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Influence of IBR vaccination timing on ovarian and uterine statuses and circulating progesterone and estradiol 17β in synchronized crossbreed heifers

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ABSTRACT

To declare the interaction between IBR vaccination and the synchronization protocol, dairy heifers (n= 24), 12.5-14.0 months old, were equally and randomly assigned according to the timing of vaccination into pre-synch group (vaccinated 3 days before synchronization), in-synch group (vaccinated five days after the start of the synchronization), and control (given sterile water same as treated groups). Ovarian and uterine ultrasound examinations were done on Day -3, 5, 8, 19 and 43. Blood samples were collected for measuring estradiol and progesterone levels after vaccination. The effect of IBR vaccination on ovarian activity appeared on Day 5 (P= 0.07) through decrease in the mean diameter of the growing follicles in pre-synch group compared to in-synch group, and on Day 8 through reduction of the total and growing follicular numbers (P= 0.05 and 0.03, respectively) in in-synch group compared to other groups. Estradiol levels were significantly (P=0.03) higher on day 8 (day of insemination) in the pre-synch group than the other groups. The uterine changes were characterized by a significant decrease in uterine diameters on Day 5 in the pre-synch group compared with control. The ventral uterine diameter in in-synch group decreased on Day 5 (P= 0.015) and re-increased on Day 8 (P= 0.007) compared to control group. In conclusion, administration of IBR vaccination prior to synchronization may possess some impacts on the ovarian and uterine activities without major adverse reactions on reproduction. Therefore, vaccination timing is recommended to be at least three days away from the beginning of the synchronization protocol.

Introduction

Bovine herpesvirus 1 (BHV-1) is a virus of the family Herpesviridae and subfamily Alphaherpesvirinae causes female reproductive failure as consequence to corruption of the ovary and/or conceptus (Wathes *et al*., 2020). Systemic disease causes abortions, up to 60%, in non-immune pregnant cows (Tizard, 2021).

BHV-1 infection in pre-pubertal heifers causes slow growth, retard breeding and delay the age at first calving (Wathes *et al*., 2020). BHV-1 infection at the time of service may reduce fertility, with the potential to cause chronic necrotizing endometritis and oophoritis, accompanied by a shortened estrous cycle. Infection later in the estrous cycle may result in a decreased conception rate, whereas infection later in pregnancy can lead to abortion, mummification, stillbirth and the birth of live calves which die shortly thereafter (Graham, 2013; Wathes *et al*., 2020).

Vaccination is the common well-known way for protection from viral infections. Generally, there are two types of vaccines; modified-live (MLV) and inactivated (IVV) virus vaccines (Tizard, 2021). Modified-live virus vaccine stimulates the immune system by actively infecting host cells, and they also carry with them the potential to revert to virulence and inflict the damage they are designed to prevent (Kelling, 2007). In comparison with MLV vaccines, the inactivated virus vaccines (IVV) less broad and short-acting and used broadly in many conditions (Kelling, 2007).

The use of some MLV vaccines in pregnant females potentially causes viral replication and transmission across the blood-uterine barrier sufficient to induce varying degrees of embryo/fetal loss (Bolin, 1995), but other MLV products (non-replicating MLV) have been altered to avoid these complications (Sprott and Forrest, 2001). Although, vaccination before breeding has been successfully reduce the side effects of BHV-1 on reproductive efficiency, the use of live BHV-1 vaccines in pregnant animals increased the risk of reproductive loss, even though the product was used according to label directions (O'Toole and Van Campen, 2010). Heifers vaccinated with an inactivated BHV-1 vaccine are more likely to have significantly higher pregnancy rates relative to heifers vaccinated with a modified live (MLV) vaccine (Perry *et al*., 2013). The reproductive performances (e.g., number of parturitions, the mean age at first calving, etc.) of cows vaccinated against IBR/BVD showed better indices in the vaccinated group when compared to unvaccinated animals (Pacheco-Lima *et al*., 2019).

