



## Influence of Mastitis Type on Reproductive Performance of Karan Fries Cows During Early Lactation

Arpita Mohapatra<sup>1\*</sup> and Manju Ashutosh<sup>2</sup>

<sup>1</sup>Animal Physiology and Biochemistry Division, ICAR-CSWRI, Avikanagar, Rajasthan, INDIA

<sup>2</sup>Dairy Cattle Physiology Division, ICAR-National Dairy Research Institute, Karnal, Haryana, INDIA

\*Corresponding author: A Mohapatra; E-mail: arpita.ndri.mohapatra1@gmail.com

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### ABSTRACT

The present study was designed to evaluate the influence of mastitis type (clinical and subclinical) on reproductive performance of crossbred cows. 50 pregnant Karan Fries (KF) cows were screened from NDRI cattle yard. Based on milk somatic cell count (SCC) and modified Californian Mastitis Test (mCMT) cows were grouped in to three groups [Healthy, Sub-clinical mastitis (SCM) occurring within 1 week of calving and SCM occurring between 7<sup>th</sup> to 8<sup>th</sup> week of calving]. Five of the selected SCM cows showed systemic signs of clinical mastitis (CM) in their later life, so formed a separate group. Blood samples were collected from these cows from day calving at weekly interval till +90th days post-calving for plasma progesterone profiling. The animals were kept under observation to find out number of days to first artificial infection (AI), inter estrus interval, services per conception, day's open and calving interval. All these parameters were unaffected in cows with SCM occurring at any time during early lactation. Cows with CM after first AI had increased services per conception, increased days open and prolonged calving interval. In brief, this study shows SCM had a less pronounced effect on reproductive performances of KF cows than CM. Therefore, reduction of CM incidence during early lactation can improve the reproductive performance of crossbred cows and ultimately give economic benefit to the cattle owner.

**Keywords:** Subclinical mastitis, clinical mastitis, reproductive performance, early lactation

Profitable and efficient milk production from a dairy cow depends upon how placidly it's early lactation period progresses. Diseases during this period affect milk yield and reproductive efficiency that may end with culling decisions. Mastitis is one of the economically most imposing diseases as it is difficult to control and lacks simple solution. The economic impact of mastitis on dairy herds is related to reduced milk production, reduced milk quality, increased costs with treatment, discarded milk and increased risk for culling (Holland *et al.*, 2015). The negative effects of mastitis during early lactation not only limited to this. It indirectly affects the reproductive performance of dairy cow (Gunay and Gunay, 2008). Reproductive efficiency is of great concern for dairy farmers. Each day an animal fails to establish pregnancy the farmer incurs monetary losses of more than 90 days

(Fetrow and Blanchard, 1987). Reproductive efficiency is essential for the maintenance of consistently high levels of milk production. There are reports showing that development of CM during early lactation make the cow less likely to conceive (Yang *et al.*, 2012). SCM on the other hand, is an invisible malady. SCM represents a significant proportion (20–25%) of the burden of mastitis in modern dairy management (Hegde *et al.*, 2013). However, little is known about the effects of SCM during early lactation on reproductive performance and also very few studies are available till date which can show association of mastitis with reproductive performance of crossbred dairy cows in tropical condition. Thus, the objective of the present study was to compare the effects of SCM and CM during early lactation on reproductive performance of crossbred cows.



## MATERIALS AND METHODS

### Selection of animals

For the study, 50 Karan Fries cows approaching parturition were selected from the experimental herd of National Dairy Research Institute (NDRI), Karnal, Haryana (latitude 29.43° N & longitude 77.2° E), India. All the experimental animals were healthy and free from any anatomical, physiological and infectious disorders. All these cows were offered ad lib green fodder and calculated amount of concentrate mixture. Fresh tap water was also made available ad lib at all times of the day.

### Blood collection schedule

Blood samples (10 ml/animal) were collected from freshly calved cows at weekly interval till 90<sup>th</sup> day post-calving for plasma progesterone assay posing minimum disturbance to animals into sterile heparinized vacutainer tubes from jugular vein puncture. Immediately after collection blood, samples were transported to the laboratory in ice for further processing.

