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Effects of vaccination against reproductive diseases on reproductive performance of lactating dairy cows submitted to AI

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ABSTRACT

Four experiments evaluated the effects of vaccination against bovine herpesvirus-1 (BoHV-1), bovine viral diarrhea virus (BVDV), and *Leptospira* spp. on reproductive performance of lactating dairy cows without (experiments 1, 2, and 3) or with previous vaccination against these diseases (experiment 4). Cows were assigned to a fixed-time AI protocol (FTAI; d −11 to 0) in all experiments, as well as AI 12 h upon estrus detection in experiment 3. Pregnancy status was determined with transrectal ultrasonography on d 30 and 71 (d 60 for experiment 3) after AI. Pregnancy loss was considered in cows pregnant on d 30 but non-pregnant on the subsequent evaluation. In experiment 1, 853 cows received (VAC) or not (CON) vaccination against BoHV-1, BVDV, and *Leptospira* spp. at the beginning of the FTAI (d −11) and 30 d after AI. Pregnancy loss was reduced ($P = 0.03$) in VAC cows compared with CON. In experiment 2, 287 cows received VAC or CON 30 d prior to (d −41) and at the beginning (d −11) of the FTAI. Pregnancy rates on d 30 and 71 were greater ($P \leq 0.03$) in VAC cows compared with CON. In experiment 3, 1680 cows with more than 28 d in milk were randomly assigned to receive VAC or CON with doses administered 14 d apart, and inseminated within 15–135 d after the second dose. Pregnancy rates on d 30 and 60 were greater ($P \leq 0.02$) in VAC cows compared with CON. In experiment 4, 820 cows received (REVAC) or not (CON) revaccination against BoHV-1, BVDV, and *Leptospira* spp. at the beginning of the FTAI protocol (d −11). Pregnancy rates and loss were similar ($P \geq 0.54$) between treatments. Hence, vaccinating naïve cows against BoHV-1, BVDV, and *Leptospira* spp. improved reproductive efficiency in dairy production systems, particularly when both doses were administered prior to AI.

1. Introduction

Productivity of dairy cattle systems is highly dependent on reproductive performance of the herd, given that the lactation cycle is initiated and renewed by pregnancy (Lucy, 2001). Reproductive efficiency of dairy herds is substantially impacted by pregnancy losses, whereas more than 50% of dairy cows that conceive lose their pregnancy during the initial 6 weeks of gestation (Santos et al., 2004). Up to 50% of pregnancy losses in cattle are associated with infectious diseases, such as infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD), and leptospirosis (Khodakaram-Tafi and Ikede, 2005; McEwan and Carman, 2005). More specifically, *Leptospira* spp. infection is known to cause fetal death, abortions, and infertility (Mineiro et al., 2007). The BVD virus (BVDV) infects reproductive tissues and interferes with follicular and embryo

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development (Grooms, 2004; Grooms et al., 2007), whereas the bovine herpesvirus-1 (BoHV-1) that causes IBR is known to directly impair ovarian function and embryo quality (Miller and Van der Maaten, 1986; Kelling, 2007). These pathogens and respective diseases are present in cattle herds worldwide (Rebhun, 1995), thus impacting reproductive and overall efficiency of the global dairy industry. As an example, seroprevalence for BoHV-1, *Leptospira* spp., and the BVDV, as well as the incidence of IBR, leptospirosis, and BVD are increased in Brazil (Takiuchi et al., 2001; Flores et al., 2005; Junqueira et al., 2006).

Management techniques to prevent pregnancy loss in dairy herds, such as hormonal manipulation, thermal comfort, and nutritional management are increasingly being implemented into dairy systems worldwide (Lucy, 2001). Conversely, immunization strategies developed to reduce the impact of reproductive diseases, such as vaccination against IBR, leptospirosis, and BVD, do not receive proper attention (Littel-Van der Hurk, 2006). Recent research from our group demonstrated that vaccination against reproductive diseases increased overall reproductive efficiency in Brazilian beef herds (Aono et al., 2012), whereas few research studies directly evaluated the effects of such vaccination programs on reproductive efficiency in dairy cattle. Hence, the objective of the present study was to evaluate the adoption of vaccination programs against IBR, BVD and leptospirosis on pregnancy rates and pregnancy losses in commercial dairy operations.

