

1 **Supplemental Material**

2 **Supplemental Table S1.** Number and percentage of cows per treatment within farm, parity group, type of semen assigned for first
 3 service, milk yield group, health disorder group, and season of AI for lactating dairy cows randomized with the Double-Ovsynch
 4 protocol with an interval of 56 h (G56 treatment) or 80 h (G80 treatment) between induction of luteolysis and induction of ovulation
 5 before TAI during the Breeding-Ovsynch portion of the protocol.

Outcome and variable	Farm A			Farm B			Total
	G56	G80	Total	G56	G80	Total	
Cows enrolled, n	1,763	1,765	3,528	575	569	1,144	4,672
Parity							
Primiparous %, n	32.3 (570)	32.0 (564)	32.1 (1,134)	35.5 (204)	37.3 (212)	36.4 (416)	33.2 (1,550)
Multiparous %, n	67.7 (1,193)	68.0 (1,201)	67.9 (2,394)	64.5 (371)	62.7 (357)	63.6 (728)	66.8 (3,122)
Type of semen assigned for first AI							
Sex-sorted %, n	37.2 (655)	38.2 (674)	37.7 (1,329)	19.0 (109)	22.1 (126)	20.5 (235)	33.5 (1,564)
Beef %, n	62.8 (1,107)	61.8 (1,091)	62.3 (2,198)	81.0 (466)	77.9 (443)	79.5 (909)	66.5 (3,107)
Milk yield tertile ¹							
Low %, n	32.6 (586)	34.3 (605)	33.8 (1,191)	36.2 (208)	35.0 (199)	35.6 (407)	34.2 (1,598)
Medium %, n	34.2 (602)	33.7 (595)	33.9 (1,197)	31.5 (181)	34.1 (194)	32.8 (375)	33.6 (1,572)
High %, n	32.6 (575)	32.0 (565)	32.3 (1,140)	32.3 (186)	30.9 (176)	31.6 (362)	32.1 (1,502)
Health disorders before first AI ²							
Yes %, n	23.0 (406)	22.7 (400)	22.8 (806)	25.6 (147)	28.7 (163)	27.1 (310)	23.9 (1,116)
No %, n	77.0 (1,357)	77.3 (1,365)	77.2 (2,722)	74.4 (428)	71.4 (406)	72.9 (834)	76.1 (3,556)

Season of AI³

Cold %, n/n	50.5 (859/1,702)	49.4 (842/1,706)	49.9 (1,701/3,408)	46.2 (255/552)	45.4 (240/529)	45.8 (495/1,081)	48.9 (2,196/4,489)
Warm %, n/n	49.5 (843/1,702)	50.6 (864/1,706)	50.1 (1,707/3,408)	53.8 (297/552)	54.6 (289/529)	54.2 (586/1,081)	51.1 (2,293/4,489)

6 ¹Milk yield tertiles were based on accumulated milk yield up to 100 DIM; for primiparous (medium tertile lower limit = 3,336.9 kg and upper limit = 3,822.7 kg)
 7 and multiparous cows (medium tertile lower limit = 4,930.4 kg and upper limit = 5,493.4 kg) in Farm A and primiparous (medium tertile lower limit = 3,436.8 kg
 8 and upper limit = 3,827.2 kg) and multiparous (medium tertile lower limit = 4,599.0 kg and upper limit = 5,152.9 kg) in Farm B.

9 ²Health disorders group = cow had at least one of the following health disorders diagnosed between parturition to the day of the first insemination: milk fever,
 10 retained placenta, metritis, mastitis, displaced abomasum, indigestion, ketosis, and pneumonia.

11 ³AI season = inseminations performed from May to September were considered to occur during the warm season whereas AI from October to April during the
 12 cold season. Percentages of cows AI in the cold and warm season were calculated out of the total of cows that completed first AI.

14 **Supplemental Table S2.** Percentage of cows that received AI at detected estrus (AIE) and
 15 expressed estrus before AI for lactating dairy cows enrolled in a randomized controlled
 16 experiment that were synchronized with the Double-Ovsynch protocol with an interval of 56 h
 17 (G56 treatment) or 80 h (G80 treatment) between induction of luteolysis and induction of
 18 ovulation before TAI during the Breeding-Ovsynch portion of the protocol. Data are presented as
 19 unadjusted means (%) and least squares means [95% CI]. Effects confounders that were forced
 20 (parity, farm) or significant, and significant interactions with treatment are also reported.

Outcome and variable	Level	n	Unadjusted mean, % (n)	LSM [95% CI], %	P-value
Cows that received AIE					
Treatment	G56	2,254	2.5 (56)	2.2 [1.7, 2.9]	< 0.001
	G80	2,235	31.4 (703)	29.7 [27.1, 32.5]	
Parity	Primiparous	1,500	23.7 (355)	13.4 [11.3, 15.9]	< 0.001
	Multiparous	2,989	13.6 (406)	5.8 [4.8, 7.0]	
Farm	A	3,408	17.2 (613)	10.7 [9.3, 12.4]	< 0.001
	B	1,081	13.7 (148)	7.4 [6.0, 9.1]	
Milk yield tertile ¹	Low	1,474	19.2 (282)	10.7 ^a [8.9, 12.7]	< 0.001
	Medium	1,534	17.7 (271)	9.2 ^a [7.6, 11.1]	
	High	1,481	14.0 (208)	7.2 ^b [5.8, 8.7]	
Health disorder ²	Yes	1,026	14.5 (149)	8.1 [6.5, 10.0]	0.05
	No	3,463	17.7 (612)	9.8 [8.5, 11.4]	
Farm × parity	A × primiparous	1,091	24.4 (266)	14.7 [12.3, 17.4]	0.07
	A × multiparous	2,317	15.0 (347)	7.7 [6.5, 9.1]	
	B × primiparous	409	21.8 (89)	12.2 [9.4, 15.7]	
	B × multiparous	672	8.9 (59)	4.4 [3.2, 5.8]	
Cows with estrus before AI					
Treatment	G56	2,254	27.4 (617)	28.0 [25.9, 30.2]	< 0.001
	G80	2,235	70.2 (1,569)	73.9 [71.8, 76.0]	
Parity	Primiparous	1,500	63.5 (952)	65.0 [62.1, 67.7]	< 0.001
	Multiparous	2,989	41.6 (1,244)	37.4 [35.2, 39.6]	
Farm	A	3,408	50.3 (1,715)	55.6 [53.5, 57.6]	< 0.001
	B	1,081	44.5 (481)	46.9 [43.5, 50.4]	
AI season ³	Cold	2,196	49.8 (1,093)	53.2 [50.4, 55.9]	0.03
	Warm	2,293	48.1 (1,103)	49.3 [46.7, 51.9]	
Milk yield tertile	Low	1,474	53.3 (785)	56.6 ^a [53.5, 59.7]	< 0.001
	Medium	1,534	50.1 (768)	52.3 ^b [49.2, 55.5]	

	High	1,481	43.4 (643)	44.8 ^c [41.7, 47.9]	
Treatment × milk yield tertile	G56 × Low	738	33.5 (247)	34.6 ^c [31.0, 38.4]	0.04
	G56 × Medium	760	25.5 (194)	26.5 ^d [23.2, 30.0]	
	G56 × High	756	23.3 (176)	23.7 ^d [20.6, 27.1]	
	G80 × Low	736	73.1 (538)	76.3 ^a [73.0, 79.4]	
	G80 × Medium	774	74.2 (574)	77.0 ^a [73.8, 79.9]	
	G80 × High	725	64.4 (467)	68.0 ^b [64.2, 71.4]	

21 ^{a-d}Means within a variable with different superscripts differ ($P \leq 0.05$) based on the LSD post hoc mean separation
 22 test.

