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REGULAR ARTICLES

Risk factors and impact of retained fetal membranes on performance of dairy bovines reared under subtropical conditions

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Abstract The risk factors and impact of retained fetal membranes (RFM) on productive and reproductive performance of crossbred cattle, Zebu cattle, and Murrah buffalos were evaluated using data spread over 12 years. Multivariable logistic regression model was used to identify risk factors and to quantify their odds ratio (OR). Overall incidence of RFM in crossbred cattle, Zebu cattle, and Murrah buffalos were 26, 16, and 13 %, respectively; and significant risk factors for RFM in crossbred cattle were abortion (OR=3.9), dead calf (OR=4.1), dystocia (OR=4.3), pluriparity (OR=1.5), and shorter gestation length (OR=4.3). In Zebu cattle, abortion (OR=4.0), dead calf (OR=3.7), dystocia (OR=3.9), lower birth weight of calf (OR=1.6), and shorter gestation length (OR=6.4) were significant risk factors for RFM. In Murrah buffalos, abortion (OR=19.2), dead calf (OR=4.4), dystocia (OR=4.7), pluriparity (OR=1.7), shorter gestation length (OR=12.7), and calving during summer season (OR=1.8) were the risk factors for RFM. Although the occurrence of RFM did not affect fertility parameters, a significant ($P<0.05$) decrease in 305-day milk yield and total milk yield was observed in RFM-affected crossbred cattle. Taken together, it may be concluded that increased parity, abnormal calving, and short gestation length were the main risk factors for RFM in dairy bovine.

Keywords Retained fetal membranes · Crossbred · Zebu buffalos · Risk factors · Productive · Reproductive

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Introduction

Dairy cattle and buffalos can suffer from several reproductive disorders; retained fetal membranes (RFM) is one of the most important since it is a risk factor for uterine and udder infections, metabolic diseases like ketosis and displaced abomasum, and reduced pregnancy rate and milk production (LeBlanc 2008). In healthy cows, fetal membranes are expelled within 3–6 h after parturition; and if it extends beyond 8–12 h, the condition is called RFM (Hanafi et al. 2011). The incidence of RFM is reported to range from 4 to 18 % of calvings (Han and Kim 2005; Hossein-Zadeh and Ardalan 2011). Negative effects of RFM include decreased milk production (Gaafar et al. 2010), longer intervals to the first estrus and first breeding, lower probability of conception at the first breeding, longer interval to conception, higher incidence of metritis (LeBlanc 2008), and increased culling rate in dairy bovines (Beagley et al. 2010). Although the etiology of RFM remains uncertain, abnormal calving (dystocia, stillbirth, twinning, length of gestation), parity, calving season, calf birth weight, and nutritional imbalance were reported as risk factors for the development of RFM in exotic cows reared under temperate regions (Sheldon et al. 2006). Although the information on the risk factors for the occurrence of RFM in exotic cattle reared under temperate regions are available for Zebus

and crossbred cattle, the information is scanty and sporadic.

Since it has been reported that the risk factors for RFM and the consequences of RFM on productive and reproductive performance vary depending on the climatic variability, managerial practices and herd health control measures followed (Han and Kim 2005). The present study was undertaken to investigate the risk factors associated with the occurrence of RFM and its effects on subsequent milk production and postpartum reproductive performance in crossbred cattle, Zebu cattle, and Murrah buffalos reared under subtropical conditions.

Materials and methods

Data collection and classification

The calving data of Zebu (Sahiwal), crossbred (Holstein-Friesian X Tharparkar) cows, and Murrah buffalos (spread over 12 years; 2001–2012) maintained at Livestock Research Centre, Dairy Cattle Breeding Division and Animal Health Complex at the National Dairy Research Institute, Karnal, were utilized for the study. The data related to milk production (305-day milk yield, total milk yield per lactation), reproduction (parity, calving date, sex and body weight of calf, days to the first AI, service period, calving interval, and services per conception), and the occurrence of reproductive disorders (retained fetal membrane, abnormal calving) were utilized for analysis.

