with the ovulatory follicle at maximum diameter (P<0.01) and at Day -1 (P<0.0001) before the first ovulation (r=0.65 and r=0.59, respectively) and the second ovulation (r=0.83 and r=0.84).

During 31 days preceding the first ovulation, main effects of day indicated increasing diameters for F1 and F2 (Fig. 1). The group-by-day interaction was significant for only F3. There was no difference between groups or an interaction of group and day for either FSH or LH concentrations. A day effect for LH reflected increasing (P<0.05) concentrations between Days -7 to -1. During the 19 days preceding the second ovulation, there were main effects for both group and day but no interaction for any of the four follicles (Fig. 1). Although a day effect was obtained for both FSH and LH, there were no differences involving groups.

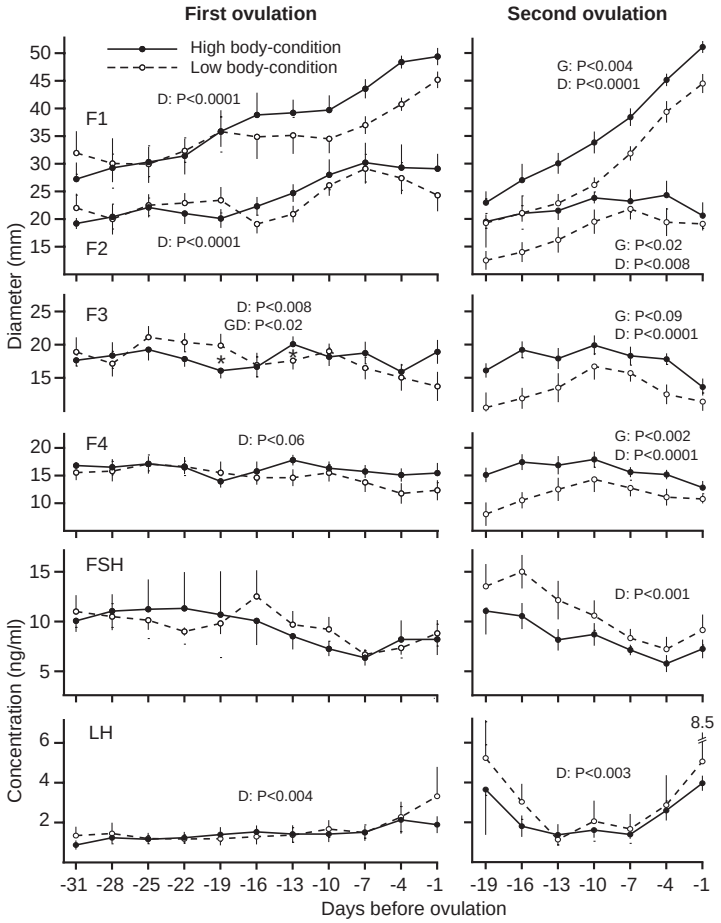

Figure 1. Mean ± SEM diameter of the four largest follicles (F1, F2, F3, and F4) before the first ovulation of the year (left panel) and the second ovulation (right panel) in mares with high (solid lines) or low (broken lines) body-condition scores. The main effects and interactions that were significant or approached significance are shown. G = group effect; D = day effect; GD = group-by-day interaction. An asterisk indicates a difference (P<0.05) within a day, when a significant interaction was obtained.

Discussion

Body condition increased in the high-condition and low-condition groups during the experiment. There was no indication that the changes in body condition were different between groups (no day-by-group interaction). The feeding program, slightly improved the initial body-condition scores and maintained body weights (no effect involving day) until the end of the experiment.

The longer interval from the beginning of the experiment to the first ovulation of the season in mares with low body condition is consistent with results of previous studies of feed restriction and/or poor body condition (Ginther 1979; Henneke *et al*., 1984; Kubiak *et al*., 1987). In addition, two mares in the low-condition group were removed because they did not ovulate within 116 days; follicles attained diameters of 28 and 40 mm in these two mares. Although mares with low body condition needed more days for the ovulatory follicle to reach ≥ 25-mm, the interval between a 25-mm ovulatory follicle and ovulation was shorter but the growth rate of the ovulatory follicle after Day -7 was similar. These findings are consistent with the smaller diameter of the preovulatory follicle in the low body-condition mares. Body condition did not alter the length of the first interovulatory interval of the reproductive season.

