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Effect of breeding during the peri-vaccination window against foot-and-mouth disease on the pregnancy rate in the cow: A retrospective study

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Foot and mouth disease (FMD) is a transboundary disease that affects all the cloven-hoofed animals. Considering the economic importance, India launched FMD control program in 2003-04 by biannual mass vaccination of cattle with inactivated oil-adjuvanted FMD vaccine covering the serotypes O, A, and Asia. Despite the fact that regular vaccination has long-term benefits in the control of FMD, potential short-term negative effects on the production and reproduction hamper the voluntary participation of farmers in the control program. It is reported that vaccination of cows against FMD caused a transient loss in the milk production (Krishnaswamy *et al*. 2021), induced allergic reactions (Yeruham *et al.* 2001), and increased pregnancy-loss (Garcia *et al.* 2021) in the cow. Vaccination can adversely affect the embryonic viability and pregnancy by inducing acute phase protein response and febrile reaction in the cow (Ferreira *et al.* 2016).

According to the Committee on Bovine Reproduction Nomenclature (1972), early embryonic mortality occurs between fertilization and gestation day 24, whereas, late embryo mortality occurs between gestation day 25 and 45 (Walsh *et al*. 2011). There is a consensus among the bovine theriogenologists that mortality is higher during the first 16 days of gestation than other periods of pregnancy (Diskin and Sreenan 1980). Recently, it is reported that administration of FMD vaccine (Bioaftogen, Bivalent inactivated oil emulsion vaccine) 2 days before AI delayed the ovulation by 4 days and resulted in significant reduction in the conception rate in the Hanwoo cows (Kim *et al*. 2021). As retrospective study is simple, quick, and inexpensive, this approach was used in present research to investigate the pregnancy outcome to artificial insemination (AI), when cows were bred within 14 days of FMD vaccination as it coincides with the window of early embryonic mortality.

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The study was conducted on lactating crossbred (Holstein-Friesian×*Bos indicus*) and Deoni (*Bos indicus*) cows under subtropical conditions maintained at the Livestock Research Centre, Southern Regional Station (SRS) of ICAR-National Dairy Research Institute (NDRI), Bengaluru (Karnataka) in the year 2021. The farm is located at an altitude of 1,200 m above the mean sea level at 12° 58′ N latitude and 77° 38′ E longitude. Briefly, vaccination for FMD (Raksha-Ovac trivalent®, Indian Immunologicals Limited, 2 mL intramuscular) was done in May-June of every year with inactivated trivalent (serotypes O, A, and Asia1) oil adjuvanted vaccine. In addition, vaccination is done for haemorrhagic septicemia, black quarter, anthrax, and calfhood vaccination of female calves for brucellosis.

Heat detection was done by a trained stockman for 20 min with a teaser bull around 08:00 h every day and cows in estrus were bred through AI with frozen-thawed semen. Confirmatory pregnancy diagnosis was done by rectal palpation between day 45 and 55 post-AI. Date of AI and the outcome of pregnancy diagnosis were recorded in the AI register and breeding card, respectively.

Date of FMD vaccination was collected from the health register for 6 years from January 2016 to January 2021. Keeping the date of FMD vaccination as day 0 post-vaccination (dpv 0), cows those were bred before 14 days of FMD vaccination (dpv -14 to -1) were categorized as AI pre-vaccination, whereas, those who were bred from dpv 0 to 14 were grouped into AI post-vaccination categories. The pregnancy outcome post-AI was retrieved from the breeding card and history sheets. In addition, records on breed, parity, and days in milk on the day of FMD vaccination were also collected. After data entry in the excel spreadsheet, repeat breeder cows with >3 AI, cows in parity ≥4, and pregnant heifers were excluded as they are known to affect the pregnancy rate. The final dataset consisted of 47 records derived from crossbred (n=26) and Deoni (n=17) cows. Out of 26 crossbred cows, four appeared twice in the final dataset accounting for the difference of four between the total records (n=47) and total

