Complete Luteolysis and Pregnancy Per Insemination After Modifying the Standard 7-day Ovsynch Program in Dairy Cows

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Summary

Two experiments were conducted with Holstein-Friesian cows in the Republic of North Macedonia and with Holstein cows in Kansas. We hypothesized that a single standard dose of $PGF_{2\alpha}$ injected on day 8 instead of day 7 in the standard Ovsynch program would increase the proportion of cows with complete luteolysis and increase the synchronization risk compared with control cows receiving a standard dose on day 7. Cows were treated with the standard program (Ov-7x1) or with Ov-8x1 experimental program in experiment 1, using only a single dose of $PGF_{2\alpha}$. In experiment 2, a third treatment was added (Ov-7x2), in which a second dose of $PGF_{2\alpha}$ was administered 24 hours after the first dose. Overall, the results in experiment 1 demonstrated a greater percentage of multiparous cows in the OV-8x1 treatment had complete luteal regression compared with multiparous Ov-7x1 cows, whereas treatments were equally effective in primiparous cows. In contrast, results in experiment 2 revealed nearly 100% of cows in the Ov-7x2 treatment receiving the second dose of $PGF_{2\alpha}$ had complete luteolysis. In both experiments, when the status of luteal function before PGF treatment was examined, the treatments were equally effective in causing complete luteal regression. Pregnancy rates, however, did not differ among treatments indicating that any of the three treatments will likely produce similar pregnancy outcomes with the flexibility of applying either the 7- vs. 8-day treatments.

Introduction

Fixed-time insemination programs have been adopted by dairy managers to facilitate 100% artificial insemination submission rate and reduce the dependence on the detection of estrus. Adoption of various on-farm reproductive technologies increased rapidly in just 7 years based on surveys conducted in 2006 and repeated in 2013 in 17 of the major U.S. dairy states representing 80% of the dairy operations and more than 80% of the dairy cows (National Health Monitoring Survey, Animal Plant Health Inspection Service, USDA). More than 12% of dairy operations used a timed AI program at first services and more than 21% used such programs to resynchronize ovulation in cows diagnosed not pregnant. The original 7-day Ovsynch program (GnRH-1 [day 0] - 7 days - $PGF_{2\alpha}$ - 56 hours - GnRH-2 - 16 hours - AI) has been modified by further research.

Maximal pregnancy rate achieved with the Ovsynch program has at least 3 limiting factors: (1) failure of GnRH-1 to induce ovulation of the dominant follicle to initiate a new follicular wave; (2) complete luteolysis after a single dose of $PGF_{2\alpha}$; and (3) failure to induce ovulation after G-2. The proportion of cows ovulating after GnRH-1 was 64% (range of 23 to 96%) and varied by stage of the estrous cycle. Ovulation after GnRH-1 is maximized when the Ovsynch program is initiated between days 5 and 12 of the estrous cycle and this is accomplished by various presynchronization programs (Presynch, PG-3-G, Double Ovsynch, and G-6-G).

Cows exposed to presynchronization and that also ovulated after GnRH-1 generally have a greater pregnancy rate than cows that did not ovulate. Although it is assumed that the latter responses are associated with increased circulating progesterone from the newly formed corpus luteum (CL), during growth of the preovulatory follicle, the progesterone concentration at $PGF_{2\alpha}$ may not differ between cows with 1 older CL compared with cows with an older and new CL (2 CL). Nevertheless, cows that ovulated after GnRH-1 and formed a new CL are more likely to have an incomplete luteolysis than cows that did not ovulate because a CL less than 7 days old is resistant to regression after a single standard dose of $PGF_{2\alpha}$.

Attempts have been made to prevent incomplete CL regression by either increasing the dose of $PGF_{2\alpha}$ or the frequency of $PGF_{2\alpha}$ treatments. Increasing the dose of $PGF_{2\alpha}$ (cloprostenol) from 500 to 750 µg for cows submitted to a 7-day Ovsynch protocol has increased the luteolytic risk, but this effect was only observed in multiparous cows. Inclusion of a second $PGF_{2\alpha}$ treatment 24 h after the first $PGF_{2\alpha}$ (days 7 and 8 after GnRH-1) increased the percentage of cows with complete luteolysis from 80 to 97%. Furthermore, a recent meta-analysis demonstrated a clear benefit of an additional $PGF_{2\alpha}$ treatment during the Ovsynch program on luteal regression (+11.6 percentage units) and on pregnancy rate (+4.6 percentage units).