The influence of BHV-1 vaccination on cow reproductive cycle is scarcely studied. Moreover, the interaction between vaccination, synchronization programs and conception are poorly studied and seemed variable (Walz *et al*., 2015b; Ferreira *et al*., 2018). Some studies showed that there is no negative interaction between them , while other studies Perry *et al*. (2013) showed there's a negative interaction between them. The present work aimed to investigate the effect of vaccinating dairy heifers with polyvalent vaccine containing a modified live chemically altered BHV-1, before and during the synchronization protocol on the ultrasound monitored ovarian and uterine statuses, and ovarian steroids hormonal levels.

Materials and methods

Ethical approval

The research protocol was approved by the Ethical Committee for Institutional Animal Use and Care of the Faculty of Veterinary Medicine, Benha University with the approval number (BUFVTM 44-06-23).

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Animals and experimental design

Heifers (n= 24), 12.5-14.0 months of age, belonged to private dairy farm were used in the present study extended from Jan. to July 2023. Animals were managed according to routine animal husbandry procedures, fed an age-appropriate balanced ration, and vaccinated with a polyvalent vaccines against the viral respiratory reproductive diseases (e.g., infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD), bovine respiratory syncytial virus (BRSV), and parainfluenze 3 (PI3)) routinely bi-annually. All heifers were examined by trans-rectal ultrasonography (CHISON ECO5, China) before starting the work to determine the health status and functionality of the reproductive organs.

Heifers were randomly and equally assigned to one of three treatment groups (n= 8 per each) according to the timing of vaccination. Animals were administered a single dose of the commercially available modified live (chemically altered) and inactivated combined vaccine (Cattle Master GOLD FP 5 L5, Zoetis Inc.) by subcutaneous injection. The 1st group (Pre-synch group) was vaccinated 3 days before start of synchronization (Day 0 = Day of start of synchronization protocol). The 2nd group (In-synch group) was given the same vaccine five days after the start of the synchronization (i.e., 3 days before AI). The 3rd group (served as a control) was given two doses of placebo (sterile water on Day -3 and 5). All heifers were daily observed after vaccination for post vaccine-adverse events. All heifers received the same synchronization protocol and bred by FTAI at the same time by the same person.

Ovarian and uterine examinations were done by transrectal ultrasonography at five times points (Day -3, 5, 8, 19 and 43). Blood samples were collected from each heifer before each medication/animal examination for measuring the ovarian steroid hormone changes (Estradiol (E2) and Progesterone (P4)) after vaccination.

Medicaments

Vaccine: Polyvalent vaccine (Cattle Master GOLD FP 5 L5, Zoetis Inc.) was used. It is a combined freeze-dried preparation of chemically altered strains of IBR and PI3 viruses, and modified live BRSV, plus a liquid adjuvanted preparation of inactivated BVDV (types 1&2) and inactivated cultures of the 5 Leptospira serovars.

Hormones for estrus synchronization: include PGF2α (Enzaprost T: Ceva Sante Animale, each 1 ml contains 5 mg dinoprost as trometamol with 16.5 mg benzyl alcohol), GnRH (Cystrolin: Ceva Sante Animale, each 1 mL contains 50 µg Gonadorelin diacetate tetrahydrate) and progesterone intravaginal implant (PRID Delta insert: Ceva Sante Animale, each device containing 1.55 g progesterone).

Synchronization and artificial insemination

All heifers were subjected to 6 days Co-synch 48 h (6dCo48) synchronization protocol according to Fernandez-Novo *et al.* (2021) as shown in Fig 1. Moreover, animals were dosed with PGF2α at 3 days before synchronization to induce regression of any ovarian luteal tissue and to unify the ovarian status before beginning of the synchronization protocol (Perry *et al.*, 2013). All heifers were subjected to fixed time artificial insemination (FTAI) on Day 8 concurrently with the last GnRH 2nd dose.

