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Post-partum follicular dynamics in beef cows calving during spring and autumn in southern Brazil*

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Abstract

Ovarian activity early post-partum in beef cows with intermediate body condition scores that calved during spring and autumn and treated with either 48 h of temporary weaning or exogenous hormones was investigated. Calving cows were given body condition scores and their ovaries were ultrasonographically scanned daily starting on day ten post-partum. The number and size of the follicles were recorded. Upon detection of a dominant follicle ($\geq$9 mm), the animals were distributed to different treatments. Over 80% of the animals (41/49) in both seasons presented a dominant follicle during the second or third week post-partum. The percentage of cows ovulating within seven days after treatment varied from 30% (3/10) for control cows to 60% (6/10) for MAP+GnRH treated cows for both spring and autumn calving cows. A reduction of 16% and 19% in body condition score was observed during the post-partum period studied for both spring and autumn calving cows, respectively. The decrease in body condition score was accompanied by a reduction in the follicular population of 43% during the fifth week post-partum only in those calving during autumn. In the spring calving cows, no change was detected in the follicular population despite the decrease in body condition score. Irrespective of the differences in environmental conditions between the two breeding seasons, cows present large follicles in their ovaries that are capable of responding to hormonal treatments, during the early post-partum period.

Introduction

The search for a method to rapidly re-establish cyclic ovarian activity in post-partum beef cows has been the objective of reproductive techniques developed during the last

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decades. Most of these techniques are related to weaning practices and hormonal induction of ovulation[1,2]. Using these techniques it is possible to control GnRH and LH secretion to stimulate ovarian follicular waves[3]. For economic reasons, and because of the extensive nature of beef production systems in southern Brazil (animals are stocked in large paddocks all year round, subjected to seasonal changes in forage growth and quality without any feed supplement) weaning has been the main method used to stimulate follicular development post-partum. However, when weaning is performed after 90 days post-partum (DPP) there is not an appreciable increase in the annual fertility rate. Earlier weaning requires increased producer inputs regarding rearing and nutrition of calves. To maximize income under these conditions requires that the cows be pregnant by 100 DPP and that they suckle their calves to at least 120 DPP (depending on the need for supplementary feeding and the desired growth rate of calves). The main breeding season in southern Brazil is during the spring and summer (November - January) when there is an increase in the natural pasture growth, however the body condition score (BCS) of the cows at calving at this time is poor because of the forage shortage during winter (June - August). Autumn breeding (April - May) results in higher fertility because the cows have a better BCS at calving. The time available for autumn breeding, however is short due to the deteriorating quality of available forage and the low growth rate of pastures during winter[4].

The interactions of BCS, calving date, and milk production are the main factors determining the duration of the post-partum anestrus period in all environmental conditions[5,6], however the physiological nadir for BCS is reached at different times for each production system, age and/or parity of cows and season of reproduction.

The objectives of this study were to investigate early post-partum ovarian follicular activity of beef cows with intermediate body condition scores calving in southern Brazil during the spring and autumn. Also, the effect of 48h temporary weaning and estradiol cypionate (ECP), GnRH, and the association of ECP and GnRH with medroxy-progesterone acetate (MAP) on the ovarian follicular population was investigated.

Materials and Methods

Experimental procedures

Calving cows were monitored twice daily until calving. At calving they were weighed and assigned a body condition score (BCS, 1 = excessively lean to 5 = fat[7]) immediately after parturition and thereafter they were weighed and assigned a BCS once weekly until nine weeks post-partum. Ovarian activity was monitored by daily transrectal ultrasound examination (Vet Scan 480, Pie Medical, 5mhz) starting on the 10th DPP. The ovarian structures visualized were photographed, drawn and numbered so as to follow their subsequent development. The criteria used to classify a follicle as dominant (DF) was a diameter of at least 9 mm and accompanied by a reduction in the size or number of the other follicles present in the ovaries. Once a DF was detected, the animals were distributed sequentially to one of the following treatments: during spring, a single IM injection of 0.5 mg gonadorelin (GnRH); a single IM injection of 2 mg of estradiol cypionate (ECP); calves fitted with nose plates to prevent them from suckling for 48h; an intravaginal sponge impregnated with 250 mg of medroxy-progesterone acetate (MAP) for nine days and at withdrawal an IM injection of 0.5 mg GnRH; an intravaginal sponge impregnated with 250 mg of MAP for nine days and at withdrawal an IM injection of 2 mg of ECP; and untreated control animals; and during autumn, calves fitted with nose plates to prevent them from suckling for 48h; an intravaginal sponge impregnated with 250 mg of MAP for nine days and at withdrawal an IM injection of 0.5 mg GnRH; an intravaginal sponge impregnated with 250 mg of MAP for nine days and at withdrawal an IM injection of 2 mg of ECP; and untreated control animals. Once the treatments began, whole blood samples were collected daily for seven days. Samples were centrifuged immediately after collection, and the plasma was separated and stored at -18°C until assayed. Plasma progesterone and FSH concentrations were determined by double antibody radioimmunoassay as described elsewhere [8,9]. The sensitivity of the FSH and progesterone assays was 0.3 ng/ml and 0.1 ng/ml, respectively. The inter and intra assay coefficients of variation were below 15%.