We hypothesized that a single standard dose of $PGF_{2\alpha}$ injected on day 8 instead of day 7 after GnRH-1 increases the proportion of cows with complete luteolysis and increases the synchronization risk compared with control cows receiving a standard dose on day 7. We included a treatment with both standard doses of $PGF_{2\alpha}$ as a positive control. Therefore, our objective was to evaluate the effect of a single standard dose of $PGF_{2\alpha}$ treatment on day 7, day 8, or on both days on the luteolytic and synchronization risks in lactating Holstein cows inseminated at first service after a standard presynchronization program.

Experimental Procedures

Experiment 1

Lactating Holstein-Friesian cows (n = 347) in one commercial herd located in the southeast area of the Republic of North Macedonia were enrolled in the study. Cows were housed in free-stall barns, and fed a total mixed ration (TMR) once daily to meet or exceed requirements for lactating cows producing 30 L of milk per day. Cows were milked twice daily and had free access to water.

Starting at day 35 ± 3 postpartum, all cows were scored for body condition (BCS) on a five-point scale (1 = emaciated and 5 = obese). Estrous cycles were presynchronized

using the PG-3-G scheme (Figure 1). The presynchronization scheme consisted of a 25-mg i.m. injection of $PGF_{2\alpha}$ (PrePG; 5 mL Lutalyse) followed by an i.m. injection of GnRH (8 µg Buserelin [GnRH agonist], PreGnRH; 2 mL of Receptal [GnRH agonist]) 3 days later. Ten days after the PrePG injection, an Ovsynch program was initiated (GnRH-1 – 7 days – $PGF_{2\alpha}$ – 56 hours – GnRH-2 – 16 hours – timed AI) and cows were allocated randomly to two treatments: (1) Ov-7x1 (n = 155) with an injection of $PGF_{2\alpha}$ on day 7 or (2) Ov-8x2 (n = 148) with an injection of $PGF_{2\alpha}$ on day 8 after GnRH-1, respectively. Cows in Ov-8x1 were inseminated 1 day later than cows in Ov-7x1. Pregnancy was diagnosed by transrectal ultrasonography.

Transrectal ultrasonography was conducted at GnRH-1 to map ovarian structures and at $PGF_{2\alpha}$ to assess ovulation in response to GnRH-1 (Figure 1). Blood samples to assess concentrations of progesterone were collected at PrePG, GnRH-1, $PGF_{2\alpha}$, at 72 hours after $PGF_{2\alpha}$ (at timed AI), and 7 days after timed AI from the coccygeal vein or artery for later measurement of progesterone.

Complete luteolysis was defined to occur when progesterone was ≥ 1 ng/mL before $PGF_{2\alpha}$ and ≤ 0.3 ng/mL 72 h later. In addition, when examining the effect of luteolysis, progesterone concentrations at $PGF_{2\alpha}$ were determined based on the luteal status: (1) cows with only a new CL (cows without a CL at GnRH-1 but ovulated after GnRH-1); (2) cows with an older CL (same CL that was detected at G-1); or (3) both a new and old CL.

Experiment 2

We enrolled lactating 454 Holstein cows weekly for 90 weeks at the Kansas State University Dairy Teaching and Research Center, Manhattan, KS. Cows were housed in free stalls with overhead roofs and fed a TMR twice or thrice (summer) daily calculated to meet nutrient requirements for lactating dairy cows producing 110 lb of 3.5% milk. The diet consisted of alfalfa hay, corn silage, triticale silage, soybean meal, whole cotton-seed, ground corn grain, corn-gluten feed, vitamins, and minerals. Cows were milked thrice daily.

Beginning at postpartum day 52 ± 3, estrous cycles were presynchronized using PG-3-G protocol as in experiment 1 (Figure 1). The scheme consisted of a 25-mg i.m. injection of $PGF_{2\alpha}$ (Pre-PG; 2 mL Lutalyse HighCon) and 3 days later a 100-µg i.m. injection of GnRH (Pre-GnRH; 2 mL Factrel). Seven days after the Pre-GnRH treatment in weekly clusters of cows calving between August 2018 through April 2020 and stratified by parity (primiparous vs. multiparous), cows were assigned randomly to 3 treatments: (1) Ov-7x1 (n = 150): GnRH - 7 d - $PGF_{2\alpha}$ - 56 h - GnRH-2 - 16 h - timed AI; (2) Ov-8x1 (n = 149): GnRH-1 - 8 d - $PGF_{2\alpha}$ - 56 hours - GnRH-2 - 16 hours - timed AI; or (3) Ov-7x2 (n = 153): GnRH-1 - 7 d - $PGF_{2\alpha}$ - 24 h - $PGF_{2\alpha}$ - 32 hours - GnRH-2 - 16 hours - timed AI (Figure 1). Onset of the presynchronization treatments occurred 1 d earlier so cows could receive assigned treatments and AI on the same day in each cluster (73 ± 3 days in milk).

