



Study on the Risk Factors Analysis for Subclinical Endometritis in Cohort of Repeat Breeder Indigenous Cattle

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ABSTRACT

Background: The subclinical endometritis (SCE) has severe negative impacts over the fertility in bovines. There are several risk factors identified which predispose animals to SCE. The model development to evaluate the risk factors and population level impact in cohort of indigenous repeat breeder cows may be helpful for identification of cows at high risk of developing SCE.

Methods: A total of 90 indigenous cattle with the history of repeat breeding were screened for subclinical endometritis (SCE) through endometrial cytology technique. The 5% cut off value of PMNs was established as threshold for SCE. Events likely to predispose SCE were taken in to consideration to perform a risk factor analysis using univariate logistic regression and multiple logistic regression model building. The population attributable fraction was calculated to chalk out the final risk factors among all.

Result: The baseline disease prevalence established was 32.22% (29/ 90). Univariate analysis and multivariable logistic regression identified parity and retained fetal membranes (RFM) as the only independent risk factors to SCE. Multiparous status increased the odds of subclinical endometritis 7.75-fold (OR=7.75; 95% CI: 1.81-33.33; RR=2.75). RFM triggered even severe risk inflation (OR=15.87; 95% CI: 2.99-83.33; RR=4.60). The population attributable fraction (PAF) metrics showed that multiparity caused 48.3% of all cases, while RFM accounted for 43.2% of the cases. The multivariable framework demonstrated strong predictive validation with area under the curve (AUC) of 0.794. The study concluded that RFM is the more deadly pathology, therefore wide preventative strategies targeting the multiparous cows may yield the highest herd-level reduction in disease burden. This model may offer an accurate, cost-effective and even early warning filter for farm managers and the farmers.

Key words: Multivariable logistic regression, Parity, Population attributable fraction (PAF), RFM, ROC curve, Subclinical endometritis.

INTRODUCTION

Subclinical endometritis (SCE), is the endometrial inflammation without overt clinical signs (Sheldon *et al.*, 2019). Inadequate detection and management of SCE lead suboptimal conception rates and prolonging the calving intervals (Barajas *et al.*, 2018). The prevalence reported for SCE is high (Sheldon *et al.*, 2019), with negative impacts on reproductive performance (Gilbert *et al.*, 2005; Barajas *et al.*, 2018). The physio-pathological mechanism includes chronic inflammatory response of endometrium to long term persistent bacterial infections. The risk factors/etiology of SCE are still under debate that may include unspecific infection in the uterus, or persistent inflammatory response of the endometrium to bacterial infections (Wagener *et al.*, 2017). Many risk factors for SCE, like Negative energy balance (NEB), metabolic diseases, uterine infections during the puerperium period, calving assistance (CA) and RFM, have been identified previously in large-scale dairies (Dubuc *et al.*, 2010; Cheong *et al.*, 2011; Wagener *et al.*, 2017; Yanez *et al.*, 2022). Therefore, the present study is undertaken based on the novelty in the development of a multivariable predictive model for SCE in cattle, establishing multiple significant risk factors to estimate disease probability at the individual animal level. This approach provides a potential decision-support tool for early identification of affected animals and may complement existing diagnostic strategies for improving reproductive management in dairy herds.

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MATERIALS AND METHODS

Selection of animals

Ninety repeat breeder (RB) indigenous cows (BCS 3 or more) from District Meerut and nearby areas were selected for the current research. Thereafter visual inspection of the discharge and rectal palpation were performed, as well as transrectal ultrasound to evaluate the reproductive tract (6.5 MHz linear-array transducer, Minitube portable USG). All these animals were screened for SCE through cytobrush technique (Wagener *et al.*, 2017). All the animals under study were managed under optimum herd and nutritional managemental conditions.