2. Materials and methods

All experiments described herein were conducted in commercial dairy operations located in Minas Gerais and Paraná, Brazil. All animals utilized 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 (FASS, 1999). Within each operation, cows were managed similarly independent of vaccination treatments, following existing nutritional, reproductive, and health-related procedures for each operation.

2.1. Vaccine

The vaccine utilized in all experiments (CattleMaster 4+L5, Pfizer Animal Health, São Paulo, Brazil) was a freeze-dried preparation containing a chemically altered live strain of BoHV-1, inactivated cytopathic and noncytopathic BVDV strains, and 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.

2.2. Experiment 1

A total of 853 lactating Gir × Holstein cows (multiparous, $n = 611$; primiparous, $n = 242$) originated from 38 operations were assigned to the following fixed-time AI protocol (FTAI): treatment of estradiol benzoate (2 mg im of Estrogin; Farmavet, São Paulo, Brazil) and insertion of a intravaginal progesterone releasing device (CIDR; containing 1.9 g of progesterone; Pfizer Animal Health) on d −11, $PGF_{2\alpha}$ treatment (12.5 mg im of Dinoprost; Pfizer Animal Health) on d −4, estradiol cypionate treatment (1.0 mg im of ECP; Pfizer Animal Health) in addition to CIDR removal on d −2, followed by fixed-time AI on d 0. None of these operations had a history of vaccinating the cowherd against IBR, leptospirosis, and BVD. Within each operation, cows were randomly assigned to receive (VAC; $n = 426$) or not (CON = 427) vaccination against IBR, BVD, and leptospirosis (5 mL im of CattleMaster 4+L5, Pfizer Animal Health,) at the beginning of the FTAI protocol (d −11) and 30 d after AI. Hence, the objective of this experiment was to evaluate the effects of vaccination against IBR, leptospirosis, and BVD, with both doses administered when cows were handled for reproductive management, on pregnancy rates and pregnancy losses in dairy operations that did not have a history of vaccinating the cowherd.

On d −11, cows were (average ± SEM) 111 ± 3 d in milk (DIM), producing 21.3 ± 0.2 kg of milk, and body condition score (BCS) of 2.89 ± 0.01 (Wildman et al., 1982). 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 d 30 and 71 after AI. Any cow diagnosed as pregnant on d 30 and then non-pregnant on d 71 was designated as having undergone pregnancy loss. Blood samples were collected from a subsample of CON cows from 17 operations ($n = 84$) on d −11 for determination of serological profile for BoHV-1, BVDV, and *Leptospira* spp. infections. Blood samples were randomly collected from an average of 5 females per operation, being 4 multiparous and 1 primiparous cow. Samples were collected via coccygeal vein or artery into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ), placed on ice immediately, maintained at 4 °C for 24 h, and centrifuged at 3000 × *g* for 10 min at room temperature for serum collection. Serum was stored at −20 °C until further analysis. Neutralizing antibodies against BoHV-1 and BVDV were detected by virus neutralization test in Madin–Darby bovine kidney cells (Ferreira et al., 2005) and 100 tissue culture infectious $dose_{50}$ of Los Angeles and NADL strains of BoHV-1 and BVDV, respectively (Pilz et al., 2005). Detection of agglutinant antibodies against leptospirosis was conducted using microscopic agglutination test (Ryu, 1970). The criteria for seropositive animals were titers ≥ 8 for BoHV-1, ≥16 for BVDV, and ≥100 for *Leptospira* spp. (Ryu, 1970; Ferreira et al., 2005; Pilz et al., 2005).

2.3. Experiment 2

A total of 287 lactating Gir × Holstein cows (multiparous, $n = 212$; primiparous, $n = 75$) originated from 28 operations were assigned to the FTAI protocol described in experiment 1. None of these operations had a history of vaccinating the cowherd against IBR, leptospirosis, and BVD. Within each operation, cows were randomly assigned to receive (VAC; $n = 153$) or not (CON = 134) vaccination against IBR, BVD, and leptospirosis (5 mL im of CattleMaster 4+L5, Pfizer Animal Health,) 30 d prior to (d −41) and at the beginning (d −11) of the FTAI protocol. Hence, the objective of this experiment was to evaluate the effects of vaccination against IBR, leptospirosis, and BVD, with both

doses administered prior to FTAI, on pregnancy rates and pregnancy losses in dairy operations that did not have a history of vaccinating the cowherd.