The data of RFM was classified according to parity, period of calving, season of calving, gestation period, sex and birth weight of calf, and assigned separate codes for statistical analysis. The animals were classified into 1st, 2nd–3rd, 4th–5th, and >6th parities. Seasons were classified into winter (December to March), summer (April to June), rainy (July to September), and autumn (October and November). Gestation period was classified into <275, 275–290, >290 days for crossbred and Zebu cows and <295, 295–315, >315 days for buffalos. Birth weights of the calves were grouped into different categories based on the average birth weight of the farm (≤21, 22–31, ≥32 kg for crossbred calves; ≤17, 18–23, ≥24 kg for Zebu calves; and ≤27, 28–38, ≥39 kg for buffalo calves). Grouping of gestation period and birth weight was carried out using the formula: mean±2 standard deviation.

Statistical analysis

The data were analyzed using general linear models in SPSS-16. Duncan test was used to determine the degree of significance between the means. The data on factors affecting RFM were analyzed using the following model:

$$\mathrm{Yijklmh} = u + \mathrm{Ai} + \mathrm{Bj} + \mathrm{Ck} + \mathrm{Dl} + \mathrm{Fm} + \mathrm{Gh} + (\mathrm{ABCDFG})\mathrm{ijklmh} + \mathrm{Eijklmh}$$

Where

Yijklmh	the intended recorded performance value for each individual cow
u	the overall mean
Ai	the effect of parity
Bj	the effect of calving season
Ck	the effect of weight of calves born
Dl	the effect of the type of calving
Fm	the effect of gestation length
Gh	the effect of calf sex

Multivariable logistic regression analysis was done using SYSTAT-12 for the prediction of RFM in relation to season, type of calving, gestation length, and sex and body weight of calf by using the following model. Variables (main effects or interaction terms) which were significant by the Wald statistic at $P<0.05$ were included in the final models.

The general equation of logistic regression model was defined as follows:

$$\mathrm{Logit}(\pi) = \alpha + \mathrm{b1X1} + \mathrm{b2X2} + \_\_\_ + \mathrm{bnXn}$$

Where π was the probability of RFM, α was the intercept parameter, and b1 to bn were the logistic regression coefficients (parameter estimates) for the explanatory effects (X1 to Xn) included in the statistical model. Final model used to analyze RFM included the fixed class effects of dystocia, dead calves, abortion (where absence or presence of each of the three conditions was scored as "0" or "1", respectively), parity, calving season, lower birth weight of calves, and gestation length. The logistic regression models produced odds ratio (OR) as estimates of the strength of the association between the potential risk factors and RFM.

Least squares analysis

Least squares analysis was used for analyzing data related to production (305-day milk yield (kg), total milk yield (kg), and lactation length) and reproduction (day of first AI, service period, calving interval, and service per conception) for comparison between normal and RFM cows. The following model was used for this purpose:

$$\mathrm{Yij} = \mu + \mathrm{Ti} + \mathrm{Eij}$$

Where Yij=different productive and reproductive traits, μ is over all mean, and Ti=condition (1 for normal calving and 2 for RFM). Eij=experimental error associated with i and j observations assumed to be randomly distributed.

Results

Risk factors for retained fetal membranes

Estimated odds ratio (OR), parameter estimates, and their 95 % confidence intervals for risk factors associated with RFM in crossbred cows, Zebu cows, and Murrah buffalos are given in Tables 1, 2, and 3, respectively. In crossbred cows, the incidence of RFM increased significantly ($P<0.05$) after dystocia (OR=4.3), abortion (OR=3.9), dead calf (OR=4.1), pluriparity (OR=1.5), and short gestation length (OR=4.3). Similarly, in Zebu cows, the chance of RFM increased significantly ($P<0.05$) after dystocia (OR=3.9), abortion (OR=4.0), dead calf (OR=3.7), lower birth weight of calf (OR=1.6), and short gestation length (OR=6.4). In Murrah buffalos, there were significant interaction effects ($P<0.05$) of RFM with dystocia (OR=4.7), abortion (OR=19.2), dead calf (OR=4.4), pluriparity (OR=1.7), short gestation length (OR=12.5), and calving during summer season (OR=1.8).

The overall incidence of RFM was 26, 16, and 13 % in crossbred cows, Zebu cows, and Murrah buffalos, respectively. The season of calving had a significant ($P<0.05$) effect on incidence of RFM in buffalos (high incidence during summer and low incidence during autumn) but not in crossbred and Zebu cows (Table 4). The incidence of RFM was significantly ($P<0.05$) lower in first parity compared to other parities. Although non-significant, the birth of male calves tended to increase the risk of incidence of RFM compared to the birth of female calves.