23 ¹Milk yield tertiles were based on accumulated milk yield up to 100 DIM; for primiparous (medium tertile lower
 24 limit = 3,336.9 kg and upper limit = 3,822.7 kg) and multiparous cows (medium tertile lower limit = 4,930.4 kg and
 25 upper limit = 5,493.4 kg) in Farm A and primiparous (medium tertile lower limit = 3,436.8 kg and upper limit =
 26 3,827.2 kg) and multiparous (medium tertile lower limit = 4,599.0 kg and upper limit = 5,152.9 kg) in Farm B.

27 ²Health Disorder = cow had at least one of the following health disorders diagnosed between parturition to the day
 28 of the first insemination: milk fever, retained placenta, metritis, mastitis, displaced abomasum, indigestion, ketosis,
 29 and pneumonia.

30 ³AI season = inseminations performed from May to September were considered to occur during the warm season
 31 whereas AI from October to April during the cold season.

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35 **Supplemental Table S3.** Pregnancies per AI (P/AI) at the first pregnancy evaluation and
36 reconfirmation of pregnancy, and pregnancy loss after first service for lactating dairy cows
37 enrolled in a randomized controlled experiment that were synchronized with the Double-
38 Ovsynch protocol with an interval of 56 h (G56 treatment) or 80 h (G80 treatment) between
39 induction of luteolysis and induction of ovulation before TAI during the Breeding-Ovsynch
40 portion of the protocol. Parity and farm were forced in all models whereas the remaining
41 covariates were retained only if $P < 0.10$. Data are presented as unadjusted means (%) and LSM
42 [95% CI].

Outcome and variable	Level	N	Unadjusted mean, % (n)	LSM [95% CI], %	<i>P</i> -value
P/AI first pregnancy evaluation					
Treatment	G56	2,191	48.0 (1,052)	51.4 [48.8, 54.0]	0.36
	G80	2,178	49.3 (1,074)	52.8 [50.2, 55.4]	
Parity	Primiparous	1,469	58.0 (852)	59.7 [56.7, 62.5]	<0.01
	Multiparous	2,900	43.9 (1,274)	44.5 [42.1, 46.9]	
Farm	A	3,312	45.7 (1,513)	46.1 [44.0, 48.3]	<0.01
	B	1,057	58.0 (613)	58.0 [54.8, 61.2]	
Type of semen ¹	Sex-sorted	1,552	48.0 (745)	49.5 [46.5, 52.4]	0.002
	Conventional beef	2,817	49.0 (1,381)	54.8 [52.3, 57.2]	
AI season ²	Cold	2,149	50.7 (1,089)	54.4 [51.6, 57.1]	0.004
	Warm	2,220	46.7 (1,037)	49.9 [47.3, 52.4]	
Milk yield tertile ³	Low	1,379	49.5 (682)	52.5 ^{ab} [49.4, 55.6]	0.04
	Medium	1,522	50.5 (769)	54.3 ^a [51.3, 57.2]	
	High	1,468	46.0 (675)	49.6 ^b [46.5, 52.6]	
Health disorder ⁴	Yes	979	43.7 (428)	49.6 [46.1, 53.0]	0.007
	No	3,390	50.1 (1,698)	54.6 [52.6, 56.6]	
P/AI confirmation of pregnancy					
Treatment	G56	2,164	44.8 (969)	47.7 [44.8, 50.6]	0.21
	G80	2,153	46.5 (1,000)	50.0 [47.2, 52.9]	
Parity	Primiparous	1,453	56.0 (814)	57.1 [54.1, 60.1]	<0.01
	Multiparous	2,864	40.3 (1,155)	40.6 [38.2, 43.1]	
Farm	A	3,273	42.7 (1,397)	43.1 [41.0, 45.3]	<0.01
	B	1,044	54.8 (572)	54.6 [51.2, 58.0]	
	Sex-sorted	1,540	45.9 (707)	46.6 [43.3, 49.9]	

Type of semen	Conventional beef	2,777	45.4 (1,262)	51.2 [48.5, 53.8]	
AI season	Cold	2,134	47.5 (1,014)	51.1 [48.3, 53.9]	0.004
	Warm	2,183	43.8 (955)	46.6 [44.1, 49.2]	
Milk yield tertile	Low	1,350	46.2 (623)	49.2 ^{ab} [46.0, 52.3]	0.08
	Medium	1,506	47.3 (713)	50.8 ^a [47.7, 53.8]	
	High	1,461	43.3 (633)	46.6 ^b [43.5, 49.7]	
Health disorder	Yes	967	39.8 (385)	45.8 [42.3, 49.3]	0.001
	No	3,350	47.3 (1,584)	51.9 [49.9, 54.0]	
Farm × treatment	A × G56	1,634	41.7 (682)	42.2 [39.5, 45.0]	0.78
	A × G80	1,639	43.6 (715)	44.0 [41.2, 46.9]	
	B × G56	530	54.1 (287)	53.2 [48.5, 57.9]	
	B × G80	514	55.4 (285)	56.1 [51.3, 60.7]	
Semen type × treatment	Sex-sorted × G56	763	44.7 (341)	44.2 [39.8, 48.6]	0.20
	Sex-sorted × G80	777	47.1 (366)	49.0 [44.5, 53.5]	
	Conventional beef × G56	1,401	44.8 (628)	51.2 [47.8, 54.6]	
	Conventional beef × G80	1,376	46.1(634)	51.1 [47.7, 54.5]	
Farm × semen type	A × Sex-sorted	1,185	42.5 (504)	41.4 [38.1, 44.3]	0.78
	A × Conventional beef	2,088	42.8 (893)	45.1 [42.5, 47.8]	
	B × Sex-sorted	355	57.2 (203)	52.1 [46.5, 57.6]	
	B × Conventional beef	689	53.6 (369)	57.2 [53.2, 61.1]	
Farm × semen type × treatment	A × Sex-sorted × G56	587	42.2 (248)	40.6 [36.5, 44.9]	0.10
	A × Sex-sorted × G80	598	42.8 (256)	43.9 [40.5, 47.3]	
	A × Conventional beef × G56	1,047	41.4 (434)	41.7 [37.5, 45.9]	
	A × Conventional beef × G80	1,041	44.1 (459)	46.4 [43.0, 49.9]	
	B × Sex-sorted × G56	176	52.8 (93)	47.8 [40.3, 55.4]	
	B × Sex-sorted × G80	179	54.2 (110)	58.5 [53.1, 63.7]	
	B × Conventional beef × G56	354	52.6 (194)	56.3 [48.6, 63.7]	
	B × Conventional beef × G80	335	52.2 (175)	55.8 [50.3, 61.2]	
Pregnancy loss					
Treatment	G56	1,046	7.4 (77)	6.1 [4.6, 8.1]	0.62
	G80	1,067	6.3 (67)	5.3 [3.9, 7.1]	
Parity	Primiparous	844	3.6 (30)	3.8 [2.6, 5.6]	< 0.001
	Multiparous	1,269	8.9 (114)	8.4 [6.5, 10.7]	
Farm	A	1,505	7.2 (108)	6.3 [4.9, 8.0]	0.96
	B	608	5.9 (36)	5.2 [3.6, 7.4]	
	Sex-sorted	740	4.5 (33)	4.8 [3.3, 6.9]	