Diagnosis of subclinical endometritis

The endometrial cytology was performed using modified cytobrush gun (Mayfair technologies, Ludhiana). The cytology gun fitted with brush was inserted aseptically into the uterine body, thereafter the plunger was pressed to externalize the cytobrush inside the uterine body, which was rotated gently against the uterine wall to collect an endometrial sample. Following collection of the sample, the cytobrush was retracted back in the rod to avoid contamination. Thereafter cytobrush was recovered and slide was prepared by rolling it on the glass slide and allowing it to air-dry. The slides were stained using field's stain and then examined at 400X magnification. The cut off value of PMNs was $\geq 5\%$ for declaring animal positive for SCE (Wagener *et al.*, 2017).

Risk factors

The puerperium and post-partum events were recorded based on the history and observation and the events of interest were recorded to determine the possible risk factors for SCE. Five age group categories were included in the present study: 4-5, 5-6, 6-7, 7-8 and 8-9 years. Five calving seasons (CS) within the year were taken into consideration for statistical analysis: the rainy season, autumn, spring, summer and winter. Two parity categories were included: primiparous and multiparous. The assisted delivery, RFM, calf sex and abortion were also recorded in the current study. Whether the animals suffered with any kind of uterine diseases (UD) first 3 weeks post-partum or not was also included in the study.

Statistical analysis

In the present study descriptive statistics using SPSS were utilized to summarize the distribution of explanatory variables and the status of subclinical endometritis (SCE). The frequencies and percentages were calculated for all categorical variables. The association between individual explanatory variables and SCE was assessed using the chi-square test, while fisher's exact test was applied when any cell's expected frequency in any of the cell was < 5 . The variables that reveal significant ($P \leq 0.35$) association with SCE in univariate analysis were further considered for multivariable analysis. Therefore, in the first stage,

univariate logistic regression was used to screen potential predictor variables using $P \leq 0.35$ as described by Villaseñor-Gonzalez *et al.* (2025) and in the second stage, these variables were entered into a multivariable logistic regression model with backward elimination and a retention criterion of $P < 0.10$. A relatively liberal screening threshold is commonly recommended in epidemiological and regression modeling studies to avoid excluding variables that may not appear significant in univariate analysis but may become important after adjustment for confounding in multivariable analysis. Under univariate binary logistic regression, crude odds ratios (OR) and their 95% confidence intervals (CI) were estimated. A backward stepwise elimination method was employed, retaining variables with $P < 0.05$ in the final model. Adjusted odds ratios (OR) with 95% confidence intervals were derived from the final model. Relative risks (RR) were estimated from adjusted odds ratios using the approach described by Zhang and Yu (1998). The proportion of exposed animals (Pe) and the population attributable fraction (PAF) were calculated to evaluate the relative contribution of significant risk factors to the occurrence of SCE. Statistical significance was determined at $P < 0.05$. The multicollinearity among the explanatory variables was evaluated using the Variance Inflation Factor (VIF) and no evidence of problematic multicollinearity was observed (all VIF values < 5).

RESULTS AND DISCUSSION

Explanatory variables and cohort baseline epidemiology

The 5-6 years age group showed dominant footprint, accounting for 35.6% ($n=32$) of the cows. Parity classification including primiparous and multiparous was relatively balanced, comprising 46.7% ($n=42$) and 53.3% ($n=48$) of the herd, respectively. The highest incidences were recorded during the rainy season (26.7%, $n=24$), followed by spring (23.3%, $n=21$) and winter (21.1%, $n=19$). The majority of animals had calving without requiring assistance (82.2%, $n=74$) and 96.7% ($n=87$) of the animals had no abortion history. The RFM and post-partum uterine diseases were diagnosed in 21.1% ($n=19$) and 22.2% ($n=20$) of the animals, respectively. The male and female calves comprised of 53.3% ($n=48$) and 46.7% ($n=42$) of the total births. The prevalence of SCE was established at 32.2% ($n=29$), leaving a baseline of 67.8% ($n=61$) consisting of negative cases (Table 1).