In both cattle and buffalos, shorter gestation period was significantly ($P<0.05$) associated with increased incidence of RFM. Birth weight of calf did not influence the incidence of RFM in crossbred and Zebu calves; however, in buffalos, lower birth weight of the calves was significantly ($P<0.05$) associated with higher incidence of RFM (16 %).

Effect of RFM on productive and reproductive performance

Effect of RFM on performance of crossbred cows, Zebu cows, and Murrah buffalos is given in Table 5. RFM-affected crossbred cows had significantly ($P<0.05$) lower 305-day milk yield and total milk yield compared to the cows that normally expelled the fetal membranes. However, such effect was not observed in Zebu cows and Murrah buffalos. The effect of RFM on reproductive performance was non-significant in crossbred cows, Zebu cows, and Murrah buffalos.

Effects of RFM on incidence of metritis

Significantly ($P<0.05$) higher incidence of metritis was observed in RFM-affected crossbred, Zebu cows, and Murrah buffalos compared to those that expelled the fetal membranes normally. In crossbred cows, 77 % (519/671) of RFM-affected cows later developed postpartum metritis while only 3 % (58/1875) of cows that expelled fetal membranes normally developed metritis ($P<0.05$). Similarly, 54 % (135/251) Zebu cows with RFM developed metritis, however, only 2 % (31/1358) developed metritis in normal calving group ($P<0.05$). Out of 210 RFM-affected buffalos, 136 (65 %) developed metritis; but out of 1349 normally calved buffalos, only 21 (2 %) developed metritis ($P<0.05$) indicating that RFM was a risk factor for metritis development.

Table 1 Estimated odds ratio, parameter estimates, and their 95 % confidence intervals (CI) for the risk factors associated with retained fetal membranes in crossbred cows (n=2547 calvings)

Variables	Class	Estimate±SE	95 % CI	Odds ratio	95 % CI	P value
Dystocia	0	–	–	–	–	<0.001
	1	1.5±0.3	0.9–2.1	4.3	2.4–7.8	–
Abortion	0	–	–			<0.001
	1	1.4±0.1	1.1–1.6	3.9	3.0–5.2	–
Dead calf	0	–	–	–	–	<0.001
	1	1.5±0.2	1.0–1.9	4.1	2.8–6.6	–
Parity	1	–	–	–	–	<0.01
	≥2	0.4±0.2	0.1–0.7	1.5	1.1–2.0	–
Gestation period	<275	1.5±0.2	1.0–1.9	4.3	2.8–6.6	<0.001
	275–290	–	–	1	–	–
	>290	0.1±0.2	−0.3–0.5	1.1	0.8–1.7	–

Table 2 Estimated odds ratio, parameter estimates, and their 95 % confidence intervals (CI) for the risk factors associated with retained fetal membranes in Zebu cows (n=1609 calvings)

Variables	Class	Estimate±SE	95 % CI	Odds ratio	95 % CI	P value
Intercept	–	−1.9±0.1	−2.1–(−1.8)	–	–	–
Dystocia	0	–	–	1	–	<0.05
	1	1.4±0.6	0.2–2.6	3.9	1.2–12.9	–
Abortion	0	–	–	–	–	<0.001
	1	1.4±0.2	1.0–1.8	4.0	2.7–5.8	–
Dead calf	0	–	–	–	–	<0.001
	1	1.3±0.4	0.6–2.0	3.7	1.8–7.6	–
Birth weight of calf	<18	0.5±0.2	0.0–0.9	1.6	1.0–2.5	<0.05
	19–26	–	–	1	–	–
Gestation period	<275	1.9±0.2	1.9–2.3	6.4	4.0–10.5	<0.001
	275–290	0.4±0.2	0.0–0.8	1.5	1.0–2.3	–
	>290	–	–	1	–	–

Discussion

Retained fetal membranes (RFM) is a substantial risk factor for metritis and endometritis, eventually leading to impaired reproductive performance in dairy animals. The incidence of RFM varies with species, breed, management practices, and agro-climatic conditions. The present study estimated the risk factors for RFM along with their odds ratio and studied the impact of RFM on performance of crossbred cattle, Zebu cattle, and Murrah buffalos.

