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Use of medroxyprogesterone acetate (MAP) in lactating Holstein cows within an Ovsynch protocol: follicular growth and hormonal patterns

D. Cavestany[a,*], A. Meikle[b], H. Kindahl[c], E. Van Lier[d], F. Moreira[e], W.W. Thatcher[e], M. Forsberg[f]

[a]*Instituto Nacional de Investigación Agropecuaria (INIA) La Estanzuela, CC 39173, 70000 Colonia, Uruguay*
[b]*Facultad de Veterinaria, Montevideo, Uruguay*
[c]*Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, SLU, Uppsala, Sweden*
[d]*Facultad de Agronomía, Montevideo, Uruguay*
[e]*DPS, University of Florida, Gainesville, FL, USA*
[f]*Department of Clinical Chemistry, Faculty of Veterinary Medicine, SLU, Uppsala, Sweden*

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Abstract

To evaluate the effects of incorporating medroxyprogesterone acetate (MAP) in an Ovsynch protocol, cyclic lactating dairy cows were assigned randomly to two groups (control and MAP, $n = 8$ each). Ovsynch treatment (Day 0: GnRH, Day 7: PG, Day 9: GnRH) was initiated at random stages of the estrous cycle (control) and an intravaginal polyurethane sponge impregnated with 300 mg of MAP was inserted intravaginally in the MAP group at Day 0 and removed at Day 7 of the Ovsynch protocol (MAP treatment). Ovaries were scanned daily from Day 0 until the second GnRH treatment on Day 9 and from then every 6 h for 36 h. Milk samples were collected three times weekly starting 17 days before the initiation of treatment to determine the stage of the cycle at the beginning of the Ovsynch protocol. Blood samples were collected to monitor estradiol (E2), progesterone (P4), LH, and 15-ketodihydro-$PGF_{2\alpha}$ (PGFM) by RIA. Response to the first GnRH treatment varied with the stage of the cycle at the time of initiation of treatment, as cows in metestrous and late diestrous did not ovulate. In cows ovulating, growth rate of the new follicle was not affected by the addition of MAP. No treatment differences were found in E2 concentrations which reached a maximum at Day 9, consistent with the maximum follicular size. At Day 7, cows with luteal concentrations of P4 had increased concentrations of PGFM, but cows with basal P4 did not show an active release of prostaglandins. There were no treatment differences in the ovulatory response to the second GnRH-induced ovulation, with 11 of the 16 cows ovulating between 16 and 32 h. The addition of MAP to the Ovsynch protocol could not mimic the normal

* Corresponding author. Tel.: +598-5748000x1409; fax: +598-5748012.
E-mail address: cavestan@inia.org.uy (D. Cavestany).

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high progesterone levels needed to prevent premature ovulations in those cows with premature CL regression.

Keywords: Reproduction; Estrous synchronization; Medroxyprogesterone acetate; GnRH; Prostaglandin

1. Introduction

The main objective of a reproductive management program is to obtain the maximum number of cows pregnant in the shortest period of time. Pregnancy rate is the product of conception rate by estrous detection rate [1]. Increasing conception rate is difficult, so the most feasible way of improving pregnancy rate is increasing the estrous detection rate. This can be done by devoting sufficient time to observe cows for estrous [2], by utilizing estrous detection aids [3], or increasing the number of cows in estrous through different synchronization methods [4]. An Ovsynch protocol, using a combination of GnRH-PG-GnRH treatments [5] precisely synchronizes ovulation in lactating dairy cows to allow insemination at a fixed time [6]. However, this method does not accurately synchronize ovulation in all animals, approximately 10% of the cows tend to show estrous earlier than the targeted time [7]. Animals treated with GnRH early in the estrous cycle produced less progesterone (P4) before PG treatment; they had a greater concentration of estradiol (E2) at estrous and had a shorter interval from the treatment of PG to estrous [8]. However, initiation of an Ovsynch (GnRH-PG-GnRH) protocol at later stages of the estrous cycle leads to regression of the corpus luteum (CL) prior to treatment with PG and asynchrony between ovulation and timed insemination [9]. Progesterone prevents ovulation of the mature follicle [10], so maintaining high concentrations of P4 between the first GnRH treatment and PG treatment may prevent premature ovulation. Natural P4 or synthetic progestins have been used in synchronization protocols in beef cows [11,12] and dairy cows [13,14]. However, there is no information about the use of progestins within an Ovsynch protocol in cyclic lactating cows and its effect on follicular growth and hormonal patterns.