Univariate screenings and predictive associations

Univariate relationship screening through chi-square and fisher's exact tests established highly significant associations ($p < 0.05$) among SCE and primary explanatory variables, excluding the abortion history ($p=0.242$) and sex of the calf ($p=0.809$) (Table 2). Fisher's exact test was rigorously substituted across parameters where low cell frequencies violated standard chi-square distributional assumptions (Age group and abortion history).

To isolate localized directional effects, individual variables were evaluated using univariate binary logistic regression (Table 3). Parity ($p=0.004$), calving season ($p=0.099$), calving assistance ($p<0.001$), abortion history ($p=0.231$), RFM ($p<0.001$) and uterine disease ($p<0.001$) exhibited strong regression footprints. Applying an expansion threshold constraint of ($p\leq 0.35$) to capture weak or confounding interactions, parity, calving assistance, abortion history, RFM and uterine disease were cleared for multivariable entry (Villasenor-Gonzalez *et al.*, 2025) through backward stepwise selection method.

Multivariable logistic regression and model diagnostics

The independent risk configuration was mapped using multivariable logistic regression utilizing a backward stepwise selection sequence. Independent variables with non-significant structural impact were iteratively stripped, whereas variables with true predictive strength ($p<0.05$) were retained in the final framework.

The final model identified parity and RFM as the independent predictors of SCE. Model diagnostic tests showed that removing parity from the equation significantly compromised the overall model fit (Change in -2 Log

Table 1: Distribution of explanatory variables and outcome status.

Variable	Category	N	(%)
Age group	4 to 5 years	15	16.7
	5 to 6 years	32	35.6
	6 to 7 years	15	16.7
	7 to 8 years	13	14.4
	8 to 9 years	15	16.7
Parity	Primiparous	42	46.7
	Multiparous	48	53.3
Season	Spring	21	23.3
	Autumn	10	11.1
	Rainy	24	26.7
	Summer	16	17.8
	Winter	19	21.1
Calving assistance	No	74	82.2
	Yes	16	17.8
History of abortion	No	87	96.7
	Yes	03	3.3
RFM	No	71	78.9
	Yes	19	21.1
Sex of calve	Male	48	53.3
	Female	42	46.7
Uterine disease	No	70	77.8
	Yes	20	22.2
Subclinical endometritis	Negative cases	61	67.8
	Positive cases	29	32.2

Abbreviations: n= Frequency; %= Percentage; RFM: Retained fetal membranes.

Table 2: Association of explanatory variables with subclinical endometritis.

Variable	Test used	p-value
Age group	Fisher's exact test	.006
Parity	Chi-square	.003
Season	Chi-square	<0.001
Calving assistance	Chi-square	<0.001
History of abortion	Fisher's exact test	.242
RFM	Chi-square	<0.001
Sex of calf	Chi-square	0.809
Uterine disease	Chi-square	<0.001

$p<0.05$ was considered statistically significant.

Fisher's exact test was applied where expected cell frequencies were less than 5.

Likelihood=9.166; $p=0.01$). Similarly, the removal of RFM caused an even more drastic collapse in model performance (Change in -2 Log Likelihood=29.047; $p<0.01$), proving that both risk parameters are independently and highly significantly ($p<0.01$) tied to SCE development.

The definitive effect points and precision estimates are mentioned in Table 4. Multiparous animals demonstrated a 7.75-fold inflation in the odds of developing subclinical endometritis relative to primiparous animals (OR=7.75; 95% CI: 1.81-33.33). Furthermore, the presence of RFM generated a profound impact, with affected animals exhibiting a 15.87-fold increase in disease odds compared to their non-retained counterparts (OR=15.87; 95% CI: 2.99-83.33).

The corresponding true epidemiological risks showed a Relative Risk (RR) of 2.75 (95% CI: 1.31-5.78) for multiparity and an RR of 4.60 (95% CI: 2.71-7.80) for RFM. The PAF for multiparity and RFM stood at 48.3% and 43.2%, respectively (Fig 1). Predictive performance assessment yielded an area under the curve (AUC) of 0.794, demonstrating a strong, internally validated diagnostic fit (Fig 2).