Ovarian follicles were classified according to their diameter into: large follicles (LF) greater than or equal to 9 mm in diameter, medium size follicles (MF) between 5 and 9 mm in diameter, and small follicles (SF) less than 5 mm in diameter. Ovulation was determined to be when a LF disappeared and an echogenic area compatible with luteal tissue appeared in its place using transrectal ultrasonography. For the purpose of statistical analysis the variables BCS, total number of follicles (NOFOL), LF, MF, SF were initially grouped according to post-partum week and subjected to one-way ANOVA. The data on follicular population before the first DF was detected was first analyzed for all the animals. The diameter of the LF within treatments and the plasma concentrations of progesterone and FSH were subjected to a repeated-measures ANOVA using the NCSS 6.0 package[10], represented by the following mathematical model:

$$Yijkl = m + Ai + Sij + Bk + ABik + eijkl$$

where A is the between treatment effect, Sij is the between group error, B is the day after treatment effect, and eijkl is the within subject error. All variable means represent the mean plus the contribution of studied effects.

Spring experiment

During the spring experiment, 28 adult purebred Hereford and Aberdeen Angus cows calving between 7 September and 5 November were used. Once a DF was detected, cows were subjected to one of the following treatments: GnRH, a single IM injection of 0.5 mg gonadorelin[i] (n=5); ECP, a single IM injection of 2 mg of estradiol cypionate[ii] (n=4); W48h, calves fitted with nose plates to prevent them from suckling for 48h (n=5); MAP+GnRH, an intravaginal sponge impregnated with 250 mg of medroxy-progesterone acetate[iii] for nine days and at withdrawal an IM injection of 0.5 mg GnRH (n=5); MAP+ECP, an intravaginal sponge impregnated with 250 mg of MAP for nine days and at withdrawal an IM injection of 2 mg of ECP (n=5); and Control, untreated control animals (n=4).

Autumn experiment
During the autumn experiment, 21 adult purebred Hereford and Aberdeen Angus cows calving between 2 March and 2 May were used. Once a DF was detected, cows were subjected to one of the following treatments: W48h, calves fitted with nose plates to prevent them from suckling for 48h (n=5); MAP+GnRH, an intravaginal sponge impregnated with 250 mg of MAP for nine days and at withdrawal an IM injection of 0.5 mg GnRH (n=5); MAP+ECP, an intravaginal sponge impregnated with 250 mg of MAP for nine days and at withdrawal an IM injection of 2 mg of ECP (n=5); and Control, untreated control animals (n=6).

Results

Spring experiment
All 28 cows calving during the spring had a DF by 29 DPP. Fifteen had DF (53.5%) during the second week, 11 (39.3%) during the third week and two (7.2%) during the fourth week.

In examining the data before the detection of the first dominant follicle, (before the treatments were applied) Duncan's multiple comparison test revealed that the BCS was lower (P<0.001) during week four than during weeks two and three post-partum, however, no difference in ovarian activity was observed (Table 1) .

During the seven day post-treatment period the percentage of cows that ovulated was 80% (4/5) for the GnRH treatment, 75% (3/4) for ECP treatment, 40% (2/5) for W48h treatment, 100% (5/5) for MAP+GnRH treatment, 80% (4/5) for MAP+ECP treatment, and 25% (1/4) for the control. The mean diameter of the LF at each day revealed no significant difference between treatments, but considering all the groups a significant decrease (P<0.05) in mean diameter was observed on days two, three, and four with respect to day zero (Figure 1a). Furthermore, a significant interaction (P<0.05) between treatments and day was detected (Table 2). The profile of the LF for the treatments that had a significant difference can be observed in Figure 1b. The mean diameter of the LF of the GnRH and MAP+GnRH treated animals on day seven was significantly greater (P<0.05) than that of the control animals from days one to seven.