Table 1. Estimates for the predictors of pregnancy in Deoni and Crossbred cows

Coefficients[$]	Estimate	Standard error	Z-value	Pr(>|z|)	Odds ratio (OR)	95% Confidence interval of OR	
						2.5%	97.5%
(Intercept)	-1.0417	0.7848	-1.327	0.184	0.35	0.06	1.51
Day_AI2	-0.7479	0.7313	-1.023	0.306	0.47	0.10	1.88
DIM_Stage2	1.3074	0.9038	1.447	0.148	3.70	0.68	25.5
DIM_Stage3	0.2174	0.9171	0.237	0.813	1.24	0.21	8.46
Breed2Deoni	0.5171	0.8655	0.597	0.550	1.68	0.30	9.69
Parity2	0.6845	1.0110	0.677	0.498	1.98	0.27	15.54
Parity3	-1.2948	1.3057	-0.992	0.321	0.27	0.01	2.77

[$]AI pre-vaccination group, crossbred, days in milk stage 1 (DIM_Stage 1) and parity 1 served as reference categories to calculate the log of odds (estimate β) and odds ratio (exponential β).

cows (n=43). Days in milk was divided into three stages of lactation for Deoni (Das *et al*. 2011) and crossbred cows (Krishnaswamy *et al*. 2021).

The objective of the analysis was to find whether the timing of AI with respect to FMD vaccination had any effect on the binary outcome variable of pregnancy. To achieve this, the collated data was initially analysed by generalized linear mixed model by fitting the categorical predictor variables such as day of AI with respect to dpv 0, (AI pre-vaccination and AI post-vaccination), breed (crossbred and Deoni), stage of lactation (stage 1, 2, and 3) and parity (1, 2 and 3) as fixed effects and cow as random effect. As the random effect of the cow was non-significant, multiple logistic regression was preferred over the generalized linear mixed model. As the full model had a lower -2 log likelihood ratio than that of the null model (intercept only model), the former model was run by including the above predictor variables. AI pre-vaccination group, crossbred, lactation stage 1 and parity 1 served as reference categories to estimate the log of odds (co-efficient, β) for the corresponding predictors. The initial analysis was done with SPSS v.21 as reported (Anonymous, 2020) and because of graphical options to convert log-of-odds into probability scale, final data analysis was done in R version 3.6.3 (2020-02-29).

Retrospective investigation was preferred as compared to prospective approach because of the non-availability of larger number of organized dairy herds in India. Further, use of fixed time AI protocol is not commonly adopted. The results of the present study are presented in Table 1 and Fig. 1. The results of binary logistic regression revealed that the probability of pregnancy was not affected when the cows were bred within 14 days of FMD vaccination though the odds of pregnancy numerically decreased in the AI post-vaccination group. When the data was reanalysed by keeping the day of AI as a continuous predictor variable (dpv -14 to +14), there was a lack of effect on the pregnancy outcome. A possible association between the effect of the