Ultrasound examination and data quantifications

Transrectal ultrasonographic scanning was conducted by the same person according to Quintela *et al.* (2012) using portable ultrasound machine veterinary device (CHISON ECO5, China) provided with 6-8 MHz linear array transducer. The ovarian structures (Graffian follicles or corpus luteum) on both ovaries were scanned and their dimensions were recorded using the device installed software. All visible follicles (≥ 2 mm) were counted, measured, and classified into growing (< 10 mm diameter) or mature (> 10 mm diameter) according to Taneja *et al.* (1995). Furthermore, the changes in uterine wall diameter (mm) along the dorsal, cranial, and ventral uterine curvature were assessed according to Gad *et al.* (2017). The changes in ovarian activity in terms of the total follicular number, number and size of growing follicles, number and size of mature follicles, follicle deviation (i.e., difference between the 1st and 2nd largest follicles)., corpus luteum size, and total follicular area (mm2) per two ovaries were recoded.

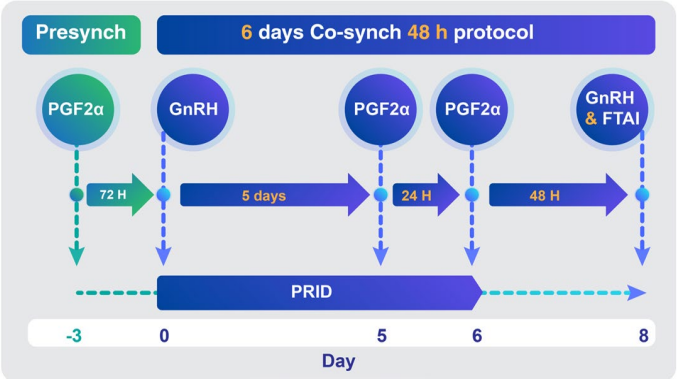

Fig. 1. Cows synchronization protocols procedures in the "6 days Co-synch 48 h" protocol according to Fernandez-Novo *et al.* (2021) with PGF2α at 3 days before synchronization according to Perry *et al.* (2013).

Blood sampling and hormonal assays

Blood samples (10 mL) were collected from all examined heifers into vacutainer tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA), via coccygeal vein. Samples were centrifuged at 3000 rpm for 10 min., for serum collection (Aono *et al.*, 2013). The harvested sera were stored at -20°C until estradiol and progesterone hormonal analysis by using ELISA reader (Stat Fax 2100, Italy – reading at wave length 450 nm) and available commercial kits. The kits used for progesterone analysis was DRG Progesterone Enzyme Immunoassay Kit with analytical sensitivity 0.045 ng/mL, intra assay variation CV 6.86 % and inter assay variation CV 5.59%). The kits used for estradiol analysis was DRG Estradiol ELISA kit with analytical sensitivity 9.714 pg/mL, intra assay variation CV 4.13% and inter assay variation CV 9.39%.

Samples collected on Day 8 (Day of FTAI represented follicular phase), and Day 12 (4 days post AI) were used for tracking the estradiol concentrations. Samples collected on Day 5 (a day before PRID device removal), Day 19 (represented luteal phase), and Day 35 post AI (Early pregnancy) were used for tracking progesterone concentrations (Perry *et al.*, 2013).

Data handling and statistical analysis

Data obtained were tabulated and statically analyzed using IBM SPSS (ver. 23) program according to Arkkelin (2014). Data were analyzed for normal distribution using the Shapiro- Wilk test. Data passed normality (Total follicular and growing follicles numbers, and progesterone) were analyzed parametrically with the One-way ANOVA and post-hoc with Dunkan multiple range test. Other parameters (Mature Follicles number, mean size of growing follicles, mean size of mature follicles, size of pre-ovulatory follicle , follicle deviation, total follicular area, corpus luteum size and estradiol levels) were analyzed non-parametrically with Kruskal-Wallis Test. P value was set at 0.05 for significant differences.