Similar to what was observed for follicle development, no significant differences were detected between treatments for circulating concentrations of both FSH and progesterone. The overall mean concentration of FSH during the eight days of sampling was 0.39 ng/ml. The lowest mean value was observed on day one (0.34±0.02 ng/ml) and the highest (0.46±0.02 ng/ml) on day two, both were statistically different (P<0.05) from the means of the other days.

The mean plasma concentrations of FSH and progesterone for the significant (P<0.05) interaction between treatments and the day after the dominant follicle was identified are shown in Figure 2. There was a significant increase in plasma FSH concentration on the second day after MAP+ECP treatment in comparison to the control group (Figure 2a). The plasma progesterone concentrations for the treatments with no progestagen supplementation, are shown in Figure 2b. Cows that received GnRH treatment ovulated early and showed a characteristic increase in progesterone after day five of treatment. There was a significant ($P<0.05$) increase in plasma progesterone concentration in the groups of animals treated with MAP+GnRH, and GnRH alone, probably indicating that luteal activity started earlier in these groups of animals. Despite the high ovulation rate in the cows treated with ECP alone or in association with MAP, no increased plasma progesterone concentrations were observed in comparison to control cows.

In the case of the cows subjected to temporary weaning during spring, the pattern was similar to that of the control group (Table 2).

Autumn experiment
All 21 cows calving during autumn presented a DF by 30 days post-partum. Eight (38%) occurred during the second week, seven (33%) during the third week, two (10%) during the fourth week, and five (19%) during the fifth week.

All cows had a significant ($P<0.05$) reduction in BCS after the fourth week post-partum (Table 3). The total follicular population also decreased during that period, and the decrease was statistically significant for NOFOL and MF.

The mean ovulation rate within seven days after treatments was 33% (2/6) for the control, 20% (1/5) for the MAP+GnRH, 40% (2/5) for the W48h, and 20% (1/5) for the MAP+ECP treatment groups.

The diameter of the LF of the cows that calved during autumn was affected by the type of treatment applied ($P<0.05$). The overall mean diameters (+SE) of the largest follicles during the observation period were 11.4±0.8, 11.2±0.8, 10.0±0.7 and 7.3±0.8 mm for the MAP+GnRH, MAP+ECP, weaning, and control treatments, respectively. No significant difference was observed for days after treatment for the diameter of the LF. The means for the significant interaction ($P<0.05$) observed between treatment and days after treatment for the diameter of the largest follicle are presented in Table 4.

The mean diameter profile of the LF for the W48h group was similar to the controls, except on the first day. The mean diameter of the LF for the MAP+ECP treated animals increased ($P<0.05$) after day four in contrast with the MAP+GnRH treatment in which follicles were bigger ($P<0.05$) than the controls from days zero to six.

The changes in circulating concentrations of FSH and progesterone were not different between treatments in relation to the day the DF was detected. No statistically significant differences were detected for FSH and progesterone. The concentrations of FSH varied between 0.33 and 0.51 ng/ml for the W48h cows, between 0.40 and 0.52 ng/ml for the MAP+GnRH cows, between 0.33 and 0.44 ng/ml for the MAP+ECP cows, and between 0.35 and 0.50 ng/ml for the control cows. Concentrations of progesterone on the seventh day post treatment were 1.95, 3.21, 0.60 and 1.54 ng/ml respectively for W48h, MAP+GnRH, MAP+ECP and Controls.

Discussion

The main objective of this work was the study of ovarian activity during the early post-partum period in cows with intermediate BCS. More than half of the cows have this BCS at the time of mating during spring, in the extensive beef cattle production systems in southern Brazil, where animals are stocked in large paddocks all year round, subjected to seasonal changes in forage growth and quality without any feed supplement. Under these conditions, cows have to be pregnant by 100 days post-partum to be economically profitable. The detection of large or dominant follicles during the second and/or third week post-partum, provides an opportunity to try to reduce the interval between calving and conception by inducing ovulation. In the present study, most of the cows (over 80%) had a large follicle during the first three weeks after parturition both in spring and autumn, but ovulation did not occur. This indicates a deficient LH secretion pattern from the pituitary[11]. Furthermore, our results are in agreement with results described in European beef and dairy systems and in grazing dairy systems in New Zealand[5,12] where early post-partum follicular waves have been detected.