A negative effect of low body condition on the ovulatory follicle during the transition between the anovulatory and ovulatory seasons and during the first interovulatory interval was indicated by reduced diameters at maximum, on Day -1, and averaged over Days -7 to -1. These findings are consistent with the results of the correlation analysis which indicated that the greater the body condition, the greater the diameter of the ovulatory follicle at the maximum and at Day -1 for the first and second ovulations. Previous studies of the relationships between nutritional deficiency and follicle dynamics in cattle indicated negative effects on the maximum diameter and growth rate of the dominant follicle (Perry *et al*., 1991; Rhodes *et al*., 1996; Mackey *et al*., 1999). However, this is the first report of a negative effect of poor body condition on diameter of the first and second ovulatory follicles of the season in mares. In contrast to the cattle results, growth rate of the ovulatory follicle in mares was not altered by body condition.

Mares with low body condition had more multiple major anovulatory waves before the first ovulation of the year, although, in this regard, the interval from beginning of experiment to the first ovulation was 15 days longer in the low-condition group. Body condition may account for some of the differences among reports on the incidence of major anovulatory waves at the end of the anovulatory season in mares (Ginther, 1992), suggesting a need for further study. The occurrence of estrous cycles with three major follicular waves increased in beef heifers subjected to a low dietary intake (Murphy *et al*., 1991), which seems similar to the present findings in mares.

Mares with low body condition had fewer medium follicles before the first ovulation of the season. This result has been reported previously in mares subjected to an inadequate nutrition (Van Niekerk and Van Heeden, 1972) or with low body condition (Gentry *et al*., 2002) during the anovulatory season. However, fewer follicles during the ovulatory season (first interovulatory interval) in mares with poor body condition have not been previously reported.

Apparently this is the first report on the changing diameter of the four largest follicles during the transition between the anovulatory and ovulatory seasons and during the first interovulatory interval in mares with different body-condition scores. From Day -31 preceding the first ovulation, the main effect of day indicated increasing diameters of F1 and F2 for both body-condition groups. However, diameter of F1 was smaller in the low-condition group between Days -7 and -1. For the 19 days before the second ovulation, all four follicles were larger in mares with high body condition as indicated by the main effects of group. The day effect for each follicle indicated increasing diameter of F1 throughout the 19 days, contrasting with a plateau or decreasing diameters of F2–F4 after 10 days before ovulation. This result is consistent with the continuing increase in diameter of a dominant follicle (F1) and the decreasing diameters of subordinates follicles (F2–F4) after follicle deviation or selection during the estrous cycle (Ginther *et al*., 2003).

In the present study, the gonadotropins were not affected by body-condition. This finding agrees with previous studies in mares subjected to feed-restriction or with low body condition. Acute (McManus and Fitzgerald, 2000) or chronic (Gentry *et al*., 2002) feed restriction did not alter the plasma concentrations of LH, FSH, TSH, GH, glucose, or insulin. In contrast, in heifers (Rhodes *et al*., 1996), sheep (Miller *et al*., 1998), and pigs (Tokach *et al*., 1992) poor nutrition or body condition was associated with altered gonadotropins concentrations. Recently, the adipocyte-derived hormone leptin was suggested as a candidate for signaling nutrient status to the reproductive axis (Bray, 1996). A decrease in endogenous leptin secretions was temporally associated with cessation of reproductive activity during the anovulatory season in mares (Fitzgerald and McManus, 2000). It was proposed that, the reproductive response to a decrease in photoperiod or a presumptive inhibitory melatonin signal is modified by energy availability,

which may be signaled to the hypothalamus-pituitary axis through a change in the circulating concentration of leptin. In addition, low leptin, IGF-I, and prolactin plasma concentrations were observed in mares with low body-condition score during the middle of the anovulatory season (Gentry *et al*., 2002). Although the present study indicated a distinct effect of low body condition on the follicles associated with the first and second ovulations of the season, additional study will be required to elucidate the underlying mechanisms. Apparently, FSH and LH were not involved directly. Studies are needed in mares on the concentrations of follicular-fluid factors during low body condition. In this regard, the concentration of estradiol-17β was reduced and the concentrations of NEFA (Nonsterified Fatty Acid), IGFBP2 and IGFBP3 (IGF - Binding Protein) were increased in the follicular fluid of dairy cows subjected to feed restriction (Comin *et al*., 2002).

In conclusion, results of this study confirmed previous findings that poor body condition during the transition between the equine anovulatory and ovulatory seasons is associated with a delayed beginning of the ovulatory season. Novel findings were that body condition affected development of the ovulatory follicle for both the first and second ovulations of the ovulatory season, as shown by smaller diameter of the follicle in mares with low body condition. In addition, mares with low body condition had fewer follicles ≥5 mm before both the first and second ovulations. In the low body-condition group, only the largest follicle was negatively affected (Days -7 to -1) before the first ovulation; however, the diameter of the four largest follicles was reduced during 19 days before the second ovulation.

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