Type of semen	Conventional beef	1,373	8.1 (111)	6.7 [5.2, 8.7]	
Health disorder	Yes	425	9.4 (40)	6.7 [4.7, 9.5]	0.06
	No	1,688	6.2 (104)	4.8 [3.7, 6.2]	
Farm × Treatment	A × G56	742	8.0 (60)	6.1 [4.3, 8.8]	0.99
	A × G80	763	6.3 (48)	5.5 [3.9, 7.8]	
	B × G56	304	5.6 (17)	6.1 [3.7, 10.0]	
	B × G80	304	6.2 (19)	5.5 [3.2, 9.4]	
Semen type × Treatment	Sex-sorted × G56	358	4.7 (17)	6.2 [3.8, 10.0]	0.39
	Sex-sorted × G80	382	4.2 (16)	4.6 [2.6, 8.0]	
Treatment	Conventional beef × G56	688	8.7 (60)	6.0 [4.1, 8.7]	
	Conventional beef × G80	685	7.4 (51)	6.5 [4.6, 9.1]	
Farm × Semen type	A × Sex-sorted	526	4.2 (22)	4.5 [2.9, 6.8]	0.09
	A × Conventional beef	979	8.8 (86)	7.5 [5.8, 9.9]	
	B × Sex-sorted	214	5.1 (11)	6.4 [3.5, 11.5]	
Farm × Semen type	B × Conventional beef	394	6.3 (25)	5.2 [3.4, 7.9]	
	A × Sex-sorted × G56	258	3.9 (10)	4.2 [2.3, 7.8]	0.06
Semen type × Treatment	A × Sex-sorted × G80	268	4.5 (12)	4.8 [2.7, 8.3]	
	A × Conventional beef × G56	484	10.3 (50)	8.9 [6.4, 12.2]	
	A × Conventional beef × G80	495	7.3 (36)	6.4 [4.5, 9.2]	
	B × Sex-sorted × G56	100	7.0 (7)	9.1 [4.4, 17.9]	
	B × Sex-sorted × G80	114	3.5 (4)	4.5 [1.7, 11.5]	
	B × Conventional beef × G56	204	4.9 (10)	4.0 [2.1, 7.6]	
	B × Conventional beef × G80	190	7.9 (15)	6.7 [3.9, 11.1]	

43 ^{a-c}Means within a column for a variable with different superscripts differed ($P \leq 0.05$) based on the LSD post hoc
44 mean separation test.

45 ¹Type of semen = category assigned based on the type of semen used for first service (sex-sorted or conventional
46 beef semen).

47 ²AI season = inseminations performed from May to September were considered to occur during the warm season
48 whereas insemination from October to April were considered to occur during the cold season.

49 ³Milk yield tertiles were based on accumulated milk yield up to 100 DIM; for primiparous (medium tertile lower
50 limit = 3,336.9 kg and upper limit = 3,822.7 kg) and multiparous cows (medium tertile lower limit = 4,930.4 kg and
51 upper limit = 5,493.4 kg) in Farm A and primiparous (medium tertile lower limit = 3,436.8 kg and upper limit =
52 3,827.2 kg) and multiparous (medium tertile lower limit = 4,599.0 kg and upper limit = 5,152.9 kg) in Farm B.

53 ⁴Cows that had at least one of the following health disorders diagnosed between parturition to the day of the first
54 insemination: milk fever, retained placenta, metritis, mastitis, displaced abomasum, indigestion, ketosis, and
55 pneumonia.

56 **Supplemental Table S4.** Effects of the interaction between farm, parity (**Pty**), and treatment (**Trt**) on the proportion of cows AIE,
57 proportion of cows with estrus before AI, pregnancies per AI (**P/AI**) at first pregnancy evaluation and pregnancy confirmation, and
58 pregnancy loss after first service for lactating dairy cows that were synchronized with the Double-Ovsynch protocol with an interval
59 of 56 h (G56 treatment) or 80 h (G80 treatment) between induction of luteolysis and induction of ovulation before TAI during the
60 Breeding-Ovsynch portion of the protocol. Results from an exploratory analysis based on a statistical model for the interaction was
61 forced in the model. Data are presented as LSM [95% CI] and unadjusted means (%).

