Acute Progesterone Administration Regresses Persistent Dominant Follicles and Improves Fertility of Cattle in Which Estrus Was Synchronized with Melengestrol Acetate

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ABSTRACT: Experiments were conducted to determine whether acute progesterone administration would regress persistent follicles and improve fertility in heifers and postpartum cows fed melengestrol acetate (MGA). In Exp. 1, heifers (n = 13) were fed MGA for 11 d (1st d of MGA = d 1). Prostaglandin F2α (PGF2α) was administered to heifers on d 2 to regress the corpus luteum (CL). On d 9, heifers were randomly assigned to receive an injection of either 200 mg of progesterone (PROG) or no hormone (vehicle; VEH). Neither growth of the persistent follicle nor plasma estradiol concentrations were altered by administration of VEH, and the persistent follicle ovulated after cessation of MGA feeding. Administration of PROG regressed the persistent follicle, reduced (P < .05) systemic estradiol concentrations, and resulted in ovulation of a newly recruited follicle. In Exp. 2, heifers were fed MGA for 14 d and were administered either PROG (n = 30) or VEH (n = 31) on d 12. When the CL was absent on d 12, synchronization conception rate (SCR) and pregnancy rate (PR) were greater (P < .05) for heifers administered PROG than for those administered VEH. Neither SCR nor PR were different among treatments when the CL was present on d 12. In Exp. 3, cows (n = 49) were fed MGA for 14 d and were administered either PROG or VEH on d 12. In cows lacking a CL (n = 32), administration of PROG increased (P < .05) SCR, but not PR. We conclude that acute PROG administration induces turnover of persistent follicles and may increase fertility when estrus is synchronized with MGA.

Key Words: Follicles, Melengestrol, Progesterone

Introduction

Melengestrol acetate (MGA) is an orally active, synthetic progestin that effectively synchronizes estrus in cattle (Patterson et al., 1989). The use of MGA in estrus synchronization programs has been limited, however, due to the reduced fertility of the synchronized estrus (Zimbelman and Smith, 1966). The reduced fertility after progestin administration is suggested to arise from altered follicular development (Savio et al., 1993b).

Follicular growth in cattle occurs in a wavelike fashion; cows usually have two or three waves of follicular development during an estrous cycle (Sirois and Fortune, 1988). This wavelike pattern of follicular development is interrupted by administration of progestins in the absence of the corpus luteum (CL; Sirois and Fortune, 1990). Progestin treatment prolongs the lifespan of the dominant follicle and increases systemic estradiol concentrations (Sirois and Fortune, 1990). Dominant follicles that have a prolonged lifespan have been termed persistent follicles.

The development of persistent follicles can be avoided during progestin administration if normal luteal progesterone concentrations are achieved (Stock and Fortune, 1993). The objective of the following experiments was to determine whether short-term increases in progesterone concentrations would regress persistent follicles and increase subsequent fertility in heifers and postpartum cows fed MGA.
Materials and Methods

Experiment 1

Crossbred heifers (Angus × Simmental), 428 ± 7 d of age, were used. All heifers were postpubertal, as verified by weekly analysis of plasma progesterone concentrations for several weeks before initiation of the experiment. Heifers were fed MGA for 11 d (1st d of MGA = d 1) in amounts typically used to suppress estrus (.5 mg of MGA-heifer⁻¹-d⁻¹; Hill et al., 1971). A supplement consisting of ground ear corn containing MGA (.22 mg/kg) was fed to supply MGA. Heifers were group-fed corn silage (9 kg/animal) daily, toppedressed with 2.3 kg of the supplement to provide the dose of MGA. Daily feeding was observed to ensure that each heifer consumed feed containing MGA.

Heifers were administered prostaglandin F₂₅₅ (PGF₂₅₅; 25 mg i.m.; Lutalyse, Upjohn, Kalamazoo, MI) on d 2 to induce luteal regression. On d 9, heifers were randomly assigned to receive an injection of either 200 mg of progesterone (PROG; n = 7) or no hormone (vehicle; VEH; n = 6). Progesterone (Sigma Chemical, St. Louis, MO) was dissolved in sesame seed oil (vehicle) at a concentration of 20 mg/mL. The 200-mg dose of progesterone was administered in a single 10-mL i.m. injection. In a preliminary dose-response experiment, we found that the dose of progesterone chosen would increase systemic progesterone concentrations to > 1 ng/mL for 24 h after injection when administered to heifers in the follicular phase.

