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Effects of vaccination against reproductive diseases on reproductive performance of beef cows submitted to fixed-timed AI in Brazilian cow-calf operations

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ABSTRACT

The objectives were to assess incidence of pregnancy losses, associate this outcome with immunization programs against reproductive diseases, and evaluate the effects of vaccination against bovine herpesvirus-1 (BoHV-1), bovine viral diarrhea virus (BVDV), and *Leptospira* spp., on reproductive efficiency of Brazilian cow-calf operations. In experiment 1, 7614 lactating Nelore cows from 18 ranches were assigned to the same estrus synchronization and fixed-time AI protocol (ESFTAI; Days −11 to 0). Pregnancy status was determined with transrectal ultrasonography on Days 30 and 120 after AI. Pregnancy loss was deemed to have occurred when cows were pregnant on Day 30 but nonpregnant on Day 120. Incidence of pregnancy loss across all ranches was 4.1%; pregnancy losses were detected ($P < 0.10$) in 14 ranches but not detected ($P > 0.11$) in four ranches. Pregnancy loss was lower ($P \leq 0.02$) in ranches that vaccinated against BoHV-1, BVDV, and *Leptospira* spp. compared with ranches that did not vaccinate, or only vaccinated against *Leptospira* spp. In experiments 2 and 3, lactating Nelore cows ($N = 1950$ and 2793, respectively) from ranches that did not have a history of vaccinating against reproductive diseases (experiment 2), or only vaccinated against *Leptospira* spp. (experiment 3), were assigned to the same ESFTAI used in experiment 1. Within each ranch, cows received (VAC) or not (CON) vaccination against BoHV-1, BVDV, and *Leptospira* spp. at the beginning of the ESFTAI (Day −11) and 30 days after (Day 41) AI. In experiment 2, VAC cows had greater ($P \leq 0.05$) pregnancy rates compared with CON on Days 30 and 120. In experiments 2 and 3, pregnancy loss was reduced ($P \leq 0.03$) in primiparous VAC cows compared with CON cohorts. In experiment 4, 367 primiparous, lactating Nelore cows previously vaccinated against *Leptospira* spp. were assigned to the same ESFTAI used in experiment 1. Cows received VAC, or the same vaccine 30 days before (Day −41) and at the beginning (Day −11) of the ESFTAI (PREVAC). Pregnancy rates on Days 30 and 120 were greater ($P \leq 0.05$) in PREVAC cows compared with VAC cows. In conclusion, pregnancy losses affected reproductive and overall efficiency of Brazilian cow-calf operations, and might be directly associated with BoHV-1, BVDV, and *Leptospira* spp. infections. Hence, vaccinating cows against these pathogens, particularly when both doses are administered before fixed-time AI, improved reproductive performance in Brazilian cow-calf systems.

1. Introduction

Reproductive failure is considered the main economic loss for beef cow-calf producers worldwide [1,2]. Geary [3]

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estimated economic losses because of embryonic and fetal losses in the US beef industry to be more than $1.2 billion yearly, with approximately 40×10^6 cattle exposed to breeding. Brazil currently exposes more than 90×10^6 beef females to breeding yearly [4]; hence, economical losses associated with embryonic death are also of great concern to the Brazilian beef industry. Approximately 37% to 50% of pregnancy losses in cattle are associated with infectious diseases, such as infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD), and leptospirosis [5,6]. More specifically, the bovine herpesvirus-1 (BoHV-1) that causes IBR is known to directly impair ovarian function and embryo quality [7,8]. The BVD virus (BVDV) infects reproductive tissues and interferes with follicular and embryo development [9,10], whereas *Leptospira* spp. infection is known to cause fetal death, abortions, and infertility [11]. Seroprevalence for BoHV-1, *Leptospira* spp., and the BVDV, as well as the incidence of IBR, leptospirosis, and BVD, are relatively high in commercial cow-calf herds in Brazil [12–14], suggesting that reproductive diseases have a major effect on reproductive and overall efficiency of the Brazilian cow-calf industry.