Final analysis through multivariable logistic regression established both parity and RFM as significant risk factors for developing SCE (Fig 3). Multiparous cows had 7.75 times higher odds of developing SCE compared to primiparous (OR=7.75; 95% CI: 1.81-33.33). Similar to this, animals affected with RFM exhibited 15.87 times

higher odds of SCE than those not (OR=15.87; 95% CI: 2.99-83.33).

The chances of encountering SCE were found 2.75 times higher among multiparous cows (RR=2.75; 95% CI: 1.31-5.78) and it was 4.60 times higher in animals affected with RFM (RR=4.60; 95% CI: 2.71-7.80). The PAF indicated that out of total, 48.3% and 43.2% of SCE cases could be attributed to the multiparity and RFM, respectively. Also, high PAF for these both conditions (aiming to reduce or treat) suggest need for preventive strategies against these two risk factors so as to deal with SCE.

Several herd-level risk factors to SCE like CS, RFM, UD, parity, metabolic diseases like negative energy balance, calving assistance, calf sex and timing of sampling after parturition have been identified in previous studies (Kasimanickam *et al.*, 2004; Dubuc *et al.*, 2010; Cheong *et al.*, 2011; Prunner *et al.*, 2014; Yanez *et al.*, 2022; Villasenor-Gonzalez *et al.*, 2025) with the prevalence reported ranging from 7 to 53% between 3rd to 7th week post-partum (Quintela *et al.*, 2018; Villasenor-Gonzalez *et al.*, 2025). Among routine screened animals, prevalence reported was 6.35% (Narwade *et al.*, 2026), while among repeat breeder cows, the prevalence reported is between 12.7 to 52.7% (Salasel *et al.*, 2010; Janowski *et al.*, 2013; Pothmann *et al.*, 2015; Pascottini *et al.*, 2017; Wagener *et al.*, 2017; Bedewy and Rahawy, 2019; Emre, 2024; Pande *et al.*, 2025; Villar *et al.*, 2025).

Table 3: Univariate logistic regression analysis of factors associated with subclinical endometritis.

Variable	Crude OR	95% CI	p-value
Age	-	-	0.460
Parity	0.236	.088-.636	0.004
Calving season (CS)	-	-	0.099
Calving assistance	0.099	0.028-0.349	<0.001
History of abortion	0.225	0.020-2.589	0.231
RFM	0.042	0.011-0.166	<0.001
Sex of calf	1.115	0.459-2.710	0.809
Uterine disease	0.057	0.016-0.199	<0.001

OR= Odds ratio; CI= Confidence interval.

Variables with $p\leq 0.35$ were considered for multivariable logistic regression analysis.

For age group and season, multiple category-specific ORs and 95% CIs were generated relative to the reference category; therefore, a single crude OR was not presented.

Table 4: Multivariable logistic regression model for risk factors associated with subclinical endometritis.

Risk factor	Comparison	OR	OR 95% CI	RR	RR 95% C.I.	Pe	Pe 95% C.I.	PAF
Parity	Multiparous vs primiparous	7.75	1.81-33.33	2.75	1.31-5.78	0.533	0.430-0.636	0.483
RFM	Yes vs no	15.87	2.99-83.33	4.60	2.71-7.80	0.211	0.127-0.295	0.432

RFM= Retained fetal membrane; OR= Adjusted odds ratio; 95% CI= 95% confidence interval; RR= Relative risk; Pe= Proportion of animals exposed to the respective risk factor; PAF= Population attributable fraction. Variables with $P\leq 0.05$ were retained in the final multivariable logistic regression model.