Transrectal ultrasonography was used to evaluate the growth of dominant follicle(s), regression of CL following PGF₂₅₅ administration, and the day of ovulation. A dominant follicle was defined as the largest growing follicle on the ovaries. The day of ovulation was defined as the day after the dominant follicle was last detected. Ovaries were scanned on alternate days from d 1 to the last day of MGA feeding (d 11) and daily from d 11 until ovulation. Ovarian structures were identified using a real-time B-mode instrument (Aloka, 500 V, Corometrics Medical Systems, Wallingford, CT) with a 7.5-MHz linear array transducer. During each examination, ovarian maps were drawn indicating the size and location of follicles and the presence of CL.

Blood samples were obtained by jugular venipuncture on d 2, 3, 9, and 10. Samples collected on d 2, 3, and 9 were analyzed for progesterone concentrations to corroborate ultrasonographic observations of luteal regression. Samples obtained on d 9 and 10 were analyzed for estradiol concentrations to evaluate acute effects of PROG and VEH administration on peripheral estradiol concentrations.

Effects of PROG and VEH on growth of the initial dominant follicle were compared with split-plot analysis of variance (Steel and Torrie, 1980) with treatment in the main plot and day in the subplot. Differences (PROG vs VEH) in systemic estradiol concentrations, diameter of the dominant follicle the day before ovulation, and the interval from the time of the last MGA feeding to ovulation were determined with one-way analyses of variance using the GLM procedures of SAS (1988).

Experiment 2

Crossbred, postpubertal heifers, approximately 15 mo of age, from two separate farms were used in this experiment. Heifers at both locations were fed MGA (.5 mg of MGA-heifer⁻¹-d⁻¹) for 14 d. On d 12 (1st d of MGA = d 1), heifers were randomly assigned, within location, to receive either PROG (n = 30) or VEH (n = 31) as described in Exp. 1. Heifers at Location A (n = 44) were maintained on access pasture and group-fed 2.3 kg of the ground ear corn supplement containing MGA described in Exp. 1. At Location B, heifers (n = 17) were maintained in drylots and fed as described for heifers in Exp. 1. Beginning on d 15, heifers at Location A were artificially inseminated during a 42-d breeding period, followed by a 21-d natural service season. At Location B, heifers were inseminated during a 10-d period and then exposed to a fertile bull for 30 d. At both locations, heifers were observed twice daily (0700 and 1900) for behavioral signs of estrus throughout the AI and natural-service breeding periods. During the AI period, heifers were inseminated approximately 12 h after first observed standing estrus. On d 60 and 100 after initiation of the breeding season, two experienced palpators determined whether heifers were pregnant. The date of conception was estimated using the pregnancy diagnosis information.

Blood samples were collected by jugular venipuncture on d −5, 3, and 12 at Location A and on d −3, 5, and 12 at Location B. Plasma was analyzed for progesterone concentrations to confirm that all heifers were cyclic and to determine whether a CL was present when PROG or VEH was administered. Heifers with a CL on d 12 and systemic progesterone concentrations ≥ 1 ng/mL would be expected to have normal follicular turnover, whereas a majority of heifers with plasma progesterone concentrations < 1 ng/mL at this time would likely have persistent follicles (Sirois and Fortune, 1990). Heifers with systemic progesterone concentrations < 1 ng/mL on the day of PROG or VEH administration were classified as nonluteal (NL), and the remaining heifers were classified as luteal (L).

Treatment effects on proportional responses of NL and L heifers were analyzed with chi-square procedures (Steel and Torrie, 1980). The responses evaluated included synchronization conception rate and pregnancy rate. Heifers were classified as synchronized if estrus was observed within 10 d after the last day of MGA feeding. This liberal definition of estrus synchronization was used to allow sufficient
time for emergence, growth, and ovulation of a new dominant follicle in heifers administered PROG. Synchronization conception rate was defined as the percentage of all heifers that conceived to an insemination during the synchronized estrus. Pregnancy rate was defined as the percentage of heifers pregnant at the end of the breeding season. The effects of treatment on the interval to breeding were analyzed with one-way analysis of variance using GLM (SAS, 1988). The interval to breeding was the number of days from the last day of MGA feeding to service for synchronized heifers.