The objectives of this study were to evaluate the effects of the addition of medroxyprogesterone acetate (MAP) to suppress ovulation during the Ovsynch protocol and to describe the hormonal concentrations, follicular dynamics, ovulation and CL formation in normally cyclic lactating dairy cows treated at random stages of the estrous cycle.

2. Materials and methods

2.1. Animals and hormone treatments

The study was conducted at an experimental dairy farm (INIA La Estanzuela, Colonia, Uruguay). Sixteen multiparous Holstein cows in late lactation with normal reproductive histories, and body condition scores of 2.2 ± 0.1 (scale from 1 to 5; [15]) were selected. The cows were grazing on improved pastures and supplemented with a mixture of concentrates and corn silage. They were assigned randomly to two groups (control and MAP, $n = 8$ each). Ovsynch treatment was initiated at random stages of the estrous cycle and all cows were

treated with 250 μg i.m. of a synthetic GnRH analog (Gonadorelin, Fertagyl, Intervet, Boxmeer, Holland) at 08:00 h (Day 0). A polyurethane sponge impregnated with 300 mg of MAP (Farmabase, Rovereto, Italy) was inserted intravaginally immediately after GnRH treatment to the cows in the MAP Group. On Day 7, all cows were treated with a synthetic PG analog (15 mg i.m. Luprostiol, Prosolvin, Intervet, Boxmeer, Holland) at 08:00 h and in the MAP group the intravaginal sponges were withdrawn. The second GnRH treatment (250 μg of GnRH i.m.) was given on Day 9 at 08:00 h to both groups.

2.2. Determination of follicular dynamics

A 500 Aloka ultrasound machine (Aloka Co. Ltd., Tokyo, Japan) with a real-time linear B mode rectal probe of 5 MHz was used, and ovarian structures were monitored daily starting on Day 0. In order to detect time of ovulation, ultrasound frequency was increased after the second GnRH treatment (Day 9), to every 6 h for 36 h. Sequential planes of the ovary were examined and ultrasonic images were recorded onto follicular maps. The size and number of follicles $\geq$5 mm and the location of the CL were recorded. Growth of the largest follicle was determined daily from emergence, which was defined as the first day when the largest follicle of a follicular wave was 5 mm in diameter [16]. Growth rate (mm/day) of the largest follicle was calculated using a procedure previously defined [17], i.e. maximum size −5 mm/number of days. The number of days was counted from the day when the largest diameter before ovulation was attained minus the day of emergence of that follicle. Ovulation was determined by the disappearance of a large follicle seen at the previous ultrasound and confirmed by visualization of a new CL and P4 concentrations of more than 3 nmol/l in the following days. If the follicle disappeared but no CL was detected, it was assumed that there was regression or turnover of the largest follicle [18].

2.3. Milk and blood sampling

Milk samples for measurement of P4 determination were taken three times per week from Day −17 to Day 0 (where Day 0 is the beginning of the treatment), to determine the stage of the cycle at the beginning of the Ovsynch treatment. Samples were refrigerated until centrifuged at 4 °C, and the fat free fraction was stored at −20 °C until assayed. Blood samples for E2, P4, LH and PGFM measurements were taken with heparin daily from Day 0 until Day 6, when the animals were catheterized for the frequent sampling that started on Day 7. On Day 7, samples were taken hourly from 2 h before to 6 h after the PG treatment. Thereafter, sampling was done every 2 h until 16 h after the second GnRH treatment (Day 9) and then every 6 h until the end of the experiment (Day 10). The samples were centrifuged immediately and plasma stored at −20 °C until hormone assays were performed.

2.4. Hormone assays

2.4.1. Estradiol-17β

Samples for E2 were determined in duplicate by a ^{125}I RIA (Estradiol double antibody, KE2D, DPC, Diagnostic Products Co., Los Angeles, CA, USA) previously validated for

bovine plasma [10]. The detection limit of the assay was 4 pmol/l. The intraassay coefficient of variation was 7%. The interassay coefficient of variation was 12%.