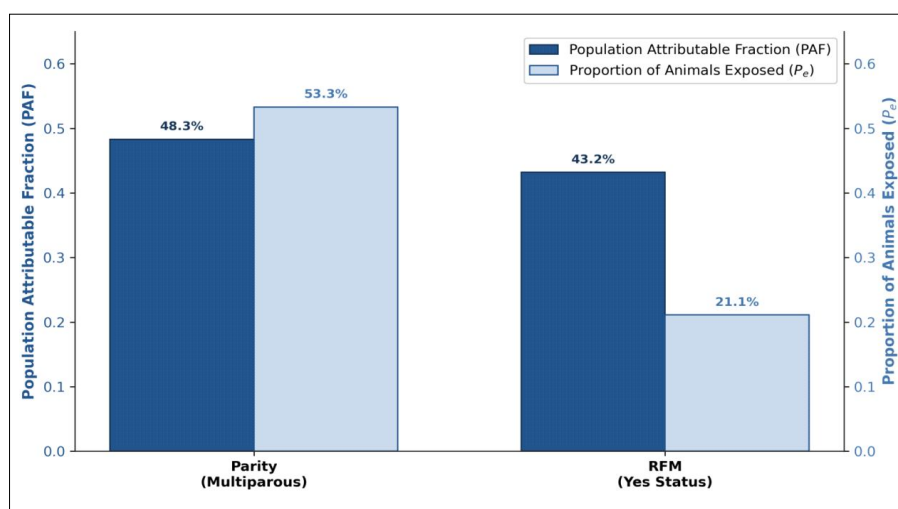


Fig 1: Population attributable factor (Multivariable logistic regression) for risk factors associated with SCE.

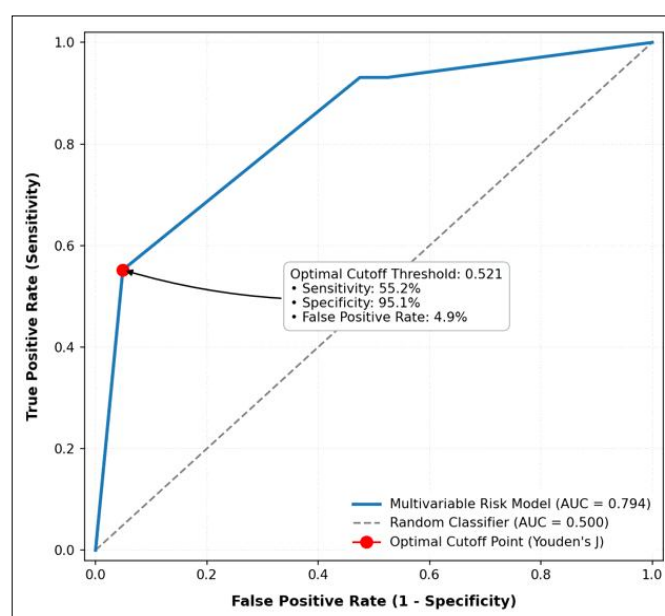


Fig 2: Receiver operating characteristic (ROC) curve validating the multivariable diagnostic risk model.

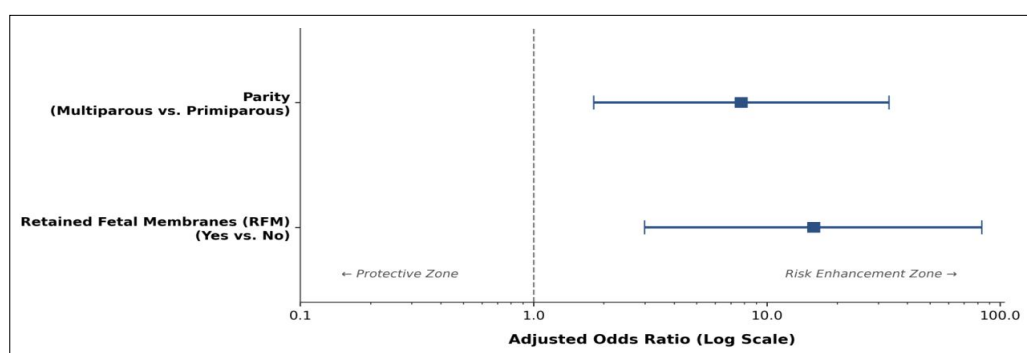


Fig 3: Multivariable logistic regression model for risk factors associated with SCE.