Management techniques to prevent pregnancy loss, such as breeding techniques, hormonal manipulation, and nutritional management, are increasingly being implemented in Brazil and other countries [3]. However, development of immunization strategies to reduce the effects of reproductive diseases, for example, vaccination against IBR, leptospirosis, and BVD, often receive less attention [15]. Furthermore, few research studies have directly evaluated the effects of such vaccination programs on reproductive efficiency in beef cattle. Hence, the objectives of the present study were to evaluate the adoption of vaccination programs against IBR, BVD, and leptospirosis on pregnancy rates and pregnancy losses in Brazilian cow-calf operations.

2. Materials and methods

All experiments described herein were conducted in commercial cow-calf ranches located in Mato Grosso do Sul and Mato Grosso, Brazil. All animals were cared for in accordance with the practices outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching [16]. Within each ranch, cows were managed similarly independent of vaccination treatments, following existing nutritional, reproductive, and immunization procedures for each ranch.

2.1. Experiment 1

Based on the lack of information regarding the incidence of pregnancy losses in Brazilian cow-calf herds, the objective was to evaluate pregnancy losses between Days 30 and 120 of gestation in beef cows assigned to fixed-time AI. Additionally, the reduction of pregnancy losses in herds with vaccination programs against reproductive diseases was evaluated. A total of 7614 lactating, multiparous Nelore cows between 50 to 80 days postpartum, originated from 18 different cow-calf ranches, were used. Each ranch adopted different strategies to control reproductive diseases: (1) no vaccination against reproductive diseases (NOIM; 13 ranches; N = 6203), (2) biannual vaccination against leptospirosis (LEPTO; two ranches; N = 738), or (3) biannual vaccination against leptospirosis and annual vaccination against IBR and BVD (ALL; three ranches; N = 673).

All cows were assigned to the following estrus synchronization protocol: estradiol benzoate (2 mg im of Estrogin; Farmavet, São Paulo, SP, Brazil) and insertion of an intravaginal progesterone releasing device (CIDR; containing 1.9 g of progesterone; Pfizer Animal Health, São Paulo, SP, Brazil) on Day −11, $PGF_{2\alpha}$ treatment (12.5 mg im of Dinoprost; Pfizer Animal Health) on Day −4, estradiol cypionate treatment (0.5 mg im of ECP; Pfizer Animal Health) in addition to CIDR and calf removal on Day −2, followed by fixed-time AI and calf return on Day 0 [17].

Cow body condition score (BCS) was recorded at AI [18]. Pregnancy status was verified by detecting a fetus via transrectal ultrasonography (Aloka SSD 500 with a 7.5 MHz linear-array transrectal transducer; Tokyo, Japan) on Days 30 and 120 after AI. Any cow diagnosed as pregnant on Day 30 and then nonpregnant on Day 120 was designated as having undergone pregnancy loss.

2.2. Experiment 2

The objective was to evaluate the effects of vaccination against IBR, BVD, and leptospirosis on pregnancy rates and pregnancy losses in Brazilian cow-calf herds that did not have a history of vaccinating the cow herd against reproductive diseases. A total of 1950 lactating Nelore cows (multiparous, N = 1643; primiparous, N = 307) between 50 and 100 days postpartum, that originated from six commercial cow-calf ranches, were assigned to the same estrus synchronization and fixed-time AI protocol described in experiment 1. Within each ranch, cows were randomly assigned to receive (VAC; N = 953) or not (CON = 1015) vaccination against IBR, BVD, and leptospirosis (5 mL im of CattleMaster 4+L5; Pfizer Animal Health) at the beginning of the estrus synchronization protocol (Day −11) and 30 days after fixed-time AI.