Experiment 3

Crossbred cows averaging 4.5 ± .03 yr of age and 63.6 ± .25 d postpartum on the 1st d of MGA feeding (d 1) were used. Cows were assigned by calving date to receive either PROG (n = 24) or VEH (n = 25) as described in Exp. 2. Cows were maintained on pasture and group-fed 2.3 kg-cow⁻¹·d⁻¹ of the MGA supplement described in Exp. 1. Beginning on d 15, the day after the final MGA feeding, cows were observed for estrus and artificially inseminated during a 10-d period followed by a 45-d natural service season. Dates of service were determined during the natural service period with the aid of a chin-ball marker fitted on a bull. Pregnancy was diagnosed and date of conception estimated as described in Exp. 2. Blood samples were collected, via the jugular vein, on d 5 and 12 and analyzed for progesterone concentrations to determine reproductive status. Cows were classified as NL if plasma progesterone concentrations were < .5 ng/mL in both samples. The group of cows classified as NL would be expected to include both anestrous cows and those cyclic cows that were first fed MGA late in their estrous cycle (i.e., d 14 to 15). Cows with ≥ .5 ng/mL plasma progesterone concentrations in either sample were classified as L. Limited numbers (PROG, n = 9; VEH, n = 8) of L cows and the variety of reproductive states represented (i.e., cows with increased progesterone concentrations on d 5 or on both d 5 and 12) precluded further classification and analysis, and the data from these cows were deleted. Chi-square analysis was performed on proportional responses (synchronization conception rate and pregnancy rate) to PROG or VEH administration in NL cows (Steel and Torrie, 1980). Treatment effects on interval to breeding were analyzed as described in Exp. 2.

Hormone Assays

All blood samples obtained in these experiments were collected in tubes containing 3% EDTA, cooled on ice, and centrifuged, and the plasma stored at −20°C until RIA. Progesterone concentrations (nanograms/milliliter) in plasma were analyzed with previously validated procedures (Hu et al., 1990). The intra- and interassay CV were 4.4 and 9.2%, respectively. Plasma estradiol concentrations (picograms/milliliter) were analyzed in a single, previously validated (Nephew et al., 1989) RIA with an intra-assay CV of 1.6%.

Results

Experiment 1

Growth and regression of dominant follicles in heifers in both treatments are shown in Figure 1. The average diameter of the largest follicle on d 2 (day of PGF₂₀, injection) was 13.4 ± .8 mm. In 10 of 13 heifers, the largest follicle on d 2 continued to grow and maintain dominance until the administration of PROG or VEH on d 9. Persistence of the dominant follicle was indicated by the regression and continued suppression of the growth of subordinate follicles. The diameter of the first dominant follicle of two heifers began to regress before d 9. In one heifer, a persistent follicle was not obvious on d 9; the largest detectable follicle was only 13 mm in diameter and was associated with systemic estradiol concentrations of 3.9 pg/mL. We assumed that a persistent follicle was absent in these three heifers on d 9, and their data were eliminated from subsequent analyses. On d 9, the diameter of the largest follicle across all heifers was 21.0 ± .6 mm. This follicular diameter is consistent with that of persistent follicles (Stock and Fortune, 1993). Further evidence for persistent follicles on d 9 was an average plasma estradiol concentration of 10.5 ± .3 pg/mL. The pattern of growth of the first dominant follicle differed (treatment × day interaction; P < .05) between heifers administered PROG and VEH from d 9 to 16. In heifers administered VEH, the dominant follicle persisted and continued to increase in diameter until ovulation. The diameter of the dominant follicle for heifers administered VEH was 22.5 ± 2.4 mm the day before ovulation. Average systemic estradiol concentrations of 12.4 ± 1.9 pg/mL on d 10 further indicated follicular persistence. In contrast, administration of PROG initiated atresia of the dominant follicle present on d 9. Systemic estradiol concentrations were markedly reduced (4.8 ± .3 pg/mL) on the day after PROG was administered (d 10). The diameter of the first dominant follicle decreased gradually after d 9, and a cohort of follicles 5 to 6 mm in diameter was first observed on d 12.5 ± .3 in heifers administered PROG. The emerging follicles detected on d 12.5 continued to increase in size, and a dominant follicle was selected from this cohort. The second dominant follicles attained an average diameter of 16.5 ± .6 mm the day before ovulation. The average diameter of the follicle that ovulated was less (P < .05) in heifers administered PROG than VEH. Also, the number of days from the last day of MGA feeding to ovulation was
Figure 1. Growth and development of dominant ovarian follicles in heifers administered either progesterone (PROG) or vehicle (VEH). All heifers were fed melengestrol acetate (MGA) from d 1 to 11. On d 2, prostaglandin F2α (PGF) was administered to all heifers. An injection of either PROG or VEH was administered to heifers on d 9. The growth of the dominant follicle in heifers administered VEH is illustrated by the dashed line, and the growth of the first dominant follicle in heifers administered PROG is indicated by the solid line. Emergence and growth of a second dominant follicle in heifers administered PROG is depicted by the dotted line. Asterisks denote the day of ovulation of the dominant follicle of each heifer administered either PROG or VEH. Pooled SEM = .45.

extended (P < .05) in heifers administered PROG (9.5 ± .4 d) compared with those given VEH (4.8 ± .5 d).