2.4.2. Luteinizing hormone

LH was measured by an RIA previously validated for bovine plasma [19]. The assay utilized a bovine monoclonal antibody (MAB 518B7 generously donated by Dr. J.F. Roser, University of California Davis, CA, USA), human ^{125}I LH (hLH double antibody RIA kit (KLHD), DPC, Diagnostic Products Co., Los Angeles, CA, USA) and ovine LH as standard (oLH AFP-95988 generously donated by A.F. Parlow, UCLA Medical Center, Los Angeles CA, USA). The sensitivity of the assay was 0.4 μg/l. The intraassay coefficient of variation was 9%. The interassay coefficient of variation was 10%.

2.4.3. Progesterone

Progesterone (P4) was determined in milk and plasma using a commercial kit (Coat-a-count, DPC, Diagnostic Products Co., Los Angeles, CA, USA). In the milk assay the intraassay and interassay coefficients of variation were 6 and 5%. In the plasma assay, the intra and intercoefficients of variation were 7 and 14%. The sensitivity was 0.1 nmol/l for milk and 0.3 nmol/l for plasma.

2.4.4. Prostaglandin metabolite (15-ketodihydro-PGF$_{2\alpha}$ PGFM)

The plasma metabolite 15-ketodihydro-PGF$_{2\alpha}$, was analyzed in unextracted plasma by RIA according to Kindahl et al. [20]. The detection limit of the assay was between 25 and 30 pmol/l. The intraassay coefficient of variation was 14% and the interassay coefficient of variation was below 11%.

2.4.5. Definitions and statistical analyses

The diameter of the largest follicle and P4 levels at the initiation of the treatment were analyzed by the GLM procedure [21] and the statistical model included the effects of the stage of the cycle.

Follicular dynamics, P4 and E2 concentrations from Day 0 to Day 9 were analyzed by the GLM procedure [21] and the statistical model included the effects of stage of the cycle at the initiation of the treatment, treatment, day, and treatment by day. Concentrations of P4 below 1 nmol/l were considered as baseline concentrations. Baseline concentrations of PGFM, E2 and LH in all animals were calculated using skewed distribution method [22]. For each animal, the average hormone concentration was calculated using all the values. After excluding values above 2 S.D. for PGFM and 1 S.D. for LH and E2 of the respective means, a new average and S.D. were calculated. This mean was considered as the baseline value. Concentrations of PGFM that were more than 2 S.D. above the baseline value were considered as significant increases, and for LH and E2 3 S.D. were used. An LH surge was defined as two consecutive LH increases with at least one of the values 10-fold greater than baseline. Analysis of variance was used to determine the difference in the interval from the end of treatment to ovulation in the two groups.

Table 1
Size of the dominant follicle (DF) and progesterone (P4) concentrations (mean ± S.E.M.) at the initiation of Ovsynch treatment (Day 0)

Stage of the estrous cycle at Day 0	*n*	Size of the DF (mm)	Plasma P4 (nmol/l)
Proestrous	4	12.5 ± 1.5[a]	0.2 ± 0.5[a]
Metestrous	2	6.5 ± 2.1[b]	1.6 ± 0.7[a]
Early diestrous	4	12.3 ± 1.5[a]	4.4 ± 0.5[b]
Mid diestrous	2	13.9 ± 2.3[a]	11.4 ± 0.7[c]
Late diestrous	4	11.3 ± 1.6[a]	11.6 ± 0.5[c]

Different superscripts (a, b, c) within columns differ ($P < 0.01$).

3. Results

3.1. Stage of the estrous cycle at the beginning of the Ovsynch treatment

The stage (day of the cycle) at the initiation of treatment was determined from the previous 17-day P4 profiles in milk samples for each cow. Cows were classified as in proestrous (Days 18–21), metestrous (Days 1–4), early diestrous (Days 5–8), mid diestrous (Days 9–13), and late diestrous (Days 14–17). This was determined by size of the dominant follicle (DF) and the plasma P4 concentrations at the beginning of the treatment (Table 1). At Day 0, cows in metestrous had a significantly smaller diameter of the larger follicle ($P < 0.01$). Cows in proestrous, metestrous and early diestrous had lower P4 concentrations than cows in mid or late diestrous ($P < 0.01$). For further analyses, the stages of the estrous cycle were classified as early cycle (proestrous and metestrous), mid cycle (early and mid diestrous) and late cycle (late diestrous).