Pregnancy status, BCS, and incidence of pregnancy losses were assessed as in experiment 1. Blood samples were collected from a subsample of CON cows from each ranch (N = 38) on Day 30 after fixed-time AI for determination of seroprevalence against BoHV-1, BVDV, and *Leptospira* spp. Blood samples were randomly collected from an average of six females per ranch (four multiparous and two primiparous cows). Blood samples 10 mL; were collected via coccygeal vein or artery into commercial blood collection tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA), placed on ice immediately, maintained at 4 °C for 24 h, and centrifuged at $3000 \times g$ for 10 min at room temperature for serum collection. Serum was stored at −20 °C until further analyzed. Neutralizing antibodies against BoHV-1 were detected by virus-neutralization techniques in Madin-Darby bovine kidney cells [19]. Neutralizing antibodies against BVDV were detected by virus-neutralization techniques in 100 tissue culture infectious $dose_{50}$ of Los Angeles and NADL BVDV strains [20]. Detection of agglutinant antibodies against leptospirosis was conducted using a microscopic agglutination test [21]. The criteria for seropositive animals were the following titers: ≥8 for BoHV-1, ≥16 for BVDV, and ≥100 for *Leptospira* spp. [19–21].

2.3. Experiment 3

The objective was to evaluate the effects of vaccination against IBR, BVD, and leptospirosis on pregnancy rates and pregnancy losses in Brazilian cow-calf herds that vaccinated the cow herd biannually against leptospirosis (2 mL im of Leptoferm 5; Pfizer Animal Health). A total of 2793 lactating Nelore cows (multiparous, N = 2432; primiparous, N = 361), 50 to 100 days postpartum, that originated from seven commercial cow-calf ranches, were assigned to the same estrus synchronization and fixed-time AI protocol described in experiment 1, and randomly assigned within each ranch to receive VAC (N = 1292) or CON (N = 1501), as described in experiment 2.

Pregnancy status, BCS, and incidence of pregnancy losses were assessed as in experiment 1. On Day 30 after fixed-time AI, blood samples were collected from a subsample of CON cows from each ranch (N = 76) for determination of seroprevalence against BoHV-1, BVDV, and *Leptospira* spp. These samples were randomly collected from an average of 11 females per ranch (eight multiparous and three primiparous). All samples were collected, processed, and analyzed for antibody detection against BoHV-1, BVDV, and *Leptospira* spp. as described in experiment 2.

2.4. Experiment 4

The objective was to determine if the timing of vaccination against reproductive diseases in relation to fixed-time AI would affect pregnancy rates and pregnancy losses. A total of 367 primiparous, lactating Nelore cows, 50 to 100 days postpartum, that originated from the same ranch were assigned to the same estrus synchronization and fixed-time AI protocol described in experiment 1. This ranch vaccinated the herd against leptospirosis biannually (2 mL im Leptoferm 5; Pfizer Animal Health), but not against IBR or BVD. Cows were randomly assigned to receive immunization against IBR, BVD, and leptospirosis (5 mL im CattleMaster 4+L5, Pfizer Animal Health) according to two schedules: (1) 30 days before (Day −41) and at the beginning (Day −11) of the estrus synchronization and fixed-time AI protocol (PREVAC; N = 232), or (2) at the beginning (Day −11) of the estrus synchronization and fixed-time AI protocol and Day 30 after fixed-time AI (VAC = 135). Pregnancy status, BCS, and incidence of pregnancy losses were assessed as in experiment 1. Blood was collected from a subsample of cows (N = 57) on Day −41, before assignment to treatments, processed, and analyzed for antibody detection against BoHV-1, BVDV, and *Leptospira* spp., as described in experiment 2.

2.5. Vaccine

The vaccine used in experiments 2, 3, and 4 (CattleMaster 4+L5, Pfizer Animal Health) was a freeze-dried preparation containing chemically-altered live strains of BoHV-1, inactivated cytopathic and noncytopathic BVDV strains, cultures of five *Leptospira* spp. serovars (L. canicola, L. grippotyphosa, L. hardjo, L. icterohaemorrhagiae, and L. pomona), with the inclusion of aluminum hydroxide as adjuvant. As recommended by the manufacturer (Pfizer Animal Health), cattle naïve to this vaccine should receive two doses (5 mL each) administered at least 4 wk apart, and this recommended method was adopted in experiments 2, 3, and 4.