On d −11, cows were (average ± SEM) 143 ± 4 DIM, producing 21.2 ± 0.4 kg of milk, and BCS of 2.94 ± 0.02 (Wildman et al., 1982). Pregnancy status and incidence of pregnancy losses were assessed as in experiment 1. Blood samples were collected from a subsample of CON cows from 10 operations ($n = 28$) on d −11 for determination of serological profile for BoHV-1, BVDV, and *Leptospira* spp. infections. These samples were randomly collected from an average of 2 females per operation, being 1 multiparous and 1 primiparous cow, processed and analyzed for antibody detection against BoHV-1, BVDV, and *Leptospira* spp. as described in experiment 1.

2.4. Experiment 3

A total of 1680 lactating Holstein cows (multiparous, $n = 1160$; primiparous, $n = 520$) originated from 17 operations were assigned to the experiment. None of these operations had a history of vaccinating the cowherd against IBR, leptospirosis, and BVD. Within each operation, nonpregnant cows with DIM greater than 28 d were randomly assigned to receive (VAC; $n = 859$) or not (CON = 821) vaccination against IBR, BVD, and leptospirosis (5 mL im of CattleMaster 4+L5, Pfizer Animal Health,), with doses administered 14 d apart. Within 15–135 d after the second dose of vaccine administration, cows were artificially inseminated 12 h upon visual estrus detection or assigned to the FTAI protocol described in experiment 1. Hence, the objective of this experiment was to evaluate the effects of vaccination against IBR, leptospirosis, and BVD, with both doses administered only 14 d apart but prior to AI, on pregnancy rates and pregnancy losses in dairy operations that did not have a history of vaccinating the cowherd.

At the time of first treatment administration, cows were (average ± SEM) 188 ± 3 DIM, producing 33.9 ± 0.2 kg of milk, and BCS of 2.77 ± 0.01 (Wildman et al., 1982). Pregnancy status and incidence of pregnancy losses were assessed as in experiment 1, but at 30 and 60 d after AI. Blood samples were collected from a subsample of CON cows from 14 operations ($n = 130$) when VAC cows received the first vaccine dose, for determination of the serological profile for BoHV-1, BVDV, and *Leptospira* spp. infection. These samples were randomly collected from an average of 10 females per operation, being 7 multiparous and 3 primiparous cows, processed and analyzed for antibody detection against BoHV-1, BVDV, and *Leptospira* spp. as described in experiment 1.

2.5. Experiment 4

A total of 820 lactating Gir × Holstein cows (multiparous, $n = 643$; primiparous, $n = 177$) originated from 15 operations were assigned to the same FTAI protocol described in experiment 1. All these operations already adopted three annual vaccinations against leptospirosis and annual vaccination against IBR and BVD. Within each operation, cows were randomly assigned to receive (REVAC; $n = 385$) or not (CON = 435) revaccination against IBR, BVD, and leptospirosis (5 mL im of CattleMaster 4+L5, Pfizer Animal Health,) at the beginning (d −11) of the FTAI protocol. Hence, the objective of this experiment was to evaluate the effects of revaccination against IBR, leptospirosis, and BVD, administered prior to FTAI, on pregnancy rates and pregnancy losses in dairy operations that had a history of vaccinating the cowherd.

On d −11, cows were (average ± SEM) 149 ± 4 DIM, producing 24.5 ± 0.3 kg of milk, and BCS of 2.95 ± 0.02 (Wildman et al., 1982). Pregnancy status and incidence of pregnancy losses were assessed as in experiment 1. Blood samples were collected from a subsample of CON cows from 11 operations ($n = 62$) on d −11 for determination of the serological profile for BoHV-1, BVDV, and *Leptospira* spp. infection. These samples were randomly collected from an average of 6 females per operation, being 4 multiparous and 2 primiparous cows, processed and analyzed for antibody detection against BoHV-1, BVDV, and *Leptospira* spp. as described in experiment 1.