	Group								<i>P</i> -value*		
	Farm A				Farm B						
	Primiparous		Multiparous		Primiparous		Multiparous		Farm	Pty	Trt
	G56	G80	G56	G80	G56	G80	G56	G80			
Cows AIE	3.2 [2.0, 5.9]	43.3 [38.9, 47.9]	2.3 [1.6, 3.3]	26.2 [23.5, 29.0]	2.8 [1.3, 6.0]	37.9 [31.5, 44.8]	1.1 [0.4, 2.8]	16.5 [12.8, 20.9]	0.02	< 0.01	<0.01
	3.5 (19/546)	45.3 (247/545)	2.4 (28/1,156)	27.5 (319/1,161)	3.0 (6/199)	39.5 (83/210)	1.1 (4/353)	17.2 (55/319)			
Cows with estrus before AI	45.4 [41.2, 49.6]	84.0 [80.7, 86.9]	20.6 [19.3, 23.0]	66.4 [63.6, 69.1]	39.3 [32.7, 46.3]	81.3 [75.4, 86.0]	14.2 [10.9, 18.2]	57.2 [51.6, 62.5]	< 0.01	< 0.01	<0.01
	45.2 (247/546)	83.8 (457/545)	20.8 (241/1,156)	66.3 (770/1,161)	39.2 (78/199)	80.9 (170/210)	14.4 (51/353)	57.0 (182/319)			
P/AI first pregnancy evaluation	50.8 [46.3, 55.2]	55.8 [51.4, 60.2]	39.3 [36.1, 42.5]	38.4 [35.3, 41.7]	66.3 [59.0, 72.8]	66.8 [59.7, 73.0]	47.2 [40.7, 53.8]	52.1 [45.3, 58.8]	< 0.01	< 0.01	0.22
	52.1 (280/537)	57.3 (303/529)	41.7 (466/1,118)	41.1 (464/1,128)	65.8 (129/196)	67.6 (140/207)	52.1 (177/340)	53.2 (167/314)			
P/AI pregnancy confirmation	49.0 [44.5, 53.5]	53.5 [49.0, 58.0]	35.2 [32.1, 38.4]	35.4 [32.3, 38.6]	64.1 [56.7, 70.9]	62.3 [55.1, 68.9]	42.5 [36.1, 49.2]	49.4 [42.6, 56.2]	<0.01	<0.01	0.21
	50.8 (269/529)	55.3 (290/524)	37.4 (413/1,105)	38.1 (425/1,115)	63.6 (124/195)	63.9 (131/205)	48.7 (163/335)	49.8 (154/309)			
Pregnancy loss	3.2 [1.6, 6.3]	3.7 [2.0, 6.8]	9.9 [6.9, 14.0]	8.3 [5.7, 11.8]	3.7 [1.4, 9.1]	5.7 [2.7, 11.6]	9.5 [5.1, 17.0]	5.8 [2.8, 12.0]	0.84	<0.01	0.91
	2.9 (8/277)	3.3 (10/300)	11.2 (52/465)	8.2 (38/463)	3.9 (5/129)	5.1 (7/138)	6.9 (12/175)	7.2 (12/166)			

62 *None of the interactions tested were significant. All had $P > 0.10$

63 **Supplemental Table S5.** Effects of the interaction between farm, type of semen (**T-S**), and treatment (**Trt**) on pregnancies per AI
64 (**P/AI**) at first pregnancy evaluation and pregnancy confirmation, and pregnancy loss after first service for lactating dairy cows that
65 were synchronized with the Double-Ovsynch protocol and were randomized to receive the last GnRH before AI at 56 h (G56
66 treatment) or 80 h (G80 treatment) after induction of luteolysis during the Breeding-Ovsynch portion of the protocol. The three-way
67 interaction was forced in all models. Data are presented as LSM [95% CI] and unadjusted means (%).

	Group								P-value		
	Farm A				Farm B				Farm	S-T	Trt
	Sex-sorted		Conventional beef		Sex-sorted		Conventional beef				
G56	G80	G56	G80	G56	G80	G56	G80				
P/AI first pregnancy evaluation	42.7 [38.6, 47.0]	44.0 [39.8, 48.2]	47.2 [43.6, 50.8]	50.2 [46.6, 53.7]	51.6 [43.7, 59.5]	59.5 [51.5, 66.9]	62.2 [55.9, 68.1]	59.7 [53.5, 65.7]	< 0.001*	0.01*	0.22*
	43.9 (259/590)	44.7 (269/602)	45.7 (487/1,065)	47.2 (498/1,055)	56.7 (101/178)	63.7 (116/182)	57.3 (205/358)	56.3 (191/339)			
P/AI pregnancy confirmation	40.7 ^d [36.6, 44.9]	41.7 ^{cd} [37.5, 45.9]	43.2 ^{cd} [39.7, 46.9]	46.9 ^{bc} [43.3, 50.5]	46.7 ^{bcd} [38.8, 54.7]	57.1 ^{ab} [49.1, 64.7]	60.2 ^a [53.8, 66.3]	54.8 ^{ab} [48.5, 60.9]	< 0.001‡	0.03‡	0.21‡
	42.2 (248/587)	42.8 (256/598)	41.4 (434/1,047)	44.1 (459/1,041)	52.8 (93/176)	54.2 (110/179)	52.6 (194/354)	52.2 (175/335)			
Pregnancy loss	4.0 ^b [2.1, 7.5]	4.8 ^{ab} [2.7, 8.3]	7.9 ^a [5.3, 11.7]	6.4 ^{ab} [4.3, 9.6]	9.1 ^{ab} [4.4, 18.2]	3.9 ^{ab} [1.4, 10.4]	3.8 ^{ab} [1.7, 8.2]	8.3 ^{ab} [4.8, 14.1]	0.84 ⁺	0.36 ⁺	0.92 ⁺
	3.9 (10/258)	4.5 (12/268)	10.3 (50/484)	7.3 (36/495)	7.0 (7/100)	3.5 (4/114)	4.9 (10/204)	7.9 (15/190)			

68 ^{a-d}Means within a variable with different superscripts differ ($P \leq 0.05$) based on the LSD post hoc mean separation test.

69 *None of the interactions tested were significant. All had $P > 0.10$.

70 ‡ Significant Farm \times Type of semen \times Treatment interaction on P/AI at pregnancy confirmation ($P = 0.03$).

71 ⁺ Significant Farm \times Type of semen \times Treatment interaction on pregnancy loss ($P = 0.03$).

Supplemental Table S6. Pregnancies per AI (P/AI) at first pregnancy evaluation and reconfirmation of pregnancy, and pregnancy loss after first service for primiparous lactating cows synchronized with the Double-Ovsynch protocol that were randomized to receive the last GnRH before AI at 56 h (G56 treatment) or 80 h (G80 treatment) after induction of luteolysis during the Breeding-Ovsynch portion of the protocol. Models included the effect of treatment, estrus status and type of AI, farm, type of semen, health disorder group, and the interaction between treatment and estrus status and type of AI. Data are presented as unadjusted means (%) and LSM [95% CI].