The present study observed a higher incidence of RFM in buffalos during summer compared to other seasons. Higher incidence of RFM during summer has already been reported in crossbred (Shivhare et al. 2013) and Friesian (Hossein-Zadeh and Ardalan 2011) cows. We observed that summer season markedly increased the chance of development of RFM by 1.75 times in Murrah buffalos. Variations in the availability of green fodders, a primary source of carotene, from season to seasons might have contributed to the seasonal variations in the incidence of RFM condition in bovines. The incidence of RFM increased with increasing parities in all the three breeds studied, which might be due to the decrease in uterine muscle tonicity with increasing age. We observed that the chance of RFM was 1.5 and 1.7 times higher in

Table 3 Estimated odds ratio, parameter estimates, and their 95 % confidence intervals (CI) for the risk factors associated with retained fetal membranes in Murrah buffalos (n=1604 calvings)

Variables	Class	Estimate±SE	95 % CI	Odds ratio	95 % CI	P value
Intercept	–	−2.4±0.1	−2.6–(−2.2)	–	–	
Dystocia	0		–	1	–	<0.01
	1	1.6±0.6	0.3–2.8	4.7	1.4–16.6	–
Abortion	0	–	–	1	–	<0.001
	1	3.0±0.2	2.5–3.4	19.2	12.5–29.5	–
Dead calf	0	–	–	1	–	<0.001
	1	1.5±0.4	0.8–2.2	4.4	2.1–9.0	–
Parity	1	–	–	1	–	<0.001
	≥2	0.5±0.2	0.2–0.9	1.7	1.2–2.4	–
Gestation period	<295	2.5±0.4	1.8–3.2	12.5	6.3–25.0	<0.001
	296–315	–	–	1	–	–
	>316	0.4±0.3	−0.3–1.1	1.5	0.7–2.9	–
Season	1	0.3±0.2	−0.2–0.7	1.3	0.8–2.0	<0.05
	2	0.4±0.3	0.1–1.1	1.8	1.1–2.9	–
	3	0.3±0.2	−0.3–0.6	1.2	0.9–1.9	–
	4	–	–	1	–	–
Birth weight of calf (kg)	<27	0.7±0.4	−0.1–1.4	2.0	1.0–3.9	<0.05
	28–38	−0.2±0.3	−0.8–0.3	0.8	0.4–1.4	–
	>39	–	–	1	–	–

Table 4 Effect of period and season of calving, parity, and type of calving on incidence of retention of fetal membranes

Factor	Crossbred (n=2549)		Zebu (n=1609)		Murrah (n=1604)	
	No. of calving	Incidence of RFM	No. of calving	Incidence of RFM	No. of calving	Incidence of RFM
Season						
Winter	914	236 (26)	683	107 (16)	443	58^{ab} (13)
Summer	586	149 (25)	446	66 (15)	263	45^{b} (17)
Rainy	661	190 (28)	300	45 (13)	605	75^{ab} (12)
Autumn	388	94 (24)	178	32 (17)	293	31^{a} (11)
Parity						
1st	777	151^{a} (19)	427	63 (15)	502	47^{a} (10)
2nd–3rd	1032	284^{b} (28)	668	101 (15)	678	97^{ab} (14)
4th–5th	448	148^{b} (33)	343	62 (18)	266	39^{ab} (15)
>6	292	87^{b} (30)	171	27 (16)	158	26^{b} (17)
Type of calving						
Normal	2154	457^{a} (21)	1423	179^{a} (13)	1444	116^{a} (8)
Abnormal	395	216^{b} (51)	186	73^{b} (39)	160	93^{b} (58)

Figures in parenthesis indicate percentage. Means with different superscript (a, b, c, d, and e) in a column within a single parameter differ significantly (P<0.05)

multiparous cows and buffalos compared to primiparous cows and buffalos. Melendez et al. (2006) also reported that primiparous cows had lower incidence of RFM than multiparous cows. Similarly, Hossein-Zadeh and Ardalan (2011) found that the chance of RFM increased by 2.69 times in multiparous cows compared to primiparous cows. However, Konyves et al. (2009) reported that parity did not influence the incidence of RFM in Holstein-Friesian cows.

An increase in the incidence of RFM with the decrease in fetal birth weight observed in the present study may be due to the lower pressure of the fetus on the fetal membrane, which possibly caused weaker attachment between the cotyledons and fetal membranes leading to difficulty in expulsion of the fetal membrane. On contrary, Gaafar et al. (2010) reported higher incidence of RFM with high birth weight of calves. Although we observed higher incidence of RFM in cows and buffalos that gave birth to male calves, the difference was statistically not significant. Earlier reports also indicate higher incidence of RFM in cows that gave birth to male calves (Majeed et al. 2009; Gaafar et al. 2010). It is possible that the androgen from the fetal testes might affect the process of fetal membrane separation, as opined by Gaafar et al. (2010).