2.6. Statistical analyses

Pregnancy data were analyzed using the GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC, USA) version 9.2 and Satterthwaite approximation to determine the denominator degrees of freedom for tests of fixed effects. In experiment 1, the model statement used for comparison of pregnancy losses across ranches contained the effects of ranch, in addition to BCS at the time of fixed-time AI as independent covariate. Data were analyzed using cow (ranch) as random variable and error term for tests of fixed effects. The model statement used for comparison of pregnancy losses according to immunization management included the effects of vaccination protocol (NOIM, LEPTO, and ALL), BCS at the time of fixed-time AI as independent covariate, and data were analyzed using cow (ranch by vaccination protocol) as a random variable and error term for tests of fixed effects. In experiments 2 and 3, the model statement for pregnancy rates on Days 30 and 120, as well as pregnancy losses, contained the effects of vaccination treatment (CON vs. VAC), ranch, parity, and all resultant interactions. Data were analyzed using cow (ranch by parity by vaccination treatment) as random variable and error term for tests of fixed effects. In experiment 4, the model statement for pregnancy rates on Days 30 and 120, as well as pregnancy losses, contained the effects of vaccination treatment (PREVAC vs. VAC), whereas data were analyzed using cow (vaccination treatment) as random variable and error term for the tests of fixed effect. Cow BCS at fixed-time AI in experiments 2, 3, and 4 were analyzed using the MIXED procedure of SAS (SAS Institute Inc.), with Satterthwaite approximation and the same models described for pregnancy analysis. Results were reported as least square means and separated using a least square difference, whereas a *t* statistic was used to determine if least square means were different than 0 in experiment 1. For all analyses, significance was set at $P \leq 0.05$, and tendencies were declared if $P > 0.05$ and ≤ 0.10. Results are reported according to treatment effects if no interactions were significant, or according to the highest-order interaction detected.

3. Results

3.1. Experiment 1

At fixed-time AI, cow BCS (± SEM) was 5.18 ± 0.01, and a significant covariate ($P < 0.01$) for analysis of pregnancy losses. Across all ranches, mean pregnancy rates on Days 30 and 120 after fixed-time AI were 46.7% (3555 pregnant and 7614 total cows) and 44.8% (3410 pregnant and 7614 total cows), respectively. Mean incidence of pregnancy loss across all ranches was 4.1% (145 nonpregnancies on Day 120 and 3555 pregnancies on Day 30 after fixed-time AI). Moreover, a ranch effect was detected ($P < 0.01$) for the incidence of pregnancy losses (Table 1). Significant

Table 1
Overall reproductive performance of beef cows assigned to fixed-time AI in Brazilian commercial cow-calf ranches.

Ranch	Vaccination program[a]	Cows, N	Pregnancy status[b]		Pregnancy loss (%)[c]	SEM[d]	P[e]
			30 days	120 days			
1	NOIM	1246	52.7	51.8	1.57	0.83	0.06
2	NOIM	501	49.3	47.1	4.23	1.22	< 0.01
3	NOIM	194	32.9	30.7	5.98	2.29	< 0.01
4	NOIM	347	36.2	33.6	7.06	1.72	< 0.01
5	NOIM	225	40.9	36.8	9.58	2.00	< 0.01
6	NOIM	544	47.1	46.1	1.93	1.34	0.15
7	NOIM	158	53.8	51.9	3.40	2.13	0.11
8	NOIM	287	49.4	44.7	8.93	1.60	< 0.01
9	NOIM	246	28.6	27.6	4.43	2.58	0.08
10	NOIM	561	42.1	39.6	5.86	1.25	< 0.01
11	NOIM	367	59.8	58.3	2.81	1.31	0.03
12	NOIM	1129	42.3	40.9	3.45	1.07	< 0.01
13	NOIM	398	60.8	57.0	6.25	1.25	< 0.01
14	ALL	95	48.2	47.9	0.89	2.76	0.74
15	ALL	293	62.3	60.9	2.40	1.44	0.09
16	ALL	285	46.7	45.9	1.62	1.69	0.34
17	LEPTO	418	28.2	26.1	5.76	1.70	< 0.01
18	LEPTO	320	49.8	47.1	5.52	1.64	< 0.01

Abbreviations: BVD, bovine viral diarrhea; IBR, infectious bovine rhinotracheitis.