Results

Ovarian changes as affected by IBR vaccination time

The total follicular number and growing follicles number (Table 1) varied numerically between treated groups, and this differences were

characterized by statistically significant (P= 0.05 and 0.03, respectively) drop in in-synch group at Day 8 after the start of synchronization compared to other pre-synchronized and control groups (6.00±0.50 vs. 8.50±1.00 and 8.50±0.78, respectively, and 4.75±0.56 vs. 7.25±0.94 and 7.63±0.75, respectively).

Vaccination with IBR showed a tendency (P= 0.07) to reduce the mean diameter of the growing follicles (Table 1) at day 5 in pre-synch group compared to in-synch group (4.29±0.26 vs. 5.23±0.37 mm, respectively).

The changes in the number and size of mature follicles (including the pre-ovulatory follicles at the day of FTAI), follicle deviation (mm), total follicular area (mm^2), and corpus luteum diameter (during luteal phase or pregnancy) showed numerical but not statistical differences between groups (Table 1)

Uterine changes as affected by IBR vaccination time.

The changes in uterine biometry of synchronized cows as affected by IBR vaccination were monitored through evaluation the changes in the dorsal (Fig 2), cranial (Fig 3) and ventral (Fig 4) uterine curvatures ultrasonographically.

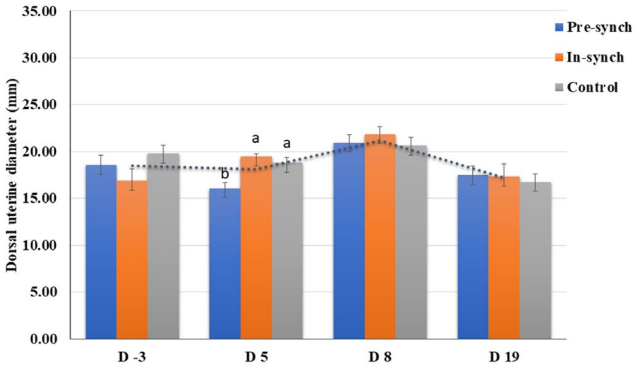

Fig. 2. Effect of IBR vaccination timing in relation to the estrus synchronization program on dorsal uterine diameter. Columns with different letters at the same time point denoted significant difference at P< 0.05.

The dorsal uterine curvature was significantly (P< 0.001) smaller in the pre-synch group at Day 5 after start of synchronization compared with the In-synch and control groups (16.08±0.65, 19.48±0.32 and 18.79±0.55 mm, respectively). Also, the cranial uterine curvature in pre-synch group tended (P= 0.08) to be smaller than control one (21.83±1.49

Table 1. Effect of IBR vaccination on ovarian activity in synchronized dairy heifers.