2.6. Statistical analysis

For all analyses, significance was set at $P \leq 0.05$, and tendencies were declared if $P > 0.05$ and ≤ 0.10. Pregnancy data were analyzed using the GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC, USA) and Satterthwaite approximation to determine the denominator degrees of freedom for tests of fixed effects. The model statement for pregnancy rates and losses contained the effects of vaccination treatment, operation, parity, AI technique (experiment 3 only; AI or fixed-time AI) and all resultant interactions, as well as BCS, DIM, and milk production as independent covariates. However, covariates were removed from the model if $P > 0.10$. Data were analyzed using cow (operation × parity × vaccination treatment) as random variable and error term for tests of fixed effects for experiments 1, 2, and 4, and cow(operation × parity × vaccination treatment × AI technique) for experiment 3. Cow BCS, DIM, and milk production were analyzed using the MIXED procedure of SAS (SAS Institute Inc.), with Satterthwaite approximation and the same models described for pregnancy analysis, but without the independent covariates. Results are expressed as least square means (adjusted to the appropriate covariates for pregnancy analysis), separated using LSD, and 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

No differences were detected between VAC and CON cows for BCS ($P = 0.16$), DIM ($P = 0.85$), and milk production ($P = 0.38$; Table 1). Results associated with seroprevalence for BoHV-1, BVDV, and *Leptospira* spp. are shown (Table 2). Cow BCS was a significant covariate for pregnancy rates on d 30 ($P = 0.03$) and 71 ($P = 0.04$). No vaccination treatment effects were detected for pregnancy rates on d 30 ($P = 0.68$) and 71 ($P = 0.41$), although pregnancy loss was

Table 1
Body condition score (BCS), days in milk (DIM), and milk production of cows assigned to each experiment.

Experiment[a]	BCS[b]	DIM	Milk production (kg/d)
Experiment 1			
VAC	2.85	117	19.0
CON	2.91	116	19.6
SEM	0.04	5	0.5
P-value	0.16	0.85	0.38
Experiment 2			
VAC	2.97	140	20.0
CON	2.95	149	18.6
SEM	0.05	8	0.9
P-value	0.75	0.29	0.15
Experiment 3			
VAC	2.81	207	31.7
CON	2.80	206	31.8
SEM	0.02	4	0.4
P-value	0.85	0.83	0.75
Experiment 4			
REVAC	2.89	126.3	22.6
CON	2.98	159.0	21.3
SEM	0.04	9.4	0.7
P-value	0.04	<0.01	0.05

[a] In experiment 1, cows received (VAC) or not (CON) vaccination against IBR, BVD, and leptospirosis on d −11 and d 30 relative to fixed-time AI (d 0). In experiment 2, cows received VAC or CON on d −41 and d −11 relative to fixed-time AI (d 0). In experiment 3, cows with DIM greater than 28 d received VAC or CON, with doses administered 14 d apart and within at least 15 d prior to AI. In experiment 4, cows received revaccination (REVAC) or not (CON) against IBR, BVD, and leptospirosis on d −11 relative to fixed-time AI (d 0). In experiments 1, 2, and 4, variables were recorded on d −11. In experiment 3, variables were assessed at the time of first treatment administration.
[b] As described by Wildman et al. (1982).

reduced ($P = 0.03$) in VAC cows compared with CON cohorts loss (Table 3).

3.2. Experiment 2

No differences were detected between VAC and CON cows for BCS ($P = 0.75$), DIM ($P = 0.29$), and milk production ($P = 0.15$; Table 1). Results associated with seroprevalence for BoHV-1, BVDV, and *Leptospira* spp. are shown (Table 2). Cow BCS was a significant covariate for pregnancy rates on d 30 ($P < 0.01$) and d 71 ($P = 0.02$), whereas DIM was a significant covariate for pregnancy loss ($P = 0.04$). Cows assigned to VAC had greater pregnancy rate on d 30 ($P = 0.01$) and 71 ($P = 0.03$) compared with CON cohorts (Table 3), whereas pregnancy loss did not differ ($P = 0.41$) between treatments (Table 3).