Outcome and variable	Level	n	Unadjusted mean, % (n)	LSM [95% CI], %	P-value
P/AI first pregnancy evaluation					
Treatment	G56	733	55.8 (409)	54.5 [46.8, 62.0]	0.44
	G80	736	60.2 (443)	57.7 [52.8, 62.4]	
Estrus Status- Type of AI	E-AIE	348	61.5 (214)	55.7 ^{ab} [44.9, 65.9]	< 0.001
	E-TAI	590	62.7 (370)	64.9 ^a [60.1, 69.4]	
	NE-TAI	531	50.5 (268)	47.4 ^b [41.6, 53.2]	
Farm	A	1,066	54.7 (583)	48.8 [43.7, 53.9]	< 0.001
	B	403	66.8 (269)	63.2 [56.9, 69.0]	
Type of semen ¹	Sex-sorted	840	55.2 (464)	51.9 [46.5, 57.3]	0.002
	Conventional beef	629	61.7 (388)	60.2 [54.5, 65.7]	
Health disorder ³	Yes	232	53.5 (124)	52.8 [45.4, 60.1]	0.07
	No	1,237	58.9 (728)	59.4 [54.9, 63.7]	
Treatment × Estrus Status- Type of AI	G56 E-AIE	25	44.0 (11)	46.3 ^{bc} [22.7, 65.9]	< 0.001
	G56 E-TAI	298	59.4 (177)	61.7 ^{ab} [55.4, 67.7]	
	G56 NE-TAI	410	53.9 (221)	55.3 ^b [49.6, 60.9]	
	G80 E-AIE	323	62.9 (323)	64.7 ^{ab} [58.6, 70.3]	
	G80 E-TAI	292	66.1 (193)	67.9 ^a [61.8, 73.4]	
	G80 NE-TAI	121	38.8 (47)	39.6 ^c [30.9, 49.0]	
P/AI confirmation of pregnancy					
Treatment	G56	724	54.3 (393)	52.6 [44.9, 60.1]	0.60

	G80	729	57.8 (421)	54.8 [49.9, 59.6]	
Estrus Status- Type of AI	E-AIE	346	58.7 (203)	53.2 ^{ab} [42.5, 63.6]	< 0.001
	E-TAI	583	60.6 (353)	61.8 ^a [57.0, 66.5]	
	NE-TAI	524	49.2 (258)	45.8 ^b [40.1, 51.7]	
Farm	A	1,053	53.9 (451)	47.3 [42.3, 52.4]	< 0.001
	B	400	58.9 (363)	59.9 [53.6, 66.0]	
Type of semen	Sex-sorted	837	53.9 (451)	50.4 [45.0, 55.8]	0.02
	Conventional beef	616	58.9 (363)	57.0 [51.2, 62.6]	
Health disorder	Yes	230	50.9 (117)	50.0 [42.7, 57.3]	0.04
	No	1,223	57.0 (697)	57.3 [52.8, 61.7]	
Treatment × Estrus Status- Type of AI	G56 E-AIE	25	44.0 (11)	45.3 ^{bc} [27.0, 65.0]	0.006
	G56 E-TAI	293	58.0 (170)	59.5 ^{ab} [53.0, 65.6]	
	G56 NE-TAI	406	52.2 (212)	52.8 ^b [47.1, 58.4]	
	G80 E-AIE	321	59.8 (192)	60.9 ^{ab} [54.7, 66.7]	
	G80 E-TAI	290	63.1 (183)	64.1 ^a [57.9, 69.9]	
	G80 NE-TAI	118	39.0 (46)	39.1 ^c [30.3, 48.5]	
Pregnancy loss ⁴ Treatment	G56	406	3.2 (13)	-	-
	G80	438	3.9 (17)	-	-
Estrus Status- Type of AI	E-AIE	212	4.3 (9)	-	-
	E-TAI	366	3.6 (13)	-	-
	NE-TAI	266	3.0 (8)	-	-
Farm	A	577	3.1 (18)	-	-
	B	267	4.5 (12)	-	-
Type of semen	Sex-sorted	463	2.6 (12)	-	-
	Conventional beef	381	4.7 (18)	-	-
Treatment × Estrus Status- Type of AI	G56 E-AIE	11	0.0 (0)	-	-
	G56 E-TAI	175	2.9 (5)	-	-
	G56 NE-TAI	220	3.6 (8)	-	-
	G80 E-AIE	201	4.5 (9)	-	-
	G80 E-TAI	191	4.2 (8)	-	-
	G80 NE-TAI	46	0.0 (0)	-	-

^{a-c}Means within a column for a variable with different superscripts differed ($P \leq 0.05$) based on the LSD post hoc mean separation test.

¹Type of semen = category assigned based on the type of semen used for first service (sex-sorted or conventional beef semen).

²Categorical variable (Estrus Status-Type of AI) with three levels (E-AIE, E-TAI and NE-TAI) created to indicate expression of estrus (i.e., estrus vs. no estrus) and type of service (i.e., AIE or TAI).

³Cows that had at least one of the following health disorders diagnosed between parturition to the day of the first insemination: milk fever, retained placenta, metritis, mastitis, displaced abomasum, indigestion, ketosis, and pneumonia.

⁴P-values not reported because statistical models did not run as no observations were available for the outcome of interest for some groups.

Supplemental Table S7. Pregnancies per AI (P/AI) at first pregnancy evaluation and reconfirmation of pregnancy, and pregnancy loss after first service for multiparous lactating cows synchronized with the Double-Ovsynch protocol that were randomized to receive the last GnRH before AI at 56 h (G56 treatment) or 80 h (G80 treatment) after induction of luteolysis during the Breeding-Ovsynch portion of the protocol. Models included the effect of treatment, estrus status and type of AI, farm, type of semen, health disorder group, and the interaction between treatment and estrus status and type of AI. Data are presented as unadjusted means (%) and LSM [95% CI].

Outcome and variable	Level	n	Unadjusted mean, % (n)	LSM [95% CI], %	P-value
P/AI first pregnancy evaluation					
Treatment	G56	1,458	44.1 (643)	49.1 [42.4, 55.9]	0.30
	G80	1,442	43.8 (631)	45.5 [42.1, 48.9]	
Estrus Status- Type of AI ¹	E-AIE	399	51.4 (205)	50.8 ^a [41.3, 60.3]	< 0.001
	E-TAI	817	52.1 (426)	54.9 ^a [50.6, 59.1]	
	NE-TAI	1,684	38.2 (643)	36.5 ^b [33.4, 39.7]	
Farm	A	2,246	41.4 (930)	40.8 [37.1, 44.6]	< 0.001
	B	654	52.6 (344)	53.9 [48.5, 59.1]	
Type of semen ²	Sex-sorted	712	39.5 (281)	45.2 [40.1, 50.5]	0.07
	Conventional beef	2,188	45.4 (993)	49.4 [45.4, 53.4]	
Health disorder ³	Yes	747	40.7 (304)	49.4 [45.3, 50.3]	0.07
	No	2,153	45.1 (970)	45.2 [40.2, 50.3]	
AI Season ⁴	Cold	1,486	46.8 (686)	49.8 [45.2, 54.4]	0.01
	Warm	1,414	40.9 (578)	44.8 [40.5, 49.2]	
Treatment × Estrus Status- Type of AI	G56 E-AIE	31	45.2 (14)	47.6 ^{ab} [30.7, 65.1]	0.06
	G56 E-TAI	253	54.9 (139)	57.1 ^a [50.6, 63.5]	
	G56 NE-TAI	1,174	41.7 (490)	42.7 ^b [39.3, 46.2]	
	G80 E-AIE	368	51.9 (191)	54.0 ^a [48.4, 59.6]	
	G80 E-TAI	564	50.9 (287)	52.6 ^a [48.0, 57.1]	
	G80 NE-TAI	510	30.0 (153)	30.8 ^b [26.6, 35.3]	