[a] Ranches adopted the following vaccination strategies to control reproductive diseases: (1) no vaccination against reproductive diseases (NOIM), (2) biannual immunization against leptospirosis (LEPTO), or (3) biannual vaccination against leptospirosis and annual vaccination against IBR and BVD (ALL).

[b] Pregnancy status was verified by detecting a fetus with transrectal ultrasonography at 30 and 120 days after fixed-time AI. Values are reported as least square means.

[c] Pregnancy loss was considered in cows that were pregnant on Day 30, but nonpregnant on Day 120. Values are reported as least square means.

[d] SEM associated with pregnancy loss.

[e] Probability level of pregnancy loss to differ from 0.

pregnancy losses were detected (different than 0%; $P < 0.05$) in 11 ranches, tendencies (different than 0%; $P < 0.10$) were detected in three ranches, whereas none (different than 0%; $P > 0.11$) were detected in four ranches.

A vaccination protocol effect was also detected ($P = 0.03$; data not shown) for pregnancy loss, given that pregnancy loss was greater ($P \leq 0.02$) in NOIM and LEPTO compared with ALL ranches (4.30%, 5.00%, and 1.60%, respectively; SEM = 0.83), but similar between ($P = 0.55$) NOIM and LEPTO ranches. Further, pregnancy losses were significant in NOIM and LEPTO ranches (different than 0%; $P < 0.01$), but not in ALL ranches (different than 0%; $P = 0.11$).

3.2. Experiment 2

At fixed-time AI, BCS was similar ($P = 0.92$; data not shown) between VAC and CON cows (5.42 vs. 5.43; SEM = 0.08). Results associated with seroprevalence for BoHV-1, BVDV, and *Leptospira* spp. are shown (Table 2). A vaccination treatment effect was detected for pregnancy rates at 30 days ($P = 0.05$) and 120 days ($P = 0.01$) after AI, being greater for VAC cows compared with CON cohorts (Table 3). There was a vaccination treatment by parity interaction for pregnancy loss ($P = 0.01$). Within primiparous cows, pregnancy losses were reduced ($P < 0.01$) in VAC compared with CON cohorts. However, no vaccination treatment effect was detected ($P = 0.39$) for pregnancy losses within multiparous cows (Table 4).

Table 2
Presence of antibodies against bovine herpesvirus-1 (BoHV-1), bovine viral diarrhea virus (BVDV), and *Leptospira* spp. in experiments 2, 3, and 4.

Pathogen	Titer	Experiment 2 (N = 38)	Experiment 3 (N = 76)	Experiment 4 (N = 57)
BoHV-1	Negative (< 8)	31.6	5.2	50.9
	8 to 64	47.3	73.7	42.1
	≥ 64	21.0	21.0	7.1
	Positive (≥ 8)	68.4	94.7	49.1
BVDV	Negative (< 16)	31.6	23.7	50.9
	16 to 64	47.3	63.1	40.0
	≥ 64	21.0	13.1	9.1
	Positive (≥ 16)	68.4	76.3	49.1
Leptospira spp.	Negative (< 100)	35.9	19.5	12.3
	100 to 200	28.2	33.7	28.1
	≥ 200	35.9	46.7	59.6
	Positive (≥ 100)	64.1	80.5	87.7

Samples were collected in experiment 2 from cows without a history of vaccination against BoHV-1, BVDV, and *Leptospira* spp. Samples were collected in experiments 3 and 4 from cows without a history of vaccination against BoHV-1 and BVDV, but receiving vaccination against *Leptospira* spp. biannually. Detection of neutralizing antibodies against BoHV-1 and BVDV was conducted using virus-neutralization techniques [19,20], whereas detection of agglutinant antibodies against *Leptospira* spp. was conducted using microscopic agglutination test [21]. The criteria for seropositive reaction expressed in titers were: ≥8 for IBR, ≥16 for BVD, and ≥100 for leptospirosis (serovar *L. hardjo*) [19–21].