3.3. Experiment 3

No differences were detected between VAC and CON cows for BCS ($P = 0.85$), DIM ($P = 0.83$), and milk production ($P = 0.75$; Table 1). Results associated with seroprevalence for BoHV-1, BVDV, and *Leptospira* spp. are shown (Table 2). Cow BCS was a significant covariate for pregnancy rates on d 30 ($P = 0.04$) and 60 ($P = 0.05$). Cows assigned to VAC had greater pregnancy rate on d 30 ($P = 0.02$) and 60 ($P = 0.01$) compared with CON cohorts (Table 3), whereas pregnancy loss did not differ ($P = 0.43$) between treatments (Table 3).

3.4. Experiment 4

Cows assigned to REVAC had reduced DIM ($P < 0.01$) and BCS ($P = 0.04$), and greater milk production ($P = 0.05$) compared with CON cows (Table 1). Results associated with seroprevalence for BoHV-1, BVDV, and *Leptospira* spp. are shown (Table 2). Cow BCS, DIM, and milk production were significant covariates ($P < 0.10$) for all pregnancy analysis. No vaccination treatment effects were detected for pregnancy rate on d 30 ($P = 0.99$) and 71 ($P = 0.90$), or pregnancy loss ($P = 0.54$; Table 3).

4. Discussion

In experiments 1, 2, and 4, a substantial number 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. In experiment 3, although BoHV-1 presence was also substantial, the

Table 2
Presence (% of total samples) of antibodies against bovine herpesvirus-1 (BoHV-1), bovine viral diarrhea virus (BVDV), and *Leptospira* spp. within each experiment.[a,b]

Pathogen	Titer	Experiment 1 ($n = 84$)	Experiment 2 ($n = 28$)	Experiment 3 ($n = 130$)	Experiment 4 ($n = 62$)
BoHV-1	Negative (<8)	0	0	4	2
	8–64	27.4	25.0	44	20
	≥64	72.6	75.0	52	78
	Positive (≥8)	100.0	100.0	96	98
BVDV	Negative (<16)	47.6	46.4	78	51
	16–64	25.0	32.1	21	30
	≥64	27.4	21.4	1	19
	Positive (≥16)	52.4	53.6	22	49
Leptospira spp.	Negative (<100)	53.6	53.6	100	57
	100–200	23.8	25.0	0	31
	≥200	21.4	17.9	0	12
	Positive (≥100)	45.2	42.9	0	43

[a] Samples were collected in experiments 1, 2, and 3 from cows without a history of vaccination against IBR, BVD and leptospirosis. Samples were collected in experiments 4 from cows already receiving three annual vaccinations against leptospirosis and annual vaccination against IBR and BVD.
[b] Detection of neutralizing antibodies against BoHV-1 and BVDV was conducted using virus-neutralization techniques (Pilz et al., 2005), whereas detection of agglutinant antibodies against *Leptospira* spp. was conducted using microscopic agglutination test (Ryu, 1970). The criteria for seropositive reaction expressed in titers were: ≥8 for IBR, ≥16 for BVD, and ≥100 for leptospirosis serovar *L. hardjo* (Ryu, 1970; Pilz et al., 2005).

Table 3
Pregnancy rates and losses in cows within each experiment.[a]

Experiment[b]	Pregnancy status (%)		Pregnancy loss (%)
	DIAG1	DIAG2	
Experiment 1[c]			
VAC	40.7 (174/426)	37.7 (160/426)	6.9 (14/174)
CON	38.5 (164/427)	33.3 (138/427)	16.0 (26/164)
SEM	3.9	3.8	3.6
P-value	0.68	0.41	0.03
Experiment 2			
VAC	55.4 (82/153)	47.8 (74/153)	8.9 (8/82)
CON	39.2 (48/134)	34.4 (44/134)	3.5 (4/48)
SEM	5.8	5.9	5.4
P-value	0.01	0.03	0.41
Experiment 3			
VAC	36.3 (302/859)	33.6 (274/859)	8.1 (28/302)
CON	30.7 (254/821)	27.5 (222/821)	10.3 (32/254)
SEM	2.1	2.0	2.5
P-value	0.02	0.01	0.43
Experiment 4			
REVAC	38.4 (131/385)	34.0 (119/385)	11.4 (12/131)
CON	38.44 (151/435)	33.5 (131/435)	14.1 (20/151)
SEM	4.2	4.1	4.9
P-value	0.99	0.90	0.54

[a] In experiments 1, 2, and 4, pregnancy status was verified by detecting a fetus with transrectal ultrasonography at 30 (DIAG1) and 71 (DIAG2) d after fixed-time AI. In experiment 3, DIAG1 = 30 d after AI, whereas DIAG2 = 60 d after AI. Pregnancy loss was considered in cows that were pregnant on DIAG1, but non-pregnant on DIAG2. Values are reported as least square means. For pregnancy status, values in parentheses represent number of pregnant cows/total inseminated cows. For pregnancy loss, values in parentheses represent number of cows non-pregnant on DIAG2/cows pregnant on DIAG1.