3.2. Ovarian response to the first GnRH treatment and progesterone concentrations

Response to GnRH (ovulation or follicle regression) occurred 2 days after treatment in eleven of sixteen cows; seven (three in the MAP group and four in the control croup) ovulated and an accessory CL formed and in the other four cows (two in each group) the largest follicle regressed. There were four cows that did not respond to the first GnRH treatment, and corresponded to one in late cycle and three in early cycle (two in metestrous, in which the largest follicle had a smaller diameter than in the other groups, and one in proestrous).

Progesterone values for cows in the late stage of the cycle at Day 0 ($n = 4$) dropped to basal levels before the PG treatment at Day 7 (Fig. 1). Cows in the early stage of the cycle at the beginning of the treatment ($n = 6$) had lower P4 concentrations during the following days, while higher concentrations of P4 were observed in mid stage cows ($n = 6$), $P < 0.01$. Regression of the CL prior to PG treatment was determined by a drop in P4 to basal concentrations before the PG treatment (Fig. 1). This occurred in cows that were in the late stage of the cycle at Day 0, which caused natural regression of the CL before Day 7 (MAP 3/3, control 1/1). The formation of accessory corpora lutea after the first GnRH treatment in cows in early stage of the cycle was consistent with the P4 increase from Day 5 to Day 7 in these animals.

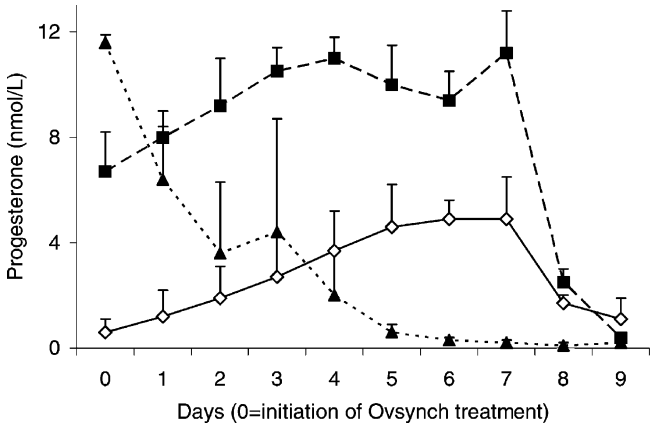

Fig. 1. Mean progesterone (± S.E.M.) concentrations corresponding to the stage of the estrous cycle (early, ◇; mid, ■; or late, ▲ cycle) at the beginning of the Ovsynch treatment.

3.3. Follicular dynamics and estradiol concentrations

After the first GnRH treatment, the largest follicle of the new follicular wave emerged at 5 mm on Day 2 (Fig. 2) and underwent selection at Day 4 when its diameter became greater than the diameter of the second largest follicle. There was no treatment difference

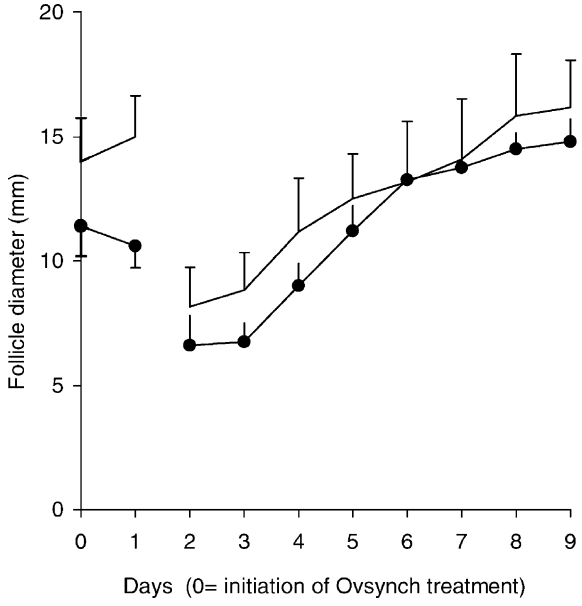

Fig. 2. Mean (± S.E.M.) follicular diameter (mm) in cows treated with an Ovsynch protocol with medroxyprogesterone acetate (MAP, —) or without medroxyprogesterone acetate (control, ●) or during the experimental period.