P/AI confirmation of pregnancy					
Treatment	G56	1,440	40.0 (576)	45.6 [38.9, 52.5]	0.31
	G80	1,424	40.7 (579)	42.0 [38.7, 45.4]	
Estrus Status- Type of AI	E-AIE	397	46.6 (185)	47.2 ^a [37.7, 57.0]	< 0.001
	E-TAI	800	48.9 (391)	51.4 ^a [47.0, 55.7]	
	NE-TAI	1,667	34.7 (579)	33.5 ^b [30.4, 36.7]	
Farm	A	2,220	37.8 (838)	37.3 [33.6, 41.1]	< 0.001
	B	644	49.2 (317)	50.6 [45.2, 56.0]	
Type of semen	Sex-sorted	703	36.4 (256)	42.2 [37.0, 47.5]	0.14
	Conventional beef	2,161	41.6 (899)	45.5 [41.5, 49.6]	
Health disorder	Yes	737	36.4 (268)	41.2 [36.2, 46.3]	0.02
	No	2,127	41.7 (887)	46.5 [42.4, 50.7]	
AI Season	Cold	1,474	43.1 (635)	46.3 [41.7, 51.0]	0.01
	Warm	1,390	37.4 (520)	41.4 [37.1, 45.9]	
Treatment × Estrus Status- Type of AI	G56 E-AIE	30	43.3 (13)	45.4 [27.0, 65.0]	0.18
	G56 E-TAI	247	51.0 (126)	53.3 [53.0, 65.6]	
	G56 NE-TAI	1,163	37.6 (437)	38.4 [47.1, 58.4]	
	G80 E-AIE	367	46.9 (172)	49.0 [54.7, 66.7]	
	G80 E-TAI	553	47.9 (265)	49.5 [57.9, 69.9]	
	G80 NE-TAI	504	28.2 (142)	28.9 [30.3, 48.5]	
Pregnancy loss					
Treatment	G56	640	10.0 (64)	7.8 [3.9, 15.3]	0.82
	G80	629	8.0 (50)	7.2 [5.0, 10.2]	
Estrus Status- Type of AI	E-AIE	205	9.8 (20)	7.8 [2.8, 20.0]	0.89
	E-TAI	423	7.6 (32)	7.0 [4.6, 10.4]	
	NE-TAI	641	9.7 (62)	7.8 [5.3, 11.4]	
Farm	A	928	9.7 (90)	9.1 [6.1, 13.3]	0.10
	B	341	7.0 (24)	6.2 [3.6, 10.6]	
Type of semen	Sex-sorted	277	7.6 (21)	6.7 [3.8, 11.4]	0.33
	Conventional beef	992	9.4 (93)	8.4 [5.6, 12.5]	
Health disorder	Yes	302	11.3 (34)	8.9 [5.4, 14.3]	0.09
	No	967	8.3 (80)	6.3 [4.1, 9.7]	
Treatment × Estrus Status- Type of AI	G56 E-AIE	14	7.1 (1)	6.9 [0.9, 36.5]	0.58
	G56 E-TAI	137	8.0 (11)	7.1 [3.8, 12.9]	
	G56 NE-TAI	489	8.1 (52)	9.9 [7.0, 13.7]	
	G80 E-AIE	191	10.0 (19)	8.9 [5.4, 14.4]	
	G80 E-TAI	286	7.3 (21)	6.8 [4.3, 10.7]	

^{a-b}Means within a column for a variable with different superscripts differed ($P \leq 0.05$) based on the LSD post hoc mean separation test.

¹Categorical variable (Estrus Status-Type of AI) with three levels (E-AIE, E-TAI and NE-TAI) created to indicate expression of estrus (i.e., estrus vs. no estrus) and type of service (i.e., AIE or TAI).

²Type of semen = category assigned based on the type of semen used for first service (sex-sorted or conventional beef semen).

³Cows that had at least one of the following health disorders diagnosed between parturition to the day of the first insemination: milk fever, retained placenta, metritis, mastitis, displaced abomasum, indigestion, ketosis, and pneumonia.

⁴AI season = inseminations performed from May to September were considered to occur during the warm season whereas insemination from October to April were considered to occur during the cold season.

Supplemental Table S8. Pregnancy per AI (P/AI) at first pregnancy evaluation and reconfirmation of pregnancy, and pregnancy loss after first service for cows that had or did not have automated estrus alerts before insemination for lactating dairy cows synchronized with the Double-Ovsynch protocol that were randomized to receive the last GnRH before AI at 56 h (G56 treatment) or 80 h (G80 treatment) after induction of luteolysis during the Breeding-Ovsynch portion of the protocol. Data are presented as unadjusted means (%) and LSM [95% CI]. Effects covariates that were forced in models (parity, farm) or significant, and significant interactions with treatment are also reported.

Outcome and variable	Level	n	Unadjusted mean, % (n)	LSM [95% CI], %	P-value
P/AI first pregnancy evaluation					
Estrus before AI	Yes	2,154	56.4 (1,215)	60.1 [57.3, 62.8]	< 0.001
	No	2,215	41.1 (911)	44.0 [41.3, 46.9]	
Parity	Primiparous	1,469	58.0 (852)	58.3 [55.3, 61.3]	< 0.001
	Multiparous	2,900	43.9 (1,274)	45.9 [43.4, 48.3]	
Farm	A	3,312	45.7 (1,513)	45.5 [43.3, 47.7]	< 0.001
	B	1,057	58.0 (613)	58.7 [55.4, 61.9]	
Type of semen ¹	Sex-sorted	1,552	48.0 (745)	49.2 [46.3, 52.2]	0.002
	Conventional beef	2,817	49.0 (1,381)	55.0 [52.5, 57.5]	
AI season ²	Cold	2,149	50.7 (1,089)	54.0 [51.5, 57.0]	0.008
	Warm	2,220	46.7 (1,037)	50.0 [47.5, 52.6]	
Health disorder ³	Yes	979	43.7 (428)	49.8 [46.3, 53.2]	0.01
	No	3,390	50.1 (1,698)	54.5 [52.4, 56.5]	
Estrus before AI × Health disorder	Estrus-Yes	449	54.6 (245)	59.5 ^a [54.7, 64.1]	0.07
	Estrus-No	1,705	56.9 (970)	60.7 ^a [54.7, 64.1]	
	No estrus-Yes	530	34.5 (183)	40.1 ^b [35.6, 44.7]	
	No estrus -No	1,685	43.2 (728)	48.1 ^c [45.4, 50.8]	
P/AI confirmation of pregnancy					
Estrus before AI	Yes	1,453	56.0 (814)	57.0 [54.1, 59.8]	< 0.001
	No	2,864	40.3 (1,155)	40.8 [38.0, 43.7]	
Parity	Primiparous	3,273	42.7 (1,397)	55.8 [52.7, 58.8]	< 0.001
	Multiparous	1,044	54.8 (572)	42.0 [39.6, 44.5]	
Farm	A	1,540	45.9 (707)	42.4 [40.3, 44.6]	< 0.001