[b] In experiment 1, cows received (VAC) or not (CON) vaccination against IBR, BVD, and leptospirosis on d −11 and d 30 relative to fixed-time AI (d 0). In experiment 2, cows received VAC or CON on d −41 and d −11 relative to fixed-time AI (d 0). In experiment 3, cows with DIM greater than 28 d received VAC or CON, with doses administered 14 d apart and within at least 15 d prior to AI. In experiment 4, cows received revaccination (REVAC) or not (CON) against IBR, BVD, and leptospirosis on d −11 relative to fixed-time AI (d 0).

[c] Cow body condition score (BCS), days in milk (DIM), and milk production obtained prior to treatment administration served as independent covariates for pregnancy analyses, and only remained in the model if $P \leq 0.10$. In experiment 1, BCS was a significant covariate for pregnancy rates on d 30 ($P = 0.03$) and 71 ($P = 0.04$). In experiment 2, BCS was a significant covariate for pregnancy rates on d 30 ($P < 0.01$) and d 71 ($P = 0.02$), whereas DIM was a significant covariate for pregnancy loss ($P = 0.04$). In experiment 3, BCS was a significant covariate for pregnancy rates on d 30 ($P = 0.04$) and 60 ($P = 0.05$). In experiment 4, BCS, DIM, and milk production were significant covariates ($P < 0.10$) for all pregnancy analysis.

incidence of seropositive cows for BVDV was not as high compared with experiments 1, 2, and 4, whereas none of the evaluated ranches had samples testing positive for *Leptospira* spp. infection. Experiments 1, 2, and 4 were conducted in the state of Minas Gerais, located in southeastern Brazil, whereas experiment 3 was conducted in Paraná, located in the southern region of the country. Incidence of BVDV and *Leptospira* spp. infections varies among Brazilian states due to several environmental, biological, and management reasons (Favero et al., 2001; Flores et al., 2005), which may help explain the differences reported herein in BVD and leptospirosis seropositive cows between experiments. Antibody titers > 64 for BoHV-1 and BVDV, and >200 for *Leptospira* spp. indicate active infections, suggesting that IBR, BVD and leptospirosis were present in the evaluated herds (Houe and Palfi, 1993; Fredriksen et al., 1999; Junqueira et al., 2006). Further, antibody titers < 64 for BoHV-1 and BVDV, and <200 for *Leptospira* spp. may be induced by vaccination, which also explains the positive serological profile for these pathogens in experiment 4, where operations recurrently vaccinated the herd against these pathogens (Junqueira et al., 2006).

Treatment effects detected on pregnancy outcomes in experiment 1 were independent of cow nutritional status (Butler, 2005), based on similar BCS, DIM and milk production between vaccination treatments, and the evaluation of these parameters as independent covariates within all pregnancy analyses. Cows vaccinated against IBR, BVD, and leptospirosis had reduced pregnancy losses after fixed-time AI compared with non-vaccinated cohorts. Supporting our rationale and hypothesis, BVDV, BoHV-1 and *Leptospira* spp. can induce pregnancy loss in cattle (Biuk-Rudan et al., 1999; Grooms and Bolin, 2005), whereas vaccination against these pathogens alleviated this outcome. Furthermore, these results corroborate with the known detrimental effects of BVD, IBR, and leptospirosis to reproductive efficiency of dairy cows, and the consequent need for proper immunization programs (Khodakaram-Tafi and Ikede, 2005; McEwan and Carman, 2005).