Transrectal ultrasonography was conducted at GnRH-1 and before the first or only $PGF_{2\alpha}$ treatment. Ovaries were scanned by transrectal ultrasonography to determine the

number and location of CL and also the number and diameter of all follicles ≥ 10 mm (mapped and recorded) as in experiment 1.

Blood samples were collected before the first or only $PGF_{2\alpha}$ treatment and on the day of insemination (70 to 72 hours after the first or only $PGF_{2\alpha}$ treatment). Complete luteolysis was assessed as in experiment 1.

Results and Discussion
Experiment 1
Mean number of lactations was 1.9 ± 1.1 with 43.8% of cows in their first lactation. Median days in milk (DIM) at AI were 68 d ranging from 52 to 80 d with a mean ± standard deviation (SD) of 67.7 ± 6.3 d. Herd milk production averaged approximately 66 lb per cow per day.

Progesterone concentrations before GnRH-1, PGF_{2a}, and AI by treatment are in Figure 2A. Delaying luteolysis by 1 day did not increase progesterone concentrations before PGF_{2a}, and progesterone did not differ between treatments before GnRH-1 or PGF_{2a}. Body condition affected progesterone concentrations before PGF_{2a} as cows with BCS ≥ 2.75 had greater ($P < 0.01$) progesterone than cows with BCS < 2.75 (8.0 ± 0.3 vs. 6.4 ± 0.3 ng/mL), respectively. In contrast, at the time of AI, regardless of parity, concentrations of progesterone did not different for cows receiving the Ov-7x1 treatment (0.65 ± 0.2 ng/mL), whereas progesterone was less ($P < 0.05$) in multiparous than primiparous cows (0.45 ± 0.2 vs. 0.17 ± 0.2 ng/mL) receiving the Ov-8x1 treatment.

As expected, progesterone concentrations differed depending on CL status before PGF_{2a}. Cows bearing only a new CL that formed after ovulation in response to GnRH-1 had the smallest concentration and cows with both an older and new CL had the largest concentration resulting in mean progesterone concentrations that differed ($P < 0.01$) among the three luteal status groups (Figure 2B).

Our hypothesis was that lengthening the period between GnRH-1 and PGF_{2a} by 1 day would increase the proportion of cows with complete luteolysis by the time of AI because new CL forming after GnRH-1 would be 24 hours older and more likely to respond to a single dose of PGF_{2a}. Of the 347 cows assigned to treatment, 87.9% were cyclic by the onset of treatment, leaving 303 cows to test this hypothesis. More ($P = 0.02$) multiparous than primiparous cows had complete luteolysis and more ($P < 0.05$) cows receiving the Ov-8x1 treatment had complete luteolysis than cows in the Ov-7x1 treatment (Table 1). An interaction ($P = 0.02$) of treatment and parity also was detected. Although complete luteolysis did not differ between treatments in primiparous cows, more ($P = 0.02$) multiparous cows had complete luteolysis when treated with Ov-8x1 than Ov-7x1 treatment (Table 1). Treatments were equally effective in causing complete luteolysis in cows with only a new CL at the time of PGF_{2a} (87.5%), an older CL (79.0%), or both (77.7%).

Pre-treatment cyclicity tended ($P = 0.10$) to affect pregnancy rate (23.8 vs. 36.8%) in anovular vs. cycling cows, respectively. Neither treatment nor parity, however, affected pregnancy rate (Table 2). Pregnancy rate in cows with a body condition score < 2.75 tended ($P = 0.09$) to be less in Ov-8x1 vs. Ov-7x1 cows (30.1 vs. 39.4%), respectively,

whereas the reverse occurred in cows with body condition score ≥ 2.75 (40.9 vs. 31.7%), respectively.