Experiment 2

Responses to PROG and VEH administration were similar (P > .1) between locations; thus, the data from both locations were combined. Equal proportions (94.8%) of heifers from both treatments were detected in estrus during the 10 d after the last feeding of MGA. Three heifers administered VEH were not observed in estrus during the synchronization period. The distribution of heifers observed in estrus after the last day of MGA feeding is shown in Figure 2. Administration of VEH resulted in 69% of the heifers inseminated 3 to 5 d after MGA withdrawal, and 72% of heifers administered PROG were inseminated 5 to 7 d after cessation of MGA feeding. The interval from the last day MGA was fed to AI during the synchronization period was greater (P < .05) in NL heifers administered PROG than in those administered VEH (Table 1). No difference (P > .1) between heifers given PROG or VEH was detected for interval to breeding in L heifers.

Synchronization conception rates in NL heifers administered PROG were three times greater (P < .05) than they were in NL heifers administered VEH (Table 1). Pregnancy rates were lower (P < .05) in NL heifers administered VEH than in NL heifers administered PROG. No difference (P > .1) in either synchronization conception rates or pregnancy rates was detected between L heifers administered either PROG or VEH.

Experiment 3

The total proportion of cows observed in estrus within 10 d after the last feeding of MGA (synchronization estrous period) was not different (75.0%) for NL cows given either PROG or VEH. The estrous cycle length for cows not conceiving on the first service (19.1 ± .4 d) was also similar across treatments. The distribution of insemination dates in cows after the

Figure 2. Percentage of heifers inseminated on each day after the cessation of feeding melengestrol acetate [MGA] (d 0 = last day MGA was fed).

Figure 3. Percentage of nonluteal cows inseminated on each day after cessation of feeding melengestrol acetate [MGA] (d 0 = last day MGA was fed).
Table 1. Synchronization conception rates, pregnancy rates, and interval from the last day of feeding melengestrol acetate (MGA) to service for heifers administered either progesterone (PROG) or vehicle (VEH)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Synchronization conception rates, %</th>
<th>Internal to breeding</th>
<th>n</th>
<th>Pregnancy rates, %</th>
</tr>
</thead>
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<td>PROG</td>
<td>16</td>
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<td>93.8f</td>
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<td>5.2 ± .4i</td>
<td>14</td>
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<td>3.7 ± .1f</td>
<td>17</td>
<td>82.4e</td>
</tr>
</tbody>
</table>

aPercentage of all heifers that conceived to an insemination during the synchronized estrus.
bNumber of days from the last day MGA was fed (d 14) to insemination for heifers in which estrus was synchronized.
cPercentage of the heifers that were pregnant at the end of the breeding season.
dNonluteal; heifers that had systemic progesterone concentrations < 1 ng/mL on d 12 of the experiment.
eLuteal; heifers that had systemic progesterone concentrations ≥ 1 ng/mL on d 12.
f,gWithin a column, means within a classification lacking a common superscript differ (P < .05).
h,iWithin a column, means within a classification lacking a common superscript differ (P < .06).

last day of MGA feeding is shown in Figure 3. All (100%) cows administered VEH that were synchronized were inseminated from 3 to 5 d after MGA removal, and 84% of the cows administered PROG that were synchronized were inseminated during this same period. Administration of PROG increased (P < .05) the interval from the last day MGA was fed to AI during the synchronization period (Table 2).

Synchronization conception rates were greater (P < .05) for NL cows administered PROG than for those administered VEH (Table 2). No difference (P > .1) was detected for pregnancy rate between NL cows receiving PROG or VEH.

Discussion

The results from Exp. 1 confirm that persistent follicles develop during the feeding of .5 mg of MGA/d in the absence of a CL. As expected, the persistent follicles ovulated after progestin withdrawal. This response is similar to that previously reported using a single norgestomet implant (Rajamahendran and Taylor, 1991; Savio et al., 1993a), progesterone-releasing intravaginal device (Sirois and Fortune, 1990), or controlled internal drug-releasing device (Savio et al., 1993b). Clearly, the concentrations of progestins typically provided by these delivery systems do not sufficiently regulate follicular growth in the absence of progesterone from the CL.