A reduction in BCS was observed during the post-partum period in both spring and autumn calving cows. This coincides with the maximum negative energy balance of the animals post-partum, after which they start a gradual recovering associated with the increasing forage availability during spring[4]. However, whilst there was no change in the follicular population for the spring calving cows, there was a decrease in follicular development during the fifth week post-partum for those cows calving during autumn. This could be due to the fact that cows calving during autumn have their peak lactational demands coinciding with declining nutrient availability in pastures as winter approaches. Another important aspect was that during autumn there were more LF and MF, and these may be more sensitive to the action of gonadotrophins. The evidence of follicular growth during the early post-partum period during both calving seasons, and the occurrence of ovulation after exogenous hormone treatments, indicates that the post-partum anestrus period is independent of the gonads, and that the ovaries are capable of responding to exogenous stimulus, as long as there is no severe nutritional restriction[5].

The significant interactions between treatments and days after the detection of a dominant follicle would be expected with respect to the different mechanisms by which the treatments elicit the liberation of luteinizing hormone. Both GnRH and estradiol treatments result in LH secretion, GnRH elicits an immediate response, because it acts directly at the anterior pituitary level, whilst estradiol acts indirectly[11]. The differences in the dynamics of the diameter of the largest follicle are in agreement with the ovulation rate observed after each treatment, as the largest follicles ovulate or luteinize and disappear from the ovaries. The small number of animals responding to treatment, and the high variability between cows, made it difficult to detect any important variations in the autumn calving cows.

Temporary weaning during the spring had no effect, because the animals had not yet started to recover BCS after parturition[4], and during autumn the temporary weaning effect was manifested by a temporary increase in follicular diameter. The effect of temporary weaning depends on the stage after parturition when applied, and also on the duration of the temporary weaning[13], as full effect on LH pulse frequency only occurs after an isolation period of 96 to 144 h[14]. In the present study the temporary weaning period was too short and applied too soon after parturition to manifest its full effect.

The effect of GnRH causing ovulation[11] was evidenced by the reduction in the mean diameter of the largest follicle on days two and three after treatment during spring (Table 2). Similar effects were observed in primiparous dairy cows undergoing anovulatory follicle turnover during the early post-partum period[16]. This decrease in LF was greater in the spring and was probably caused by a higher frequency of ovulation. The pattern was similar when the treatment was supplemented with the progestagen, only ovulation occurred one day later, which was confirmed by an increase in plasma progesterone concentrations (Figure 2b).

During autumn no differences between treatments in circulating concentrations of FSH and progesterone were detected in relation to the day the DF was observed. The lack of any detectable differences was attributed to the significant variance between individuals in each group (Sij).

Using estradiol cypionate with MAP resulted in an increase in circulating concentrations of FSH starting on day two (Figure 2a). These events could be explained by the regression, ovulation or luteinization of the largest follicles, thereby reducing the negative effect of inhibin and estradiol on FSH secretion.

Only treatments with GnRH caused ovulation and/or luteinization as evidenced by increased concentrations of progesterone after treatment. Although ovulation may have occurred later in the other treatments, the corpora lutea were most likely not secreting sufficient amounts of progesterone before the sampling period finished (Figure 2b).

It can be concluded that irrespective of the differences in environmental conditions and ovulation rates between the two breeding seasons, cows with similar BCS presented similar ovarian follicular activity. Although the results obtained show that it is possible to induce ovulation, the uterine involution of the cows would probably not be adequate to result in high fertility this early post-partum, as 35% conception rates have been reported for cows bred at 20 days after calving[15]. The results contribute to the formulation of ovulation induction schemes during the early post-partum period. They also indicate that the procedures to promote the induction of ovulation or to initiate a breeding season should coincide with the recovery of the BCS post-partum during spring. Also, the increase in ovarian activity in extensive production systems, which is dependent on climatic conditions and forage availability, can be estimated with reasonable efficiency using BCS[4]. Both spring and autumn calving cows loose BCS during the early post-partum period. Spring calving cows are subjected to increasing planes of nutrition because of the increasing quantity and quality of native pastures during spring. Autumn calving cows are subjected to decreasing planes of nutrition because native pasture quality and availability deteriorate as winter approaches. This requires that autumn calving cows must become pregnant earlier during the post-partum period than spring calving cows, because of a decreasing plane of nutrition due to winter climatic conditions.