Experiment 2

Mean number of lactations was 1.9 ± 1.1 with 44% of cows in their first lactation. Median DIM at AI was 68 d ranging from 52 to 80 d with a mean ± SD of 67.7 ± 6.3 d. Rolling herd average was 31,324 ± 458 lb during the experimental period.

Delaying luteolysis by 1 day did not increase progesterone concentrations before PGF_{2a} in the Ov-8x1 treatment. In fact, concentrations of progesterone were less ($P = 0.06$) in the cows treated with Ov-8x1 compared with the other two treatments (Figure 3A). Furthermore, progesterone concentrations were greater ($P < 0.001$) in primiparous cows compared with the multiparous cows (5.2 ± 0.2 vs. 4.3 ± 0.2 ng/mL).

As in experiment 1, progesterone concentrations differed depending on CL status before PGF_{2a}. Cows bearing only new CL had the smallest concentration and cows with both an older and new CL had the largest concentration resulting in mean progesterone concentrations that differed ($P < 0.01$) among the three luteal status groups (Figure 3B).

Cows receiving the second dose of PGF_{2a} had nearly perfect complete luteolysis, which was greater ($P < 0.05$) than that observed in cows receiving the other two treatments. Although not different, complete luteolysis was numerically greater in primiparous than multiparous cows. Complete luteolysis did not differ among cows having different CL status: new CL (91.7%), old CL (95.1%), and both new and old CL (93.3%).

Cows not cycling (n = 18) had numerically few pregnancies than cycling cows (n = 429; 27.8 vs. 41.7%), respectively. Neither treatment nor parity affected pregnancy rate (Table 4). Some variation in pregnancy rates occurred during each season with fall and winter (43.9%) tending ($P = 0.08$) to be greater than pregnancy rate during spring and summer (36.3%).

The present study aimed to determine the efficacy of injecting $PGF_{2\alpha}$ on day 8 instead of day 7 to produce more complete luteal regression in the standard Ovsynch protocol in lactating dairy cows. Overall, the results in experiment 1 in Holstein-Friesian cows demonstrated a greater percentage of multiparous cows in the OV-8x1 treatment had complete luteal regression compared with Ov-7x1 cows, whereas treatments were equally effective in primiparous cows. In contrast, results in experiment 2 in Holstein cows, revealed nearly 100% of cows in the Ov-7x2 treatment receiving the second dose of $PGF_{2\alpha}$ 24 hours after the first dose, had complete luteolysis. In both experiments, when the status of luteal function before PGF treatment was examined, the treatments were equally effective in causing complete luteal regression.

The resulting pregnancy rates achieved were the most important outcomes of these experiments. Pregnancy rates did not differ among treatments, indicating that any of the three treatments will likely produce similar outcomes with the flexibility of the 7- vs. 8-day treatments.

Table 1. Percentage of cows with complete luteolysis by 72 h after the PGF$_{2a}$ treatment injection (time of AI) in primiparous and multiparous cows (experiment 1)[1]

Treatment[2]	Parity		
	Primiparous	Multiparous	Overall
Ov-7x1	75.4 (46/61)	75.5 (71/94)	75.5[a] (117/155)
Ov-8x1	74.0 (57/77)	93.0 (66/71)	83.1[b] (123/148)
Overall	74.6[a] (103/138)	83.0[b] (137/165)	79.2 (240/303)

[a,b] Means bearing different superscript letters differ ($P < 0.05$). A treatment by parity interaction ($P = 0.03$) also was detected.
[1] Complete luteolysis was defined to occur when progesterone was ≥ 1 ng/mL before PGF$_{2a}$ treatment and ≤ 0.3 ng/mL 72 h later (time of AI). The table excludes 44 cows without a CL at PGF$_{2a}$ treatment having progesterone < 1 ng/mL.
[2] See Figure 1.

Table 2. Pregnancy rates (%) in primiparous and multiparous cows at first AI after treatment (experiment 1)

Treatment[1]	Parity		
	Primiparous	Multiparous	Overall[2]
Ov-7x1	38.4 (26/68)	32.7 (35/107)	34.9 (61/175)
Ov-8x1	34.9 (29/83)	36.4 (32/88)	35.7 (61/171)
Overall[2]	36.4 (55/151)	34.4 (67/195)	35.3 (122/346)[3]

[1] See Figure 1.
[2] Neither parity nor treatment differences were detected.
[3] One cow was culled before pregnancy diagnosis.