Ovarian activity index	Group	Day post-synchronization start time				
		-3	5	8	19	43
Total follicle number	Pre-synch	7.13±1.04	7.38±1.05	8.50±1.00a	10.13±1.25	6.38±1.03
	In-synch	5.38±1.40	6.63±1.13	6.00±0.50b	9.50±1.00	5.75±0.92
	Control	7.69±0.90	8.06±0.59	8.50±0.78a	10.44±0.84	6.44±0.65
	P value	0.34	0.58	0.05	0.81	0.83
Number of growing follicles (< 10 mm diameter)	Pre-synch	5.88±0.85	6.63±1.13	7.25±0.94a	9.50±1.27	5.50±1.04
	In-synch	4.75±1.36	6.25±1.19	4.75±0.56b	8.63±0.92	5.25±0.94
	Control	6.88±0.85	7.81±0.67	7.63±0.75a	9.88±0.90	6.19±0.58
	P value	0.38	0.54	0.03	0.69	0.74
Mean size of growing follicles (< 10 mm diameter)	Pre-synch	4.91±0.32	4.29±0.26b	4.98±0.29	5.13±0.43	4.45±0.34
	In-synch	3.94±0.93	5.23±0.37a	5.48±0.57	4.91±0.28	5.21±0.41
	Control	4.76±0.20	4.81±0.15ab	4.64±0.44	4.81±0.14	4.61±0.32
	P value	0.83	0.07	0.57	0.99	0.45
Number of mature follicles (> 10 mm diameter)	Pre-synch	1.25±0.25	0.75±0.37	1.25±0.16	0.63±0.18	0.88±0.30
	In-synch	0.63±0.18	0.38±0.18	1.25±0.25	0.88±0.13	0.50±0.27
	Control	0.88±0.23	0.25±0.16	0.88±0.13	0.63±0.18	0.25±0.16
	P value	0.16	0.52	0.19	0.46	0.24
Mean size of mature follicles (> 10 mm diameter)	Pre-synch	14.88±1.39	12.56±0.42	13.14±0.45	12.98±1.01	11.60±0.56
	In-synch	14.27±1.80	14.30±1.72	12.15±0.48	12.36±0.65	13.90±0.91
	Control	12.83±0.94	12.87±0.77	12.03±0.66	11.74±0.93	11.23±0.50
	P value	0.73	0.67	0.19	0.55	0.12
Follicle deviation (difference between 1st and 2nd largest follicle)	Pre-synch	4.36±1.03	4.66±1.28	4.70±0.71	3.23±1.11	3.35±1.30
	In-synch	2.83±0.90	4.33±1.18	4.23±0.83	3.71±1.02	3.40±0.89
	Control	3.70±0.87	2.63±0.93	4.90±1.19	1.78±0.46	2.61±0.49
	P value	0.63	0.32	0.84	0.42	0.88
Follicular area (mm^2)	Pre-synch	1896.11±670.38	975.68±344.96	978.25±345.86	1259.80±445.41	881.14±311.53
	In-synch	1306.10±493.66	869.11±307.28	757.72±267.90	1434.97±507.34	908.22±321.10
	Control	885.22±312.97	398.68±140.95	902.84±319.20	869.83±307.53	631.67±223.33
	P value	0.98	0.95	0.21	0.98	0.68
Corpus luteum diameter (mm)	Pre-synch	19.04±2.99	18.83±2.33		22.86±1.55	24.89±2.38
	In-synch	17.95±1.88	25.99±3.65		23.49±1.86	21.99±1.76
	Control	22.41±0.83	18.06±1.35		26.88±1.19	23.16±1.35
	P value	0.19	0.09		0.17	0.56

Data (Mean ± SE, n=8/group) with different letters in the same column were significantly different.

vs. 24.21±1.50 mm). On the other hand, the ventral uterine curvature was markedly smaller in pre-synch (P= 0.007) and in-synch (P=0.015) groups compared to control. Besides, the in-synch group at Day 8 (Day of insemination) showed a marked (P= 0.007) increase in ventral uterine curvature compared to control group.

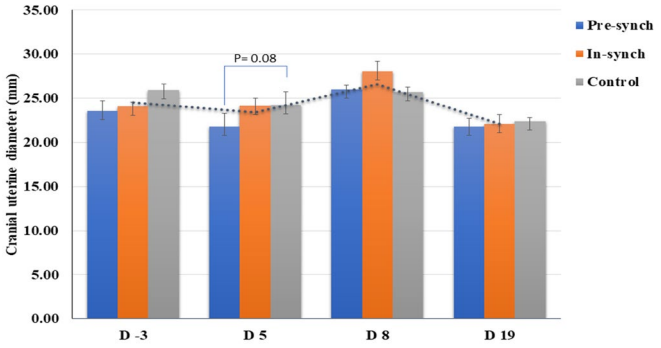

Fig. 3. Effect of IBR vaccination timing in relation to the estrus synchronization program on cranial uterine diameter.