Risk factors analysis in a previous study for SCE in RB cows including, season, parity, reproductive pathologies, no variable revealed significant effect, however in the present study the parity and RFM showed significant footprints. However similar to previous study numerical difference in case of season and reproductive pathology were noticed in present study too (Villar *et al.*, 2025). However, in other studies over effect of reproductive pathologies, statistically higher incidence of SCE was noticed among animals suffering with post-partum reproductive pathologies (Salasel *et al.*, 2010). The effect of parity in occurrence of SCE has also been observed in previous studies similar to the present study. Also, in those studies during first artificial insemination the month/season of AI also seem to affect significantly the occurrence of SCE (Pascottini *et al.*, 2017; Diaz-Lundahl *et al.*, 2021) however in the present study season during calving does not seem to affect occurrence of SCE similar to previous study (Prunner *et al.*, 2014) however another study differs with this opinion (Villasenor-Gonzalez *et al.*, 2025). In previous studies uterine infections and CA were identified as risk factors (Dubuc *et al.*, 2010; Cheong *et al.*, 2011; Villasenor-Gonzalez *et al.*, 2025) but the present study CA didn't seem to affect occurrence of SCE. RFM may be a sequence from CA and obstetrical procedures (Montiel-Olguin *et al.*, 2019). RFM, altering the immunity status (Chebel, 2021) may be the reason for it being the predisposing factor for SCE. The multiparity in cows lead to increased uterine trauma and slower involution following repeated calvings. Also, reduced postpartum immune competence, higher post-partum disorders (e.g. RFM), greater metabolic stress and impaired bacterial clearance may potentially predispose multiparous cows to SCE (Kasimanickam *et al.*, 2004; Sheldon *et al.*, 2006; LeBlanc, 2008; Sheldon *et al.*, 2009; LeBlanc, 2012).

The indigenous cattle are often raised by marginal/smallholders, where routine postpartum reproductive examinations are often impractical because of limited veterinary access and financial constraints, the established risk factors may be used to prioritize the animals at risk for timely examination and proper intervention. This model/targeted strategy may enable efficient use of veterinary services, may reduce unnecessary diagnostic costs and may support reproductive efficiency and farm profitability.

LIMITATIONS

The limitation of the present study includes relatively small sample size ($n = 90$), which may have limited the statistical analytical power and the precision of the estimated variable associations i.e. wide confidence interval. Also, the animals were based out of a single geographical region, which may have limited the generalizability of the findings to other geographical conditions. The indigenous cattle were included in the present study, therefore the established risk factors may not directly applicable to other crossbred or exotic breeds. The cross-sectional observational design

allows identification of associations but does not permit causal inferences. It is recommended that future studies involving larger sample size, multi-regional sampling comprising different breeds and longitudinal study designs are warranted to further validate and extend the current findings.

CONCLUSION

There is significant prevalence of SCE among indigenous repeat breeder cattle. Preliminary univariate analysis established a wide array of seasonal and management risks factors, while multivariable modelling demonstrates that parity and RFM are the important cow level drivers of endometrial inflammation. Population-level metrics has established that although RFM appears to be more severe anomaly, the widespread distribution of multiparous animals within the herds causes multiparity to exert a greater overall burden on the herd's reproductive health (PAF=48.3%). Finally, the strong internal predictive performance (AUC=0.794) of our multivariable model confirms that combining simple, farm-gate operational records provides a highly reliable early screening tool. This diagnostic approach allows managers/farmers to identify cows at high-risk early enabling preventive strategies to optimize reproductive health management without immediately requiring, invasive diagnostic interventions.

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Disclaimers

The views and conclusions included in the present study belong to the authors and authors are solely responsible for the accuracy of the information provided, but do not accept liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All the animal procedures for experiments were approved by the Institutional Animal ethical Committee as per approval number (IAEC/SVPUAT/2025/177).

Conflict of interest

The authors declare no conflict of interest regarding publication of the present article. No funding or sponsorship influenced the design of the present study, data collection analysis, decision to prepare and publish the manuscript.

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