Table 3. Percentage of cows with complete luteolysis by 72 h after the PGF$_{2a}$ treatment injection (time of AI) in primiparous and multiparous cows (experiment 2)[1]

Treatment[2]	Parity		
	Primiparous	Multiparous	Overall
Ov-7x1	96.8 (60/62)	86.7 (65/75)	91.2[a] (125/137)
Ov-7x2	100 (60/60)	98.7 (74/75)	99.3[b] (134/135)
Ov-8x1	90.4 (47/52)	90.6 (58/64)	90.5[a] (105/116)
Overall	96.0 (167/174)	92.1 (197/214)	93.8 (364/388)

[a,b] Treatments differed ($P = 0.05$). No parity or treatment by parity interaction was detected.
[1] Complete luteolysis was defined to occur when progesterone was ≥ 1 ng/mL before PGF$_{2a}$ treatment and ≤ 0.3 ng/mL 72 h later (time of AI). Excludes 66 cows without a CL at PGF$_{2a}$ treatment having progesterone < 1 ng/mL.
[2] See Figure 1.

Table 4. Pregnancy rates in primiparous and multiparous cows (experiment 2)

| Treatment[1]	Parity		Overall[2]
	Primiparous	Multiparous	
Ov-7x1	44.8 (30/67)	38.6 (32/83)	41.3 (62/150)
Ov-7x2	48.5 (32/66)	38.6 (32/83)	43.0 (64/149)
Ov-8x1	33.8 (22/65)	43.4 (36/83)	39.2 (58/148)
Overall[2]	42.4 (84/198)	40.2 (100/249)	41.1 (184/447)[3]