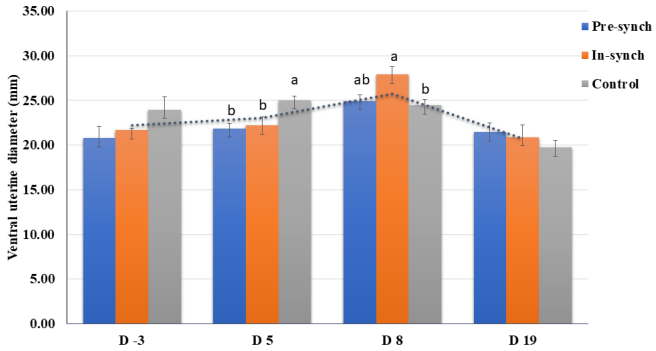

Fig. 4. Effect of IBR vaccination timing in relation to the estrus synchronization program on ventral uterine diameter. Columns with different letters at the same time point denoted significant difference at P< 0.05.

Hormonal changes as affected by IBR vaccination time

The effect of IBR vaccination on ovarian hormonal steroids (E2 and P4) is presented in Table 2. Regarding estradiol levels, there was a significant increase (P=0.03) at day 8 (day of insemination) in Pre-synch group vs. in-synch and control groups (44.36±3.59 vs. 36.24±1.85 and 34.08±1.47 pg/ml, respectively) and this significant difference subsides at day 12 (4 days post AI).

Regarding progesterone concentrations there were no significant differences between vaccinated and control groups at day 5 (Day before PRID removal), 19 (luteal phase) or 43 (early pregnancy).

Table 2. Effect of IBR vaccination on circulating hormonal levels in synchronized dairy heifers.

Groups	Estradiol (pg/ml)		Progesterone (ng/ml)		
	Day post-synchronization start time				
	8	12	5	19	43
Pre-synch	44.36±3.59a	48.03±4.34	11.50±0.42	11.24±0.51	11.83±0.21
In-synch	36.24±1.85b	47.81±1.93	11.65±0.60	11.66±0.53	11.75±0.34
Control	34.08±1.47b	50.55±5.49	11.01±0.46	11.50±0.34	11.73±0.31
P value	0.03	0.94	0.64	0.82	0.97

Data (Mean ± SE) with different letters within the same column were significantly different.

Discussion

Many strategies are adopted to reduce the time and labor involved in rearing by vaccinating heifers concomitant with synchronization. However, the impact of vaccination on estrus synchronization and conception are inconsistent. In the present study, there was some drawbacks of IBR vaccination on the physiology of synchronized animals. The interaction between IBR vaccination and estrus synchronization on ovarian activity was evident in the in-synch group at Day 5 and Day 8 through affecting growing follicles (size and number) in association with decrease ventral uterine diameter at Day 5 and re-increase at Day 8. On the other hand, the pre-synch group showed no effect on ovarian activity, but had a marked reduction in all uterine measures on Day 5 in comparison with control.