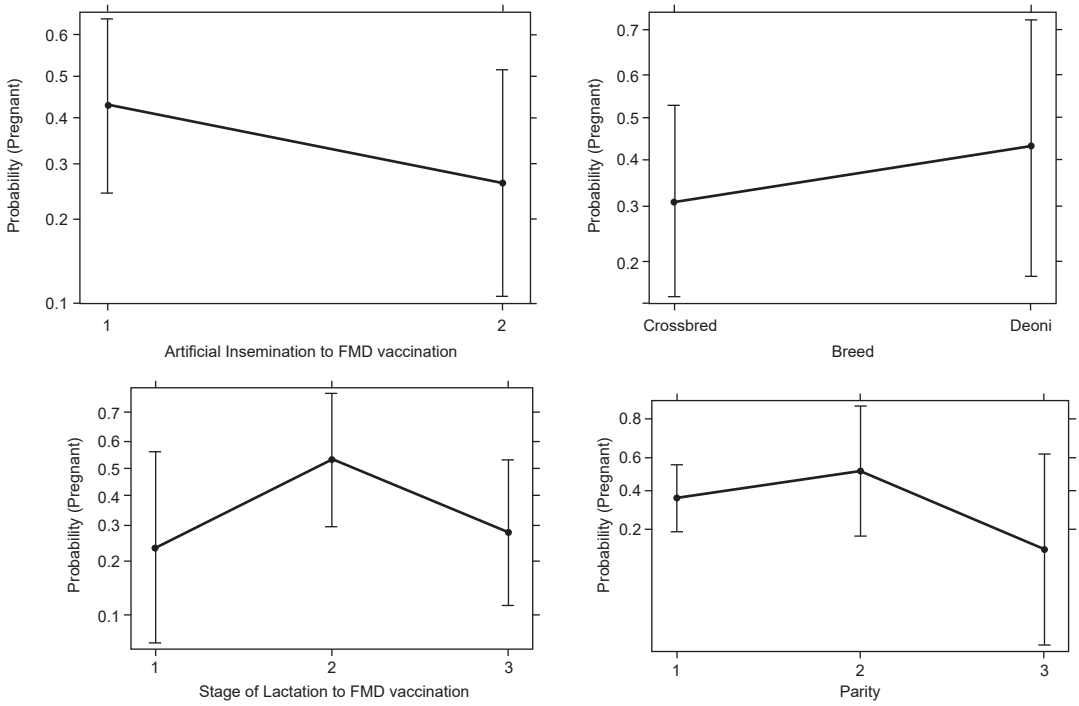

Fig. 1. Effect of FMD vaccination on the pregnancy outcome in the Deoni and Crossbred cow. *In the x axis of first graph 1 indicate 2 represent AI done before FMD vaccination and 2 represents) and AI done after FMD vaccination.

FMD vaccine on pregnancy loss was originally proposed based on the observation of pregnancy wastage within 16 days post-FMD vaccination in the cow (Von Ahlers *et al*. 1985). It is reported that administration of FMD vaccine (Bioaftogen®, Bivalent inactivated oil emulsion vaccine) 2 days before AI delayed the ovulation by 4 days and resulted in significant reduction in the conception rate in the Hanwoo cows (Kim *et al*. 2021). A Brazilian study reported that administration of bivalent inactivated FMD vaccine (Ourovac® Aftosa; bivalent foot-and-mouth vaccine in oil emulsion, Brazil, 5 mL subcutaneous) significantly increased the pregnancy loss by 4-fold when the Nelore beef cows were vaccinated 30 days after timed-AI as compared with 31 days before timed AI and it was recommended FMD vaccination of the beef herds one month before the commencement of breeding season (Ferreira *et al*. 2016). Similarly, in a randomized controlled study conducted in Argentina, administration of FMD vaccine (Bioaftogen®; Biogénesis Bagó S.A., Buenos Aires, Argentina) to pregnant Aberdeen Angus beef heifers on gestation day 33 (n=311) resulted in a relative risk of pregnancy loss by 2.8 as compared to the non-vaccinated group, however, the study recommended repetition of the experiment using a larger sample size as the 95% confidence interval was wide (Marques *et al*. 2019). In a recent experiment with 3,379 beef cows in Uruguay, vaccination against FMD from gestation day 0 to 44 significantly increased the pregnancy failure rate by 2.4 to 7.8% (Garcia *et al.* 2021). Vaccination against bovine herpesvirus 1, bovine viral diarrhea virus, and leptospirosis between gestation day 30 and 90 did not affect the pregnancy rate in the parous Nelore cows (Ferreira *et al*. 2018). Considering the small sample size of n=47 and the vulnerability of the elongating conceptus to the vaccine-induced febrile response, the results of the study need to be tested with a larger dataset.

SUMMARY

As emerging evidence indicates that FMD vaccination causes variable degrees of pregnancy loss in the beef cow, the study was conducted at NDRI SRS in sub-tropical conditions in the year 2021 to investigate its effects on the pregnancy rate in the dairy cow when they were bred within 14 days of FMD vaccination. Retrospective data on AI done within 14 days post FMD vaccination (dpv -14 to +14) and the corresponding pregnancy outcome were collected for 6 years (January 2016 to January 2021) and each record was categorized into AI pre-vaccination (dpv -14 to -1) or AI post-vaccination (dpv 0 to 14). After excluding repeat breeding cows and pregnant heifers, the final dataset consisted of 47 records derived from crossbred and Deoni cows. The binary outcome variable pregnancy was regressed on the categorical predictor variables such as day of AI with respect to dpv 0, stage of lactation and parity. The results indicated that the odds of pregnancy in the AI post-vaccinated group was 0.47 (95% confidence interval: 0.10-1.88) value) as compared with AI pre-vaccinated group. It can be concluded that FMD vaccination has no significant effect on pregnancy outcome if the animals are bred 14 days before and 14 days after FMD vaccination (irrespective of breed, age, parity, days in milk).

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