[1] See Figure 1.
[2] Neither parity nor treatment differences were detected.
[3] Seven cows were culled before pregnancy diagnosis.
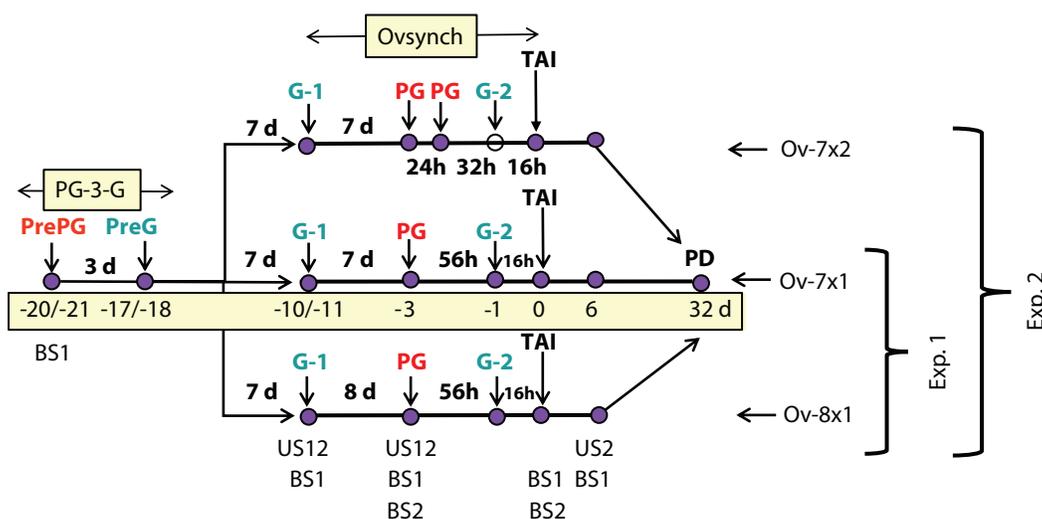
Figure 1. Experimental scheme for experiments 1 and 2. Ovulation was presynchronized in all cows before treatments were applied to facilitate first AI after calving. In both experiments presynchronization consisted of PG-3-G presynchronization (Pre-PGF$_{2a}$ [PrePG] – 3 days – Pre-GnRH [PreG]). Seven days after PreG, cows were treated with either Ovsynch (Ov-7x1 or Ov-8x1; GnRH-1 [G-1] – 7 or 8 days – PGF$_{2a}$ – 56 hours – GnRH-2 [G-2] – 16 hours – timed AI [TAI]) in experiment 1. In experiment 2, an additional Ovsynch treatment was included (Ov-7x2) in which two doses of PGF$_{2a}$ were administered 24 hours apart followed by G-2 in 32 hours and timed AI 16 hours after G-2. Pregnancy diagnosis (PD) occurred 30 to 32 days after timed AI. US12 = transrectal ultrasonography of ovaries (1 or 2 designates experiment 1 or 2, respectively); BS = blood sample.
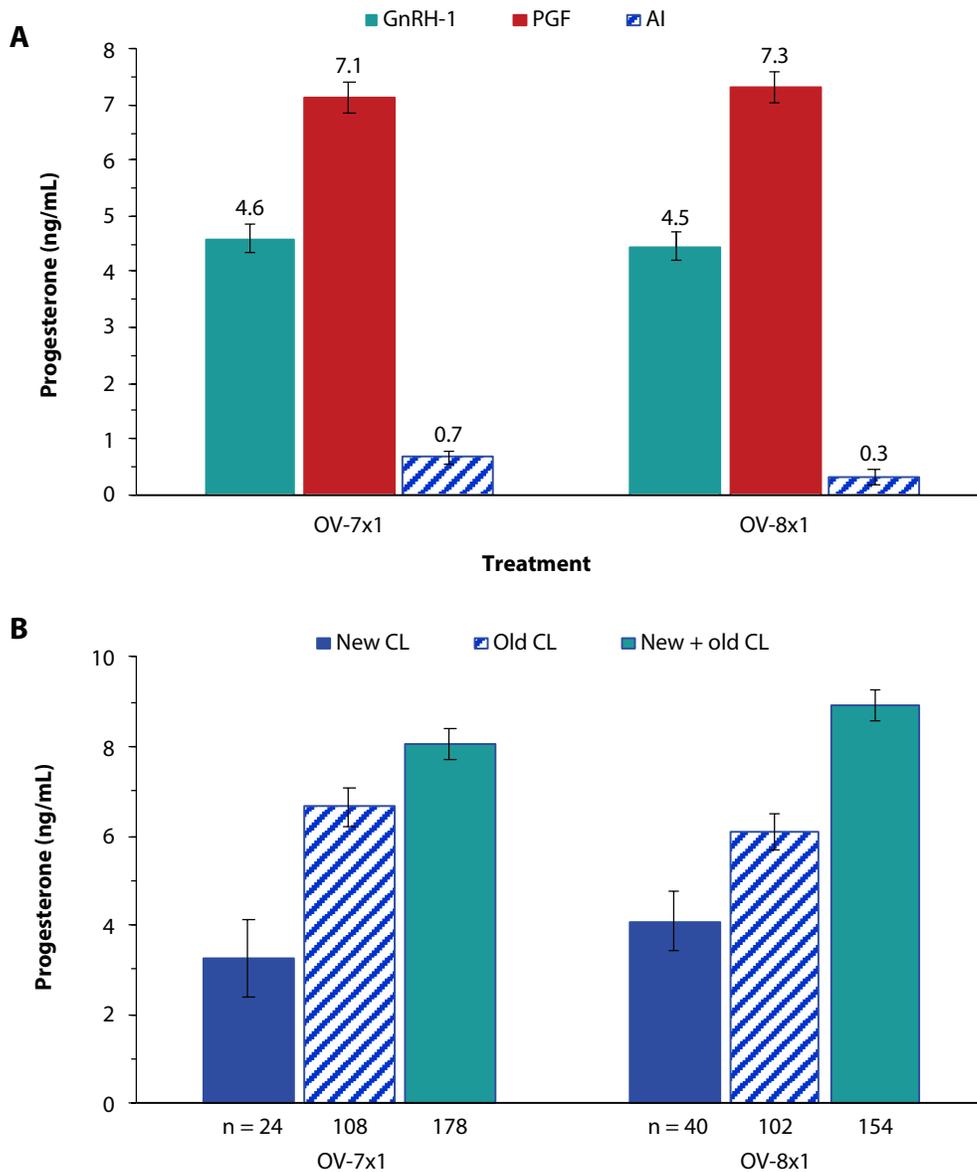
Figure 2. (A) Concentrations of progesterone (LSM ± SEM) before GnRH-1, PGF$_{2\alpha}$ (PGF), and timed AI in experiment 1. (B) Concentrations of progesterone (LSM ± SEM) before PGF$_{2\alpha}$ treatment injection in cows receiving the Ov-7x1 or Ov-8x1 treatment. Cows were classified at the time of PGF$_{2\alpha}$ treatment injection as having a new CL (formed in response to ovulation after GnRH-1), an older CL existing at GnRH-1 and present at PGF$_{2\alpha}$ or both a new and older CL.