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Table 1. Means (± SE) of BCS and number of follicles in various categories during the early post-partum period in spring calving cows determined from 10 days post-partum to the detection of the first dominant follicle.

Weeks post-partum	BCS	NOFOL	LF	MF	SF
2	3.1±0.1[a]	11.3±0.3	0.6±0.2	2.2±0.2	8.6±0.3
3	3.1±0.1[a]	9.9±0.5	0.5±0.1	2.0±0.3	7.4±0.5
4	2.6±0.2[b]	10.0±1.0	0.5±0.2	1.6±0.7	7.9±1.0

Different letters in columns ($P<0.05$).
BCS, body condition scores; NOFOL, total number of follicles; LF, number of follicles > 9 mm; MF, number of follicles between 5 and 9 mm; and SF, number of follicles < 5 mm.

Table 2. Adjusted means (± SE; mm) of the largest follicle of cows calving in the spring during the seven days after treatment.

Day	GnRH	ECP	W 48h	MAP+GnRH	MAP+ECP	Control
0	11.2±0.1	12.2±0.1	10.8±0.1	10.7±0.1	10.7±0.1	10.7±0.1
1	10.4±0.1	10.8±0.1	11.5±0.1	11.2±0.1	10.2±0.1	9.6±0.1
2	7.4±0.1	11.2±0.1	9.7±0.1	9.2±0.1	11.0±0.1	8.7±0.1
3	7.9±0.1	10.4±0.1	10.8±0.1	8.2±0.1	7.5±0.1	9.8±0.1
4	10.7±0.1	8.8±0.1	9.9±0.1	9.0±0.1	6.7±0.1	8.5±0.1
5	12.9±0.1	9.6±0.1	10.7±0.1	11.8±0.1	7.4±0.1	10.2±0.1
6	11.9±0.1	9.5±0.1	9.2±0.1	12.7±0.1	8.9±0.1	10.0±0.1
7	14.8±0.1[a]	8.7±0.1	8.6±0.1	14.0±0.1[a]	7.8±0.1	10.4±0.1[b]

Comparisons were made between each treatment and control, different letters in rows ($P<0.05$).

GnRH: cows treated with 0.5 mg of gonadorelin; ECP: cows treated with 2 mg of estradiol cypionate; W48h: cows weaned for 48 h; MAP+GnRH: cows treated with an intravaginal sponge with 250 mg of medroxy-progesterone acetate (MAP) for nine days, and at withdrawal 0.5 mg of GnRH; MAP+ECP: cows treated with intravaginal sponges with 250 mg of MAP for nine days, and at withdrawal 2 mg of ECP; Control: untreated cows.

Table 3. Adjusted means (± SE) of BCS and number of follicles in various categories during the early post-partum period in autumn calving cows determined from 10 days post-partum to the detection of the first dominant follicle.

Week post-partum	BCS	NOFOL	LF	MF	SF
2	3.2±0.1[a]	6.1±0.3[a]	0.4±0.1	2.5±0.2[a]	3.3±0.2
3	3.0±0.1[a]	6.3±0.3[a]	0.4±0.1	3.0±0.2[a]	2.9±0.3
4	2.8±0.2[ab]	6.4±0.4[a]	0.2±0.1	3.1±0.3[a]	3.1±0.4
5	2.6±0.2 [b]	3.5±0.8 [b]	0.3±0.2	1.6±0.5[b]	1.6±0.6

Different letters in columns ($P < 0.05$).

BCS, body condition scores; NOFOL, total number of follicles; LF, number of follicles > 9 mm; MF, number of follicles between 5 and 9 mm; and SF, number of follicles < 5 mm.

Table 4. Adjusted means (± SE; mm) of the largest follicle of cows calving in the autumn during the seven days after treatment.

Day	W 48h	MAP+GnRH	MAP+ECP	Control
0	10.9±0.7[a]	11.6±0.7[a]	9.0±0.7[b]	7.5±0.7[b]
1	10.7±0.7[b]	12.6±0.7[a]	8.8±0.7[b]	8.6±0.7[b]
2	10.2±0.7[b]	13.0±0.7[a]	9.9±0.7[b]	7.5±0.7[b]
3	10.9±0.7[b]	11.7±0.7[a]	10.1±0.7[b]	7.5±0.7[b]
4	9.4±0.7[b]	10.0±0.7[a]	11.8±0.7[a]	6.4±0.7[b]
5	9.5±0.7[b]	11.3±0.7[a]	11.7±0.7[a]	6.8±0.7[b]
6	9.5±0.7[b]	10.6±0.7[a]	13.2±0.7[a]	6.7±0.7[b]
7	8.6±0.7[b]	10.4±0.7[b]	15.1±0.7[a]	7.1±0.7[b]

Comparisons were made between each treatment and control, different letters in rows ($P<0.05$).