Endemic viral diseases have been encountered in cows in many countries. It is clearly understood that many of them are placental transmitted and can induce abortion or fetal anomalies. There is also strong evidence that viral infections can cause other manifestations in dairy cows, which are signaled in dropped animal fertility (Miller and Van der Maaten, 1986). Ovarian structures changes (follicular and luteal) can be used as an indicative tool for monitoring the ovarian health and any variation in the ovarian activity between vaccinated and control group (Taneja *et al.*, 1995). In the present study, the impact of IBR vaccination on synchronized heifers was seen through the reduction in the growing follicle number (by 19% at Day 5 and 37% at Day 8) and this affected the total follicle population (8% and 29% reduction rate at Day 5 and 8, respectively). Nevertheless, there was no significant impact of vaccination on other ovarian parameters. This effect perhaps due to local viral effects on the ovaries, which is limited to the presence of luteal tissue on the ovaries. When animals vaccinated against IBR, no hindrance of fertility are expected when given at 10 or 31 preceding synchronized natural insemination (Walz *et al.*, 2015a). Miller and Van der Maaten (1985) showed that heifers exposed IBR virus infection had necrotic follicles and a diffuse mononuclear cell accumulation in the ovarian stroma as well as focal necrosis and infiltration of mononuclear cells to diffuse hemorrhage and necrosis in the corpus luteum. Also, IBR was isolated form heifers ovaries vaccinated with modified-live IBR virus on Day 9 post vaccination, and the ovaries showed necrotic oophoritis characterized by multifocal areas of ovarian tissue necrosis and hemorrhage, and there were varying degree of necrosis and inflammatory change in the corpora lutea and surrounding ovarian tissues (Smith *et al.*, 1990). These findings match our hypothesis that the IBR vaccination could adversely affect ovarian function when animals were vaccinated around the time of synchronization. A similar effect was noticed after acute BVD infection which affected the maximum diameter and growth rate of dominant and subordinate follicles during the first two cycles post-infection (Grooms *et al.*, 1998). The little match between our study and the former findings may be related to the nature of used vaccines and level of their attenuation, as vaccination with chemically altered vaccines has less effect on reproductive parameters comparing to modified live vaccines (Perry *et al.*, 2017).

To double check and go through for more investigations about the direct effect of the vaccine on the ovarian activity, which done via analysis of the ovarian steroid hormonal profile. Our study showed a significant elevation in E2 levels on Day 8 in Pre-synch group compared to control, but no other effects were noticed on progesterone levels during luteal phase or pregnancy. These findings matched with (Perry *et al.*, 2013), who revealed that plasma concentrations of both E2 and P4 were affected by vaccination during the synchronization period. Miller *et al.* (1989) showed that heifers vaccinated on Day 14 post-breeding had lower progesterone within 10 days after inoculation. On the other hand, Walz *et al.* (2015a) found that there were no clear significant differences in E2 and P4 concentrations detected among vaccinated synchronized groups.

The transrectal ultrasound is a valuable tool to measure the uterine diameters (Gad *et al.*, 2017) that refers to endometritis (Salah and Yimer, 2017). This study investigated the influence of MLV-IBR vaccination before and during the synchronization protocols on the reproductive performance in dairy heifers. Our hypothesis is that the vaccination may be carry some side effects, like natural infection (Kelling, 2007). Tracking the changes in uterine horns through several ultrasound measurements revealed substantial decrease in uterine diameter on Day 5 in pre-synch and in-synch groups, though there was an increase in ventral uterine diameter on Day 8 in the in-synch group. We assume that the mild uterine changes were due to post vaccination reaction as a result of secretions of inflammation cytokines same as in mild viral infection (Sheldon *et al.*, 2009). Former studies showed that the BHV-1 infection can cause chronic necrotizing endometritis and oophoritis (Graham, 2013). Animals inseminated 30-60 days after vaccination showed protection from abortion (85.7%) without adverse post-vaccinal reaction (Zimmerman *et al.*, 2007). Interestingly, BHV-1 infection could be retained in latent form after infection and re-activated by stress of parturition or milk production (Wathes *et al.*, 2020). Also, they can promote bacterial infection e.g., *E. coli* causing

endometritis or delay uterine involution (Wathes *et al*., 2020).

Conclusion

The interaction between IBR (MLV chemically altered) vaccines and synchronization programs possessed some effects on the ovarian activity and uterine diameters but with no major adverse reactions on reproduction were encountered. However, to avoid any interaction between them, we recommend that vaccination timing be more than three days away from the beginning of the synchronization protocols. This recorded effect may need further investigations on a large scale for its validation, and to determine its long-lasting effect on conception and other reproductive parameters.

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Conflict of interest

The authors declare that they have no conflict of interest.

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