A

B
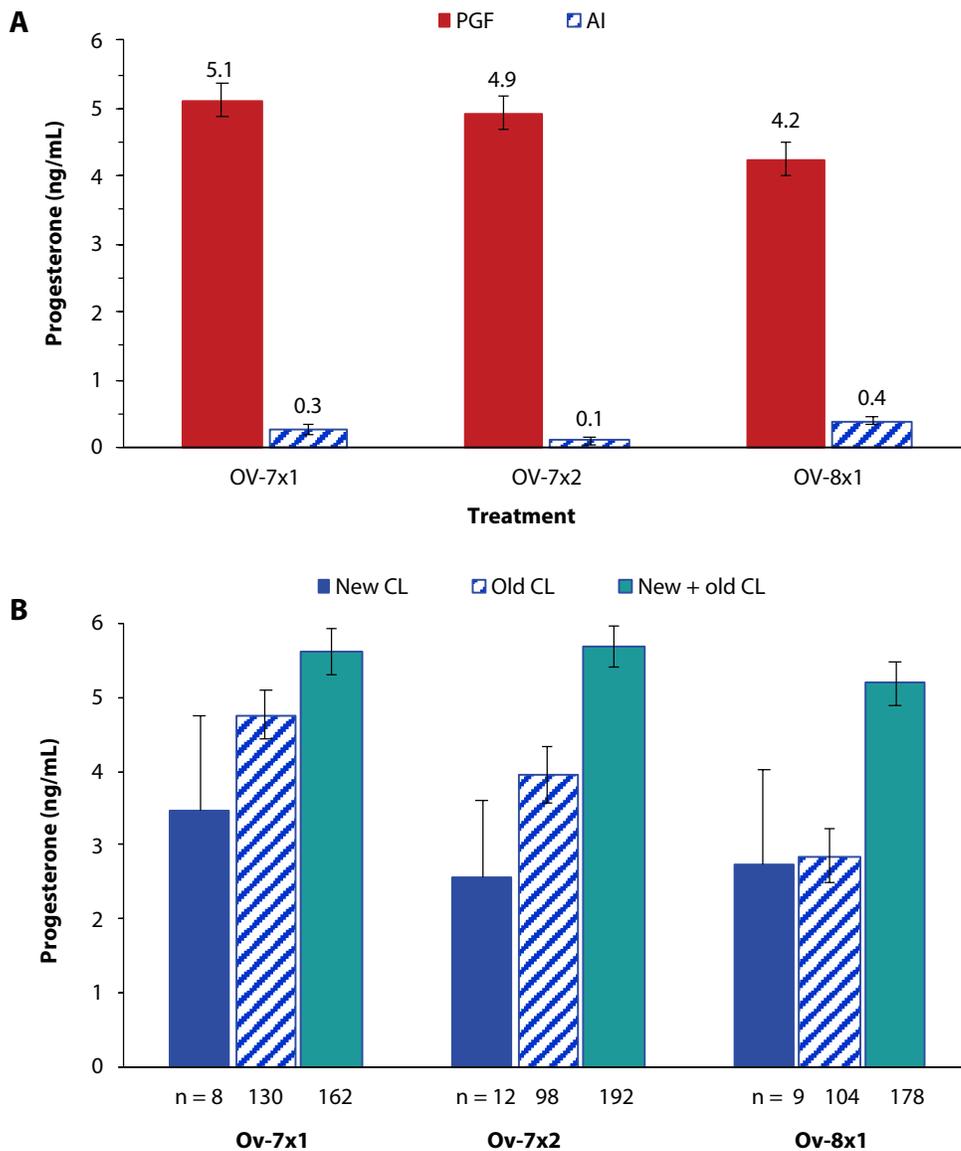
Figure 3. (A) Concentrations of progesterone (LSM ± SEM) before PGF2α (PGF) and timed AI in experiment 2. (B) Concentrations of progesterone (LSM ± SEM) before PGF$_{2\alpha}$ treatment injection in cows receiving the Ov-7x1, Ov-7x2, or Ov-8x1 treatment. Cows were classified at the time of PGF$_{2\alpha}$ treatment injection as having a new CL (formed in response to ovulation after GnRH-1), an older CL existing at GnRH-1 and present at PGF$_{2\alpha}$ or both a new and older CL.