3.3. Experiment 3

At fixed-time AI, BCS was similar ($P = 0.24$; data not shown) between VAC and CON cows (5.76 vs. 5.72;

Table 3
Pregnancy rates 30 and 120 days after fixed-time AI in cows from experiments 2, 3, and 4.

Experiment[a]	Pregnancy status[b]	
	30 days	120 days
Experiment 2		
VAC	55.1 (546/935)	53.5 (532/935)
CON	49.8 (548/1015)	45.9 (523/1015)
SEM	2.8	2.8
P	0.05	0.01
Experiment 3		
VAC	47.3 (599/1292)	46.8 (579/1292)
CON	46.7 (726/1501)	44.7 (692/1501)
SEM	4.8	4.9
P	0.84	0.45
Experiment 4		
PREVAC	55.6 (129/232)	54.7 (127/232)
VAC	45.2 (61/135)	42.9 (58/135)
SEM	3.8	3.8
P	0.05	0.03

Pregnancy rates to fixed-time AI are reported as least square means. Values in parentheses represent number of pregnant cows/total inseminated cows.

[a] In experiment 2 and 3, cows received (VAC) or not (CON) vaccination against infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD), and leptospirosis on Day −11 and Day 30 relative to fixed-time AI (Day 0). In experiment 4, cows received vaccination against IBR, BVD, and leptospirosis (at two different schedules relative to fixed-time AI (Day 0): (1) Day −41 and Day −11 (PREVAC), or (2) Day −11 and Day 30 (VAC = 135). In experiment 3 and 4, cows already received biannual vaccination against leptospirosis.

[b] Pregnancy status was verified by detecting a fetus with transrectal ultrasonography at 30 and 120 days after fixed-time AI.

Table 4
Pregnancy losses after fixed-time AI in primiparous and multiparous cows from experiments 2 and 3.

Experiment[a]	Primiparous	Multiparous
Experiment 2		
VAC	3.53 (1/78)	2.51 (13/468)
CON	13.55 (7/62)	3.52 (18/486)
SEM	2.65	1.33
P	< 0.01	0.40
Experiment 3		
VAC	0.32 (1/69)	3.21 (19/530)
CON	6.95 (6/68)	4.11 (28/658)
SEM	2.71	1.26
P	0.03	0.43

Pregnancy status was verified by detecting a fetus with transrectal ultrasonography at 30 and 120 days after fixed-time AI. Pregnancy loss was considered in cows that were pregnant on Day 30, but nonpregnant on Day 120, and is reported as least square means. Values in parentheses represent number of cows nonpregnant on Day 120/cows pregnant on Day 30.

[a] In experiment 2 and 3, cows received (VAC) or not (CON) vaccination against IBR, BVD, and leptospirosis at Day −11 and Day 30 relative to fixed-time AI (Day 0). Further, cows already received biannual vaccination against leptospirosis in experiment 3.

SEM = 0.13). Results associated with seroprevalence for BoHV-1, BVDV, and *Leptospira* spp. are shown (Table 2). There was no vaccination treatment effect for pregnancy rates at 30 days (P = 0.84) and 120 days (P = 0.45) after AI (Table 3). However, there was a vaccination treatment by parity interaction for pregnancy loss (P = 0.05). Within primiparous cows, pregnancy losses were reduced (P = 0.03) in VAC compared with CON cohorts. However, no vaccination treatment effect was detected (P = 0.43) for pregnancy losses within multiparous cows (Table 4).