In experiments 2 and 3, treatment effects on pregnancy outcomes also were independent of cow nutritional status (Butler, 2005), based on similar BCS, DIM and milk production between vaccination treatments, and the evaluation of these parameters as independent covariates within all pregnancy analyses. In both experiments, cows vaccinated against IBR, BVD and leptospirosis had greater pregnancy rates on d 30, which remained greater until the second pregnancy diagnosis compared with non-vaccinated cohorts, whereas no treatment effects were detected for pregnancy loss. These results support that BVD, IBR, and leptospirosis also impair fertility parameters and pregnancy maintenance during the first 30 d of gestation (Miller and Van der Maaten, 1986; Grooms et al., 1998; Kelling, 2007), whereas vaccination against these diseases alleviated these outcomes. Yet, infertility and pregnancy losses before d 30 of gestation were not directly evaluated herein, but also contribute significantly to reproductive and economic losses in dairy production systems (Santos et al., 2004).

Experiment 4 demonstrated that in dairy herds receiving proper vaccination with BVDV, BoHV-1 and *Leptospira* spp., revaccination against these pathogens prior to AI did not improve reproductive parameters. However, REVAC cows had reduced DIM, BCS, and greater milk production compared to CON cohorts at the beginning of the experiment, which have been negatively associated with reproductive performance of lactating dairy cows (Butler, 2005) and may have hindered any potential benefits of revaccination on pregnancy outcomes. Nevertheless, pregnancy rates and losses were similar between REVAC and CON cows when DIM, BCS, and milk production were included in the analysis as independent covariates, suggesting that differences in these production traits between treatment groups did not impact the effects of revaccination treatment on reproductive parameters.

Therefore, three annual vaccinations against leptospirosis and annual vaccination against IBR and BVD appear to be adequate to ensure immunological protection, and prevent reproductive losses caused by these diseases in dairy herds.

In this series of experiments, administration of the first vaccine dose prior to AI and the second dose 30 d after AI did not improve pregnancy rates, but reduced pregnancy losses during the second month of gestation (experiment 1). Conversely, administration of both doses of the vaccine prior to AI increased pregnancy rates, but did not impact pregnancy loss during the second month of gestation (experiments 2 and 3). These results can be attributed to the profile and timing of antibody responses upon vaccination using the vaccine tested herein. More specifically, vaccination with chemically altered live strain of BoHV-1 moderately increase antibody titers 14 d after the first dose, which peaks within 96 h after the second dose, and remains elevated for 180 d after the second dose (Sutton, 1980; Fulton et al., 1995). Vaccination with inactivated cytopathic and noncytopathic BVDV strains only increases antibody titers 14 d after the second dose, which also remains elevated for 180 d after the second dose (Fulton et al., 1995; Vogel et al., 2002; Lime et al., 2005). Vaccination with five inactivated *Leptospira* spp. serovars utilized herein often causes immediate increases in antibody titers after the first dose, remaining elevated for 150 d if the second dose was administered (Arduino et al., 2009). Hence, cows from experiment 1 had elevated antibody titers against *Leptospira* spp., BoHV-1, and BVDV after the second vaccine dose, whereas cows from experiments 2 and 3 already had elevated antibody titers during breeding and the initial 30 d of gestation. Therefore, proper antibody response and immunological protection against leptospirosis, BVD, and IBR probably began in cows from experiment 1 during the second month of gestation, leading to vaccination treatment effects detected for pregnancy loss from d 30 to 71 relative to AI. Alternatively, cows from experiments 2 and 3 likely experienced proper antibody protection against these pathogens at the period of expected ovulation, AI, and early pregnancy maintenance, resulting in increased pregnancy rates 30 d after AI.

5. Conclusions

Vaccination against IBR, BVD and leptospirosis using a commercial vaccine (CattleMaster 4+L5, Pfizer Animal Health) improved reproductive efficiency parameters in dairy herds without a history of vaccinating the cowherd against these reproductive pathogens. These results, in conjunction with the elevated incidence of cows testing seropositive for BoHV-1, BVDV, and *Leptospira* spp. in the herds evaluated herein, demonstrate the importance of reproductive diseases and proper immunization programs to reproductive and overall efficiency of dairy systems exposed to these pathogens. Moreover, cows should receive both doses of the vaccine prior to AI to ensure maximum antibody response and optimal reproductive outcomes during conception, as well as early- and mid-gestation.

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