W48h: cows weaned for 48 h; MAP+GnRH: cows treated with an intravaginal sponge with 250 mg of MAP for nine days and at withdrawal 0.5 mg of GnRH; MAP+ECP: cows treated with intravaginal sponges impregnated with 250 mg of MAP for nine days and at withdrawal 2 mg of ECP; Control: untreated cows.

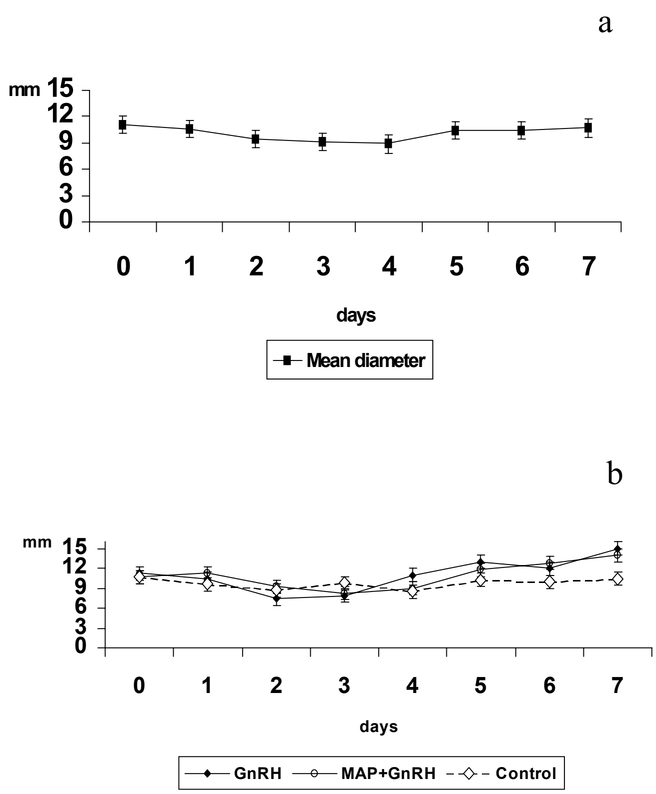

Figure 1. Variation in diameter (mean ± s.e.) of the major ovarian follicle after treatment in "a" overall means in each day for all treatments and in "b" with GnRH, supplemented (MAP+GnRH) or not with progestagen (GnRH) during spring.

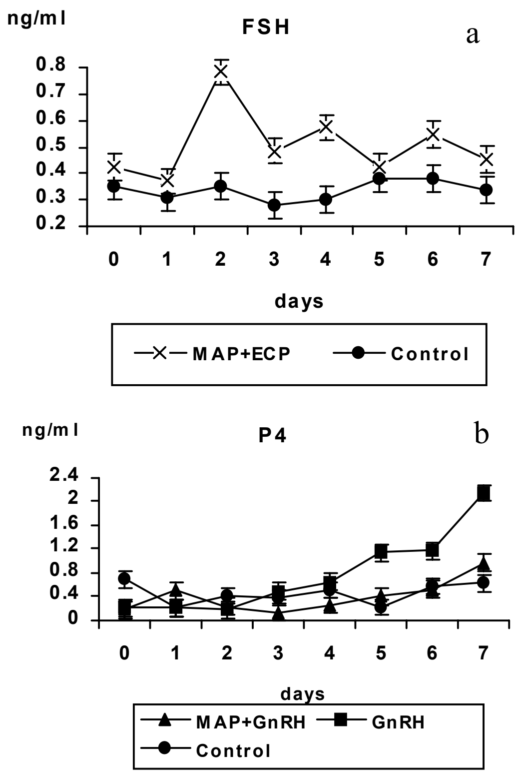

Figure 2. Means (±s.e.) of FSH and progesterone levels in treatments with respect the day of DF detection, in "a" FSH after estradiol cypionate and in "b", treatments containning GnRH in comparison with the control group during spring.

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[ii]ECP, estradiol cypionate, Rhódia-Mérieux Veterinária Ltda, Paulínia, SP, Brazil.
[iii]MAP, medroxiprogesterone acetate, C24H34O4, Purifarma, São Paulo, SP, Brazil.