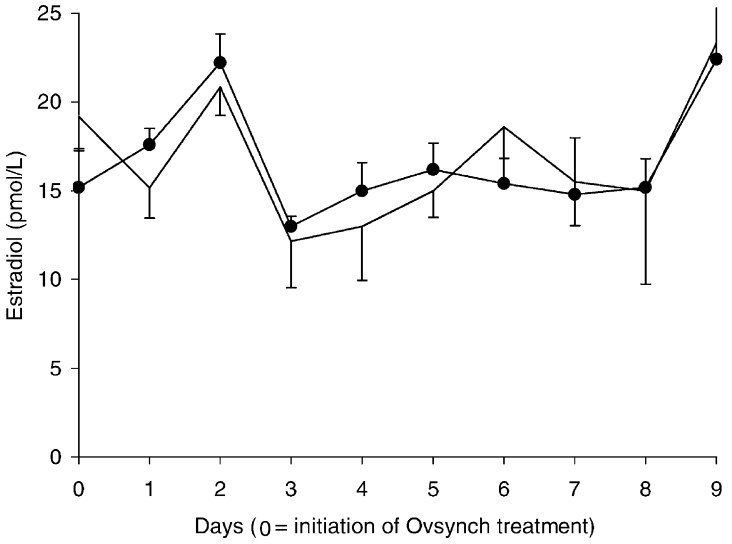

Fig. 3. Mean (± S.E.M.) estradiol concentrations in cows treated with an Ovsynch protocol with medroxyprogesterone acetate (MAP, —) or without medroxyprogesterone acetate (control, ●) during the experimental period.

($P > 0.1$) in the growth rate of the DF (1.34 mm/day, in the MAP group and 1.25 mm/day in the control group). The largest size of the DF of the new wave was attained at the day of the second GnRH treatment (Fig. 2). The diameter of the DF on Day 9 was 16.2 ± 1.9 mm in the MAP group and in the control group was 14.8 ± 0.9 (mean ± S.E.M.) ($P > 0.1$).

Estradiol mean values (above the baseline) were not different between groups, but differed among days (Fig. 3). Estradiol mean values were elevated on Days 2 and 9 with lower values on Day 3 following the GnRH treatment ($P < 0.05$).

3.4. Luteolysis and premature ovulations

Concentrations of P4 and PGFM are shown in Fig. 4. Animals were classified according to P4 levels at Day 7 (PG injection) and according to treatment (MAP versus control): luteal P4 levels in control (Fig. 4A, $n = 7$) and MAP cows (Fig. 4B, $n = 5$), and basal P4 levels (Fig. 4C; control $n = 1$ + MAP $n = 3$). Cows with luteal concentrations of P4 at Day 7 presented PGFM peaks (Fig. 4A and B), without treatment differences. In contrast, cows with premature regression of the CL did not show elevated PGFM levels (Fig. 4C).

The occurrence of premature ovulations (before the second GnRH treatment) was related to the drop in P4 concentrations (premature CL regression). With the exception of one cow, all were in late diestrous at Day 0.

3.5. LH and ovulatory response to the second GnRH treatment

Mean LH profiles during the 20 h before to 20 h after the second GnRH treatment are shown in Fig. 5. Increases of LH were detected 2 h after the second GnRH treatment and