	B	2,777	45.4 (1,262)	55.4 [52.0, 58.6]	
Type of semen	Sex-sorted	2,134	47.5 (1,014)	46.5 [43.5, 49.6]	0.008
	Conventional beef	2,183	43.8 (955)	51.2 [48.6, 53.7]	
AI season	Cold	967	39.8 (385)	51.0 [48.2, 53.8]	0.008
	Warm	3350	47.3 (1,584)	46.8 [44.2, 49.4]	
Health disorder	Yes	444	51.1 (227)	45.9 [42.4, 49.5]	0.002
	No	1,682	53.8 (905)	51.8 [49.8, 53.9]	
Estrus before AI × Health disorder	Estrus-Yes	523	30.2 (158)	56.3 ^a [51.4, 61.0]	0.02
	EstrusNo	1,668	40.7 (679)	57.7 ^a [55.1, 60.3]	
	No estrus-Yes	1,453	56.0 (814)	35.9 ^c [31.5, 40.5]	
	No estrus-No	2,864	40.3 (1,155)	45.9 ^b [43.2, 48.7]	
Pregnancy loss					
AEA before AI	Yes	1,206	6.1 (74)	6.1 [4.6, 8.1]	0.46
	No	907	7.7 (70)	5.3 [3.8, 7.4]	
Parity	Primiparous	844	3.6 (30)	3.8 [2.6, 5.6]	< 0.001
	Multiparous	1,269	8.9 (114)	8.4 [6.5, 10.7]	
Farm	A	1,505	7.2 (108)	6.3 [4.9, 8.1]	0.28
	B	608	5.9 (36)	5.1 [3.6, 7.3]	
Type of semen	Sex-sorted	740	4.5 (33)	4.8 [3.3, 6.8]	0.08
	Conventional beef	1,373	8.1 (111)	6.8 [5.2, 8.8]	
Health disorder	Yes	425	9.4 (40)	6.7 [4.7, 9.5]	0.05
	No	1,688	6.2 (104)	4.8 [3.7, 6.2]	
Milk yield tertile ⁴	Low	672	7.3 (49)	5.9 [4.2, 8.2]	0.75
	Medium	767	7.0 (54)	6.0 [4.3, 8.3]	
	High	674	6.1 (41)	5.2 [3.6, 7.4]	
Estrus before AI × Farm	Estrus-Farm A	898	5.8 (52)	5.3 ^{ab} [3.9, 7.2]	0.01
	Estrus-Farm B	308	7.1 (22)	7.0 ^{ab} [4.5, 10.7]	
	No estrus-Farm A	607	9.2 (56)	7.4 ^a [5.4, 10.2]	
	No estrus- Farm B	300	4.7 (14)	3.7 ^b [2.1, 6.4]	
Estrus before AI × Milk yield tertile	Estrus-Low	411	5.4 (22)	4.9 [3.1, 7.6]	0.09
	Estrus-Medium	440	6.8 (30)	7.2 [4.8, 10.5]	
	Estrus-High	355	6.2 (22)	6.5 [4.1, 10.0]	
	No estrus-Low	261	10.3 (27)	7.0 [4.5, 10.9]	
	No estrus-Medium	327	7.3 (24)	5.0 [3.1, 8.0]	
	No estrus-High	319	6.0 (19)	4.1 [2.5, 6.9]	

^{a-c}Means within a column for a variable with different superscripts differed ($P \leq 0.05$) based on the LSD post hoc mean separation test.

¹Type of semen = category assigned based on the type of semen used for first service (sex-sorted or conventional beef semen).

²AI season = inseminations performed from May to September were considered to occur during the warm season whereas insemination from October to April were considered to occur during the cold season.

³Cows that had at least one of the following health disorders diagnosed between parturition to the day of the first insemination: milk fever, retained placenta, metritis, mastitis, displaced abomasum, indigestion, ketosis, and pneumonia.

⁴Milk yield tertiles were based on accumulated milk yield up to 100 DIM; for primiparous (medium tertile lower limit = 3,336.9 kg and upper limit = 3,822.7 kg) and multiparous cows (medium tertile lower limit = 4,930.4 kg and upper limit = 5,493.4 kg) in Farm A and primiparous (medium tertile lower limit = 3,436.8 kg and upper limit = 3,827.2 kg) and multiparous (medium tertile lower limit = 4,599.0 kg and upper limit = 5,152.9 kg) in Farm B.

Supplemental Table S9. Ovarian responses at the time of the first GnRH (**GnRH1**) and the first PGF2 α (**PGF2-L**) of the Breeding-Ovsynch portion of the Double-Ovsynch protocol for lactating dairy cows synchronized with the Double-Ovsynch protocol that were randomized to receive the last GnRH before AI at 56 h (G56 treatment) or 80 h (G80 treatment) after induction of luteolysis during the Breeding-Ovsynch portion of the protocol. Data are presented as least squares means and SEM or 95% CI.

Item	Treatment		P-value
	G56 (n = 179)	G80 (n = 178)	
GnRH1			
Largest follicle ¹ , mm	15.0 \pm 0.2	14.8 \pm 0.2	0.47
Follicle \geq 10 mm, % (n)	99.4 (178)	99.4 (177)	0.95
Putative cystic cow ² , %	5.4 [2.9; 10.1]	5.7 [3.1; 10.4]	0.90
Cows with CL ³ , %	96.3 [92.1, 98.3]	96.0 [91.7, 98.3]	0.90
Total luteal volume, mm ³	8,221 \pm 291	7,563 \pm 293	0.11
Functional CL ⁴ , %	93.3 [88.3, 96.3]	93.8 [89.0, 96.6]	0.83
P4 ⁵ , ng/mL	2.54 \pm 0.09	2.59 \pm 0.09	0.72
P4 functional CL ⁶ , ng/mL	2.69 \pm 0.09	2.76 \pm 0.09	0.60
Ovulatory follicle, mm	14.7 \pm 0.2	14.6 \pm 0.2	0.70
Ovulatory risk, %	68.3 [61.0, 74.8]	71.5 [64.4, 77.7]	0.51
Double ovulation, %	3.0 [1.3, 6.9]	2.6 [1.0, 6.3]	0.81
PGF-L			
Largest follicle, mm	13.9 \pm 0.3	13.6 \pm 0.3	0.35
Cows with CL, % (n)	97.2 (174)	98.3 (175)	0.72
Total luteal volume, mm ³	10,496 \pm 374	10,380 \pm 378	0.83
Functional CL, %	93.6 [88.8, 96.4]	93.3 [88.3, 96.2]	0.89
P4, ng/mL	4.63 \pm 0.16	4.79 \pm 0.16	0.49
P4 functional CL, ng/mL	4.93 \pm 0.14	5.14 \pm 0.14	0.28

¹Largest follicle, mm = diameter of the largest follicle present at the time of ovulation. Only includes follicles \leq 25 mm.