The development of a persistent follicle induced during progestin administration can be circumvented. Increasing the systemic concentration of progesterone by the administration of an additional intravaginal device (Stock and Fortune, 1993) or administration of a second norgestomet implant (Savio et al., 1993a) reduces LH secretion and regresses the persistent follicle. However, increasing the dose of MGA fed is not effective (Kojima et al., 1993).

In the present study, acute administration of a bolus dose of progesterone regressed the dominant follicle in all cows.
folicule in every heifer with a persistent follicle. Regression of the persistent follicle was followed by the emergence and ovulation of a newly recruited dominant follicle. Although not directly studied in this experiment, PROG treatment likely reduced LH pulse frequency, thus allowing the dominant follicle to undergo atresia. Apparently, only a transient reduction in LH secretion is needed to allow the persistent follicle to undergo atresia. Regression of the dominant follicle was indicated by both the temporal reduction in follicular diameter and the marked reduction in systemic estradiol concentrations in heifers administered progesterone.

The use of progestins in estrus synchronization programs has been limited due to the associated reduction in fertility. The exact cause(s) of the infertility have not been determined. The infertility has been suggested to arise from the following: 1) altered follicular development (Hill et al., 1971; Savio et al., 1993a), 2) prolonged exposure to abnormally high estrogen concentrations (Henricks et al., 1973; Randel et al., 1973; Savio et al., 1993a), 3) reduced sperm transport (Lauderdale and Ericsson, 1970), 4) abnormal time of ovulation relative to estrus (Wiltbank et al., 1967), 5) alterations in the surface glycogen content and morphology of uterine glandular epithelial cells (Wordinger et al., 1974), and 6) reduced embryonic development (Hill et al., 1971; Wishart and Young, 1974). Many, if not all, of these dysfunctions could stem from prolonged follicular dominance and the associated increase in systemic estradiol concentrations. Thus, regression of the persistent follicle with PROG was expected to increase fertility in heifers with persistent follicles. In Exp. 2, the predominant effect of PROG was to increase the synchronization conception rates of heifers with low concentrations of endogenous progesterone (50 vs 17% for NL heifers administered PROG and VEH, respectively). Heifers classified as NL likely had persistent follicles, and the greatest effect of PROG was realized in these heifers. The hypothesis that the infertility associated with progestin synchronization techniques stems from the persistent follicle is indirectly supported by these results.

Administration of PROG also increased fertility of NL cows in Exp. 3. Synchronization conception rates were approximately 2.5 times greater in NL cows administered PROG than in those administered VEH. The proportion of NL cows that were anestrous compared to those that were cyclic is unknown. It is likely that a portion of these cows were anestrous at the start of MGA feeding. Conceivably, treatment of postpartum cows with progestins near the end of the anestrous period may result in follicular persistence. As with cyclic females, follicular development in postpartum cows is characterized by the wavelike recruitment and dominance of follicles, and dominant follicle diameter and lifespan increases with each successive postpartum follicular wave (Murphy et al., 1990; Savio et al., 1990). Administration of progestins to NL postpartum cows could lead to the development of persistent follicles if LH secretion is increased during the progestin exposure. Regardless of whether cows were cyclic or anestrous, administration of PROG was clearly beneficial to fertility.

In conclusion, acute administration of progesterone stimulates the regression of persistent follicles in heifers. When used in a 14-d MGA estrus synchronization scheme, progesteronene administration increased fertility in heifers and postpartum cows. Enhanced fertility was most obvious when progesterone was administered to animals likely to have persistent dominant follicles (NL heifers and anestrous cows). Research to more accurately define the effects of progesterone administration on fertility is ongoing.

Implications

Widespread use of estrus synchronization programs has been hindered because of the inability of available programs to couple effective synchrony of estrus and normal fertility. These experiments provide initial evidence for the ability of a short-term exposure to increased progesterone to markedly improve fertility when incorporated into estrus synchronization programs that use progestins. The estrus synchronization system reported herein resulted in effective synchronization of estrus with normal fertility. Further investigation should provide additional information to complete the development of this method to synchronize estrus.

Literature Cited


Patterson, D. J., G. H. Kiracofe, J. S. Stevenson, and L. R. Corah.