3.4. Experiment 4

At fixed-time AI, BCS was similar (P = 0.45; data not shown) between PREVAC and VAC cows (5.46 vs. 5.50, respectively; SEM = 0.04). Results associated with seroprevalence for BoHV-1, BVDV, and *Leptospira* spp. are shown (Table 2). There was a vaccination treatment effect for pregnancy rates at 30 days (P = 0.05) and 120 days (P = 0.03) after AI, being greater for PREVAC cows compared with VAC cohorts (Table 3). No vaccination treatment effect was detected (P = 0.17; data not shown) for pregnancy loss (4.92 vs. 1.55% of pregnancy losses for VAC and PREVAC cows, respectively; SEM = 1.73). However, it was noteworthy that there were pregnancy losses in VAC cows (different than 0%; P = 0.01), but not in PREVAC cows (P = 0.27).

4. Discussion

The specific goal of experiment 1 was to characterize the incidence of pregnancy losses in Brazilian cow-calf systems. Although expected to economically affect the local cow-calf industry [3] and be directly influenced by reproductive diseases [5,6], the incidence of pregnancy losses has not been properly documented in Brazilian herds [4]. Indeed, results from experiment 1 demonstrated that pregnancy losses between Days 30 and 120 of gestation often occurred in Nelore beef cows, with an average of 4.1% across all ranches evaluated herein. Pregnancy losses before Day 30 of gestation also contribute significantly to reproductive failure in beef females, but were not determined in the present study, as transrectal ultrasonography cannot be reliably used as diagnostic tool until Day 26 of gestation [22]. Further, the rate of pregnancy losses varied within the 18 ranches evaluated, being statistically significant in 11 ranches, but not in the remaining seven ranches. Several factors influence pregnancy maintenance in beef cows, including nutritional management and BCS [23–25], which was accounted for in experiment 1 via covariate analysis, as well as reproductive diseases such as IBR, BVD, and leptospirosis [5,6]. Accordingly, the incidence of pregnancy losses was reduced, as well as statistically insignificant, in ranches that vaccinated cattle against IBR, BVD, and leptospirosis compared with ranches that did not vaccinate or only vaccinated against leptospirosis. Hence, results from experiment 1 demonstrated that pregnancy losses are an existing concern within Brazilian cow-calf systems, independent of cow BCS, and might be associated with the immunization program against reproductive diseases adopted by each operation.

In experiments 2, 3, and 4, the majority of CON cows were seropositive for BoHV-1, BVDV, and *Leptospira* spp.; therefore, we inferred that the evaluated herds were indeed exposed to these pathogens. Antibody titers >64 for BoHV-1 and BVDV, and >200 for *Leptospira* spp. indicate active infections, suggesting that IBR, BVD, and leptospirosis were also present in the evaluated herds [13,26,27]. Further, *Leptospira* spp. titers <200 might be induced by vaccination, which might also explain the elevated positive seroprevalence for *Leptospira* spp. in experiments 3 and 4, where ranches adopted biannual vaccination against leptospirosis [13,26,27]. In experiments 2, 3, and 4, cow BCS was similar between vaccination treatments. Hence, all vaccination treatment effects reported and discussed herein seemed independent of cow nutritional status [23].

In experiment 2, cows vaccinated against IBR, BVD, and leptospirosis had greater pregnancy rates on Day 30, which remained greater until Day 120 after fixed-time AI compared with nonvaccinated cohorts. These outcomes supported the known detrimental effects of these diseases to reproductive function of beef cows, and the consequent need for proper immunization programs [5,6]. Furthermore, we inferred that IBR, BVD, and leptospirosis negatively affected fertility parameters and pregnancy maintenance during the first 30 days of gestation [7,8,28], whereas vaccination against these diseases alleviated these outcomes. Nevertheless, infertility and pregnancy losses before Day 30 of gestation were not evaluated in this series of experiments, but also contribute significantly to reproductive and economic losses in beef cow-calf operations [3]. In contrast to our original hypothesis, vaccination only alleviated pregnancy losses from Days 30 to 120 in primiparous cows. Older cattle are known to be less susceptible to IBR, BVD, and leptospirosis by having a greater chance of being exposed to pathogens during their productive lives, hence developing immunological memory against these diseases [29–31]. Based on this rationale, perhaps primiparous cows were susceptible to BoHV-1, BVDV, and *Leptospira* spp. infections until Day 120