²Putative cystic cow = percentage of cows that presented \geq 1 follicle with an average diameter $>$ 25 mm.

³Cows with CL = percentage of cows with a corpus luteum (**CL**) present confirmed by transrectal ultrasonography.

⁴Functional CL = percentage of cows that presented \geq 1 CL and circulating progesterone concentration (**P4**) \geq 1 ng/mL.

⁵P4 = circulating progesterone concentration of all cows.

⁶P4 functional CL = circulating progesterone concentration of cows that presented a functional CL.

Supplemental Table S10. Ovarian responses, body condition score, and uterine features of primiparous and multiparous cows at the time of the first GnRH (**GnRH1**), the first PGF_{2α} (**PGF-L**) and the second GnRH (**GnRH2**) before TAI, and 6 d after AI (**6d after AI**) for lactating dairy cows synchronized with the Double-Ovsynch protocol that were randomized to receive the last GnRH before AI at 56 h (G56 treatment) or 80 h (G80 treatment) after induction of luteolysis during the Breeding-Ovsynch portion of the protocol. Cows AIE were removed from the analyses at GnRH2 and 6 d after AI. Data are presented as least squares means and SEM or 95% CI.

Item	Parity		P-value
	Primiparous (n = 149)	Multiparous (n = 208)	
GnRH1			
Largest follicle ¹ , mm	14.7 ± 0.2	15.1 ± 0.2	0.18
Follicle ≥ 10 mm, % (n)	100.0 (149)	99.0 (206)	0.51
Putative cystic cow ² , %	3.3 [1.4, 7.8]	9.1 [5.9, 13.9]	0.04
Cows with CL ³ , %	98.0 [93.9, 99.4]	92.8 [88.4, 95.6]	0.04
Total luteal volume, mm ³	7,537 ± 313	8,248 ± 272	0.09
Functional CL ⁴ , %	96.6 [92.2, 98.6]	88.0 [82.8, 91.8]	0.007
P4 ⁵ , ng/mL	2.87 ± 0.10	2.25 ± 0.09	< 0.001
P4 functional CL ⁶ , ng/mL	2.96 ± 0.10	2.69 ± 0.09	< 0.001
Ovulatory follicle, mm	14.4 ± 0.2	15.0 ± 0.2	0.06
Ovulatory risk, %	74.4 [66.8, 80.8]	65.0 [58.3, 71.2]	0.06
Double ovulation, %	2.0 [0.6, 6.1]	3.8 [1.9, 7.5]	0.34
PGF-L			
Largest follicle, mm	13.5 ± 0.3	14.0 ± 0.3	0.19
Cows with CL, % (n)	100.0 (149)	96.2 (200)	0.02
Total luteal volume, mm ³	10,359 ± 405	10,517 ± 349	0.77
Functional CL, %	96.0 [91.3, 98.2]	89.4 [84.4, 92.9]	0.03
P4 ng/mL	5.26 ± 0.17	4.15 ± 0.14	< 0.001
P4 functional CL, ng/mL	5.46 ± 0.15	4.61 ± 0.13	< 0.001
GnRH2			
Body condition score	3.5 ± 0.0	3.2 ± 0.0	< 0.001
Endometrial thickness, mm	9.7 ± 0.1	9.9 ± 0.1	0.33
Uterine tone score	2.9 ± 0.2	2.7 ± 0.1	0.16
Cows with uterine tone, %	69.3 [60.4, 77.0]	61.8 [54.5, 68.6]	0.18
Uterine fluid score	0.7 ± 0.1	0.6 ± 0.1	0.22
Cows with uterine fluid, %	38.1 [31.2, 45.3]	47.4 [38.4, 56.5]	0.11
Putative cystic cow, %	3.4 [1.3, 8.7]	7.0 [4.1, 11.7]	0.20
Luteal regression ⁶ , %	98.3 [93.3, 99.6]	96.5 [92.3, 98.4]	0.37

P4, ng/mL	0.15 ± 0.15	0.21 ± 0.77	0.15
P4 CL regressed ⁷ , ng/mL	0.12 ± 0.06	0.11 ± 0.05	0.03
Ovulatory risk, %	98.3 [93.4, 99.6]	89.8 [84.5, 93.4]	0.01
Double ovulation, %	2.4 [1.3, 8.7]	5.3 [2.9, 9.7]	0.43
6d after AI			
Cows with CL, %	97.6 [92.3, 99.3]	94.1 [89.6, 96.7]	0.19
Total luteal volume, mm ³	7,331 ± 346	7,565 ± 281	0.60
Functional CL, %	96.4 [91.3, 98.5]	80.6 [73.9, 85.9]	< 0.001
P4, ng/mL	2.15 ± 0.07	1.55 ± 0.06	< 0.001
P4 functional CL, ng/mL	2.23 ± 0.06	1.81 ± 0.06	< 0.001
P/AI ⁸ , % (n/n)	65.3 (96/147)	45.3 (92/203)	-

¹Largest follicle, mm = diameter of the largest follicle present at the time of ovulation. Only includes follicles ≤ 25 mm.

²Putative cystic cow = percentage of cows that presented ≥ 1 follicle with an average diameter > 25 mm.

³Cows with CL = percentage of cows with a corpus luteum (CL) present as confirmed by transrectal ultrasonography.

⁴Functional CL = percentage of cows that presented ≥ 1 CL and circulating progesterone concentration (P4) ≥ 1 ng/mL.

⁵P4 = circulating progesterone concentration of all cows.

⁶Luteal regression = percentage of cows that had a functional CL at PGF-L and P4 ≤ 0.5 ng/mL at GnRH2.

⁷P4 functional CL = circulating progesterone concentration for cows that presented a functional CL.

⁸P4 CL regressed = circulating progesterone concentration for cows that had a complete luteal regression (P4 ≤ 0.5 ng/mL).

⁹P/AI = percentage of cows pregnant after first service at the first pregnancy evaluation. Presented as unadjusted means.