Table 5 Effect of RFM on productive and reproductive performance in crossbred, Zebu cows, and Murrah buffalos

Parameter	Crossbred		Zebu		Murrah	
	With RFM	Normal calving	With RFM	Normal calving	With RFM	Normal calving
305-day milk yield (kg)	3369^{a}±77 (526)	3640^{b}±44 (1525)	1649±79 (147)	1723±38 (841)	1902±77 (125)	2034±44 (988)
Total milk yield (kg)	3844^{a}±110 (526)	4125^{b}±61 (1525)	1705±89 (147)	1789±43 (841)	2013±57 (125)	2335±61 (988)
Lactation length (days)	350±10 (505)	343±6 (1484)	251±4 (166)	243±4 (941)	293±10 (125)	285±6 (1021)
Day of first AI (days)	97±5 (407)	85±4 (1215)	93±4 (167)	86±2 (891)	98±8 (95)	89±7 (755)
Service period (days)	165±9 (407)	151±5 (1215)	147±9 (167)	135±5 (891)	157±9 (95)	145±5 (755)
Service per conception (no.)	2.3±0.5 (407)	1.9±0.4 (1873)	2.2±0.4 (250)	1.8±0.7 (897)	2.5±0.5 (199)	2.1±0.4 (1235)
Calving interval (days)	458±10 (378)	432±6 (1215)	431±10 (142)	414±5 (845)	445±10 (87)	418±6 (786)

Figures in parenthesis indicate no. of observation. Means with different superscript (a and b) in a row differ significantly (P<0.05)

Adverse effect of abnormal caving on the incidence of RFM was more marked in buffalos than in crossbred or Zebu cows. Risk factors like dystocia, abortion, and dead calves (still birth) were also associated with increased chance of RFM in both cows and buffalos, but risk were higher in buffalos compared to cows. Abortion and dystocia may cause circulatory disturbances and thereby impair the normal detachment of fetal membranes resulting in RFM. Earlier reports also suggest that calving problems including dystocia and stillbirths were associated with RFM (Hossein-Zadeh and Ardalan 2011). Increased incidence of RFM in cows with dystocia might also be due to the lack of tonicity and slow involution or damage to the uterus by mechanical stress resulting from calving difficulties. Similar to our results, other researchers also reported increased chance of RFM in cows with abortion, stillbirth, and dystocia (Majeed et al. 2009).

The results of the present study indicate that the interval from calving to first service, service period, service per conception, and calving interval were higher in RFM cows compared to normal cows, but the difference was not statistically significant. Melendez et al. (2006) and Konyves et al. (2009) also reported that RFM did not exert a significant influence on fertility parameters. However, Bar and Ezra (2005) reported that RFM had a significant negative effect on milk yield for several weeks after calving. We also observed a significantly lower milk production in RFM-affected crossbred cows compared to normal cows; however, such difference was not observed in Zebu cows and buffalos. Higher incidence of metritis in RFM-affected animals, as observed in the present study, might also contribute for reduced milk production in affected animals. It has been reported that RFM delays uterine involution and lead to endometritis, metritis, and subfertility (Han and Kim 2005; Hossein-Zadeh and Ardalan 2011). After RFM, the chance of bacterial infection increases since RFM maintains the patency of the cervix, which is otherwise a strong physical barrier to the infections in unaffected animals. RFM also delays uterine involution, expulsion of lochia, and the regeneration of the endometrium (Sheldon et al. 2006). Another possibility is the compromised immune function in RFM-affected cows (Boro et al. 2014). However, the proportion of RFM-affected animals turning into metritis was significantly high in crossbred cattle compared to Zebu cattle and Murrah buffalos, indicating that these animals might have strong mechanisms to combat the infectious agents, a hypothesis yet to be proved.

Conclusions

It may be concluded that the period of calving, parity, calving condition, and gestation length significantly influence the incidences of RFM in dairy bovines. Although the occurrence of RFM did not affect fertility parameters, the proportion of RFM-affected animals developing metritis were high in crossbred compared to Zebu cows and Murrah buffalos. Similarly, the negative effect of RFM on milk production was prominent in crossbred cattle compared to Zebu cows and Murrah buffalos.

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Conflict of interest The authors declare that there are no conflicts of interests.

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