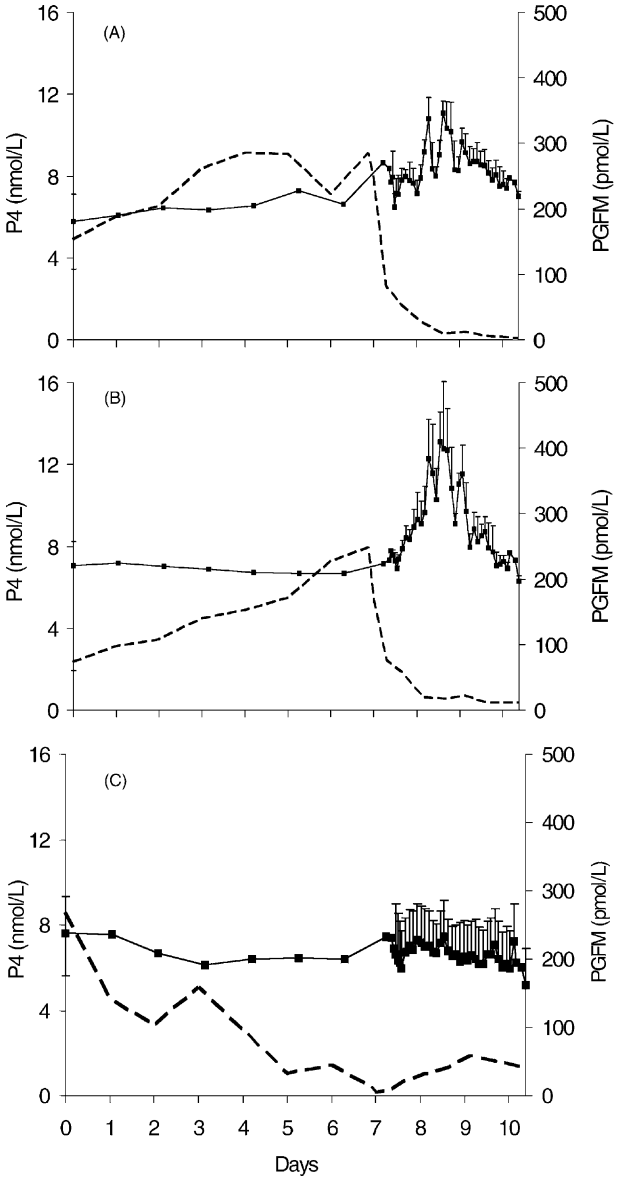

Fig. 4. Mean ± S.E.M. progesterone (P4, - - -) and 15-ketodihydro-$PGF_{2\alpha}$ (PGFM, ■) concentrations during the experimental period, in cows with luteal concentrations of P4 (A: control; B: MAP) or with basal concentrations of P4 (C) on Day 7.

concentrations remained elevated for 4 h in both groups. Surges of LH were observed in 11 cows; 5 in the MAP group and 6 in the control group. Ovulations after the second GnRH treatment occurred in six of the eight cows of the MAP group and in five of the eight cows of the control group. In the MAP group, two ovulations occurred between 16 and 22 h after

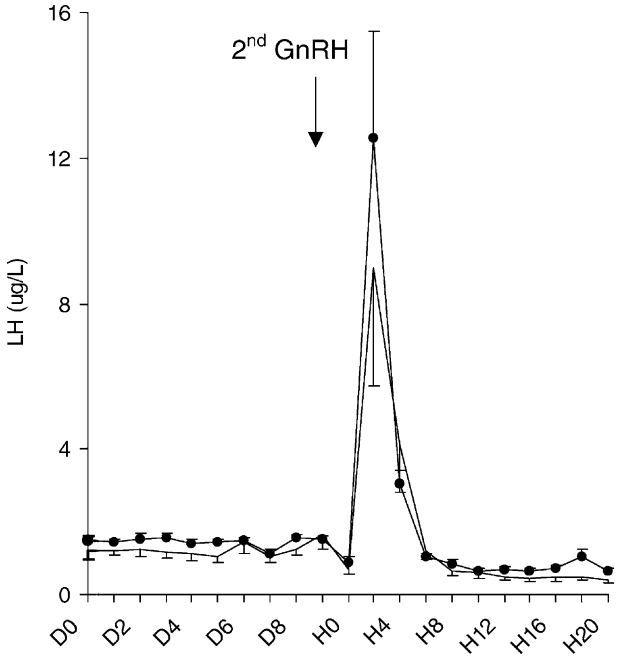

Fig. 5. Mean LH ± S.E.M. profiles in cows treated with an Ovsynch protocol with medroxyprogesterone acetate (MAP, —) or without medroxyprogesterone acetate (control, ●). Days (D0, initiation of Ovsynch treatment) and hours (H0, second GnRH treatment).

the second GnRH treatment, two between 22 and 28 h and two between 28 and 32 h. In the control group this time-span was more compressed with one ovulation occurring at 22–28 h after the second GnRH treatment and the remaining four at 28–32 h.