of gestation and thus benefited from the vaccination treatment. Conversely, perhaps multiparous cows were capable of controlling infections earlier, which prevented pathogen-stimulated pregnancy losses after Day 30 of gestation by mounting a prompt and robust immune response based on immunological memory.

Differing from experiment 2, no treatment effects were detected on pregnancy rates on Days 30 and 120 in experiment 3, suggesting that *Leptospira* spp. and vaccination against these pathogens had a greater impact on fertility and early pregnancy maintenance compared with BoHV-1 and BVDV [11,28]. Hence, immunization against IBR and BVD did not improve Day 30 pregnancy rates in cows already receiving biannual leptospirosis vaccination. Although pregnancy rates on Day 120 were similar between vaccination treatments, pregnancy losses from Days 30 to 120 were affected by vaccination treatment in primiparous but not in multiparous cows. This outcome was attributed to the same reasons described for experiment 2. Furthermore, we inferred that primiparous cows were susceptible to pregnancy losses caused by BoHV-1 and BVDV until Day 120 of gestation.

Based on results from experiment 4, beginning the vaccination program before estrus synchronization further increased its benefits on pregnancy rates on Days 30 and 120, whereas it reduced, to some extent, pregnancy losses. This outcome was attributed to the profile and timing of antibody responses upon vaccination using the vaccine tested herein. More specifically, vaccination against chemically-altered live strains of BoHV-1 moderately increased antibody titers 14 days after the first dose, which peaked within 96 h after the second dose, and remained elevated for 180 days after the second dose [32,33]. Vaccination against inactivated cytopathic and noncytopathic BVDV strains only increased antibody titers 14 days after the second dose, which also remained elevated for 180 days after the second dose [32,34,35]. Vaccination against the five inactivated *Leptospira* spp. serovars used herein often caused immediate increases in antibody titers after the first dose, and remained elevated for 150 days if the second dose was administered [36]. Hence, cows receiving vaccination on Days −11 and 30 relative to fixed-time AI had elevated antibody titers against *Leptospira* spp., but moderate antibody titers against BoHV-1, and no antibody response against the BVDV during breeding and the initial 30 days of gestation. Conversely, cow vaccination on Days −41 and −11 had elevated antibody titers against *Leptospira* spp., BoHV-1, and BVDV during breeding and the initial 30 days of gestation. Hence, PREVAC cows likely had increased antibody response and immunological protection against reproductive diseases at the period of expected ovulation, fixed-time AI, and early pregnancy compared with VAC cohorts, resulting in the treatment differences detected for pregnancy rates at 30 days. After the second vaccination dose in VAC, antibody response was likely similar between treatments, resulting in the lack of substantial treatment effects on pregnancy losses from Days 30 to 120.

In summary, pregnancy losses from Days 30 to 120 affected reproductive and overall efficiency of Brazilian cow-calf operations. Further, these pregnancy losses might be directly associated with reproductive diseases such as IBR, BVD, and leptospirosis, based on the decreased occurrence of pregnancy losses in herds vaccinated against these diseases, and the elevated incidence of cows that tested seropositive for BoHV-1, BVDV, and *Leptospira* spp. Accordingly, vaccinating cows against BoHV-1, BVDV, and *Leptospira* spp. using a commercial vaccine (CattleMaster 4+L5, Pfizer Animal Health) increased pregnancy rates on Days 30 and 120 after fixed-time AI in herds naïve to BoHV-1, BVDV, and *Leptospira* spp. vaccination. Further, cows should receive both doses of the vaccine before fixed-time AI to ensure maximum antibody response and optimal reproductive outcomes during conception, as well as early- and midgestation.

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