4. Discussion

Irrespective of group, one-third of the cows did not respond to the first GnRH treatment of the Ovsynch treatment. The overall ovulation rate to the first GnRH treatment did not differ between treatments and the response varied with stage of the estrous cycle of the cows. The highest ovulation rate was found in cows in early and mid diestrous and in proestrous, in agreement with Schmitt et al. [23] and Moreira et al. [9]. Cows in metestrous had a smaller follicle that did not respond to the GnRH treatment and continued its natural growth. Cows in late diestrous had a DF that was already in the regression phase and underwent atresia. Thus, the ability to ovulate the largest follicle present at the moment of the GnRH treatment depends on its stage of development as shown previously [18,24]. The response to the first GnRH treatment was similar to that observed in previous studies [5,25]. In cows that responded to the first GnRH treatment, the DF ovulated or regressed and a new synchronized follicular wave emerged. Formation of an accessory CL after the first GnRH treatment was observed in cows in proestrous and in early and mid

diestrous, in accordance with Wolfenson et al. [26], Schmitt et al. [23] and Rajamahendran et al. [27]. Cows in metestrous and late diestrous did not form an accessory CL in response to GnRH.

Progesterone concentrations during Days 0–7 were a reflection of the stage of the estrous cycle at the beginning of the treatment. Cows in late diestrous at Day 0 underwent premature regression of the CL and P4 concentrations decreased during the following 7 days. Progesterone concentrations at the time of PG treatment affected the pattern of PGFM. In cows with luteal P4 concentrations, PGFM levels showed episodic patterns; while in those with basal P4 concentrations the PG treatment did not evoke an endogenous PGFM elevation. It has previously been demonstrated that the duration of the luteolytic PG release is dependent on circulatory concentrations of P4 [28], and PG release continues for as long as the P4 concentrations are elevated, above a low critical level [28,29]. After the PG treatment (Day 7), P4 concentrations declined to basal concentrations on Day 8 in all cows of both groups, except for the cows in metestrous. This observation is in agreement with Lauderdale et al. [30] and Tsai and Wiltbank [31] who found that a single treatment with PG does not cause CL regression when given early in the cycle.

The growth rate of the largest follicle of the new follicular wave was similar in both groups, in contrast to the results reported by Sanchez et al. [32] using norgestomet. Cows treated with norgestomet had an increased size of the largest follicle, which was associated with higher circulating concentrations of E2. The ovarian response to MAP seems, therefore, to be different than to norgestomet. In both groups in our study, the DF reached its maximum size at Day 9, similar to findings of Pursley et al. [5].

Estradiol concentrations decreased to a nadir 3 days following the first GnRH treatment, as a reflection of ovulation or regression of the largest follicle present at Day 0. Final development of the new follicular wave was accompanied by a consistent increase in E2 concentrations following the treatment with PG and is indicative of an estrogenic preovulatory follicle [8,23]. There were no treatment differences in the E2 concentrations after the new follicular wave was initiated and this is consistent with the observation that there were no differences in the diameters of the largest follicles between groups. The profiles were similar to those reported by Pursley et al. [33] using a protocol without progestins. Sanchez et al. [32] however, reported an increase in E2 concentrations after a treatment with a single implant of a progestin.

In the present experiment, with a 2 h sampling frequency, LH surges were identified at the 2 h sampling following the second GnRH treatment, and LH concentrations remained high until the next sampling, at 4 h after the GnRH-challenge. The interval from the second GnRH treatment to ovulation was similar to that reported by Pursley et al. [5], but while in their study all the cows ovulated, in the present experiment ovulation was detected in only 11 of the 16 cows.

Detection of premature LH surges associated with premature ovulations before Day 7 was not possible because of the sampling schedule. All cows with premature ovulations also had premature CL regression. These premature ovulations are probably a consequence of the low progesterone environment. The addition of MAP to the Ovsynch protocol could not mimic a normal high progesterone environment needed to completely prevent premature ovulation. Of the four MAP-treated cows with premature CL regression, two had a premature ovulation. However, as noted above, Sanchez et al. [32] reported

similar results with the use of a different synthetic progestin. Furthermore, at the following insemination a low P4 environment has been proven to depress fertility [34].

The results of this trial confirm the importance of stage of the estrous cycle at the beginning of a synchronization treatment relative to subsequent ovarian responses. Ovulation to the first GnRH treatment and maintenance of high P4 concentrations are important for an adequate response. This should be considered in a fixed artificial insemination program and should be optimized via presynchronization or concurrent use of an effective progestin.

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