### Grouping of cows

Milk from different udders of each cow was tested for screening cows with mammary infection i.e. SCM. Animals were screened by conducting cow side Modified California mastitis test (mCMT) by commercially available kit Masti Check (Avantor Performance Materials India Ltd., New Delhi, India) as per manufacturer's protocol and further confirmed by performing milk SCC microscopically ( $5.28 \pm 0.45 \times 10^5$  cells/ml in SCM compared to  $1.65 \pm 0.16 \times 10^5$  cells/ml in healthy cows). Out of these SCM animals five animals showed the signs of Clinical Mastitis (CM) between 90<sup>th</sup> to 120<sup>th</sup> days of lactation. These animals were selected from animal health complex of institute, as they develop SCM after the study period, by a varying degree of clinical symptoms exhibited, e.g. swelling of udder, fever, anorexia, and clinical changes in physical characters of milk (consistency, colour, etc.) with a high milk somatic cell counts (SCC) ( $9.1 \pm 0.91 \times 10^5$  cells/ml).

The cows were further grouped based on the time of development of mastitis. Group I cows (n=10; out of healthy ones only 10 numbers are randomly taken in

group I) were healthy during the entire period of study without sign of any postpartum complications; Group II cows (n=07) were those who developed SCM within one week of calving; Group III cows (n=08) were those who developed SCM in 7<sup>th</sup> to 8<sup>th</sup> week of calving. Group IV cows (n=05; 1 from group I and 4 from group III) were those who developed CM between 90-120 days (these animals). Plasma progesterone was analyzed by commercially available ELISA kit (Mybiosource. com) for 90 days. Reproductive performance included calving to first service interval, inter-estrus interval, calving to conception interval/days open, the number of services per conception and calving interval. Pregnancy was confirmed by rectal palpation 60–65 days after artificial insemination (AI).

### Statistical analysis

All analysis was done using SYSTAT software package. Data from different experiments are presented as mean  $\pm$  SE. Significance was tested by employing one way ANOVA and two way ANOVA.

## RESULTS AND DISCUSSION

The mean  $\pm$  SE values of plasma progesterone levels during early lactation period for all the four groups have been presented in Table 1. All these depict that plasma progesterone levels on the day of calving in all the four groups were at their nadir which was requisite for commencement of parturition and are similar to the findings of Takagi *et al.*, (2002). Mallick and Prakash, (2011) observed that the plasma progesterone levels declined significantly from 15<sup>th</sup> day prepartum ( $4.38 \pm 0.67$  ng/ml) to minimal level on the day of calving ( $0.44 \pm 0.03$  ng/ml) which are similar to the observations of present study. The plasma progesterone levels remain almost constant till 21<sup>st</sup> day postpartum in all the groups followed by gradual increase.

Table 2 represents different indications of plasma progesterone profile which include appearance of increased plasma progesterone concentrations after calving, the duration of elevated plasma progesterone, the peak concentration of plasma progesterone and the cycle lengths for first and second cycle of healthy and mastitis crossbred cows postpartum. The first increase

in progesterone levels were seen between 22-27 days in postpartum period in all the groups. Regular cyclic activity was assumed by the appearance of increased plasma progesterone concentrations which was initiated by 22-27 day's post-partum in all the groups.

**Table 1:** Plasma progesterone concentrations (Mean  $\pm$ SEM) in healthy and mastitic crossbred cows at different days with respect to calving

Days	Plasma Progesterone (ng/ml)			
	Group-I (n=10)	Group-II (n=7)	Group-III (n=8)	Group-IV (n=5)
-15	5.17 <sup>ax</sup> $\pm$ 0.24	5.19 <sup>ax</sup> $\pm$ 0.16	5.60 <sup>ax</sup> $\pm$ 0.36	5.49 <sup>ax</sup> $\pm$ 0.30
-7	3.28 <sup>bx</sup> $\pm$ 0.37	3.58 <sup>bx</sup> $\pm$ 0.32	4.36 <sup>abx</sup> $\pm$ 0.23	4.47 <sup>abx</sup> $\pm$ 0.23
0	0.43 <sup>fx</sup> $\pm$ 0.06	0.49 <sup>fx</sup> $\pm$ 0.10	0.59 <sup>gx</sup> $\pm$ 0.13	0.69 <sup>fx</sup> $\pm$ 0.20
7	0.53 <sup>fx</sup> $\pm$ 0.04	0.70 <sup>efx</sup> $\pm$ 0.15	0.67 <sup>gx</sup> $\pm$ 0.15	0.74 <sup>efx</sup> $\pm$ 0.24
15	0.56 <sup>fx</sup> $\pm$ 0.05	0.73 <sup>efx</sup> $\pm$ 0.18	0.73 <sup>fx</sup> $\pm$ 0.05	0.72 <sup>efx</sup> $\pm$ 0.06
21	0.85 <sup>efx</sup> $\pm$ 0.09	0.80 <sup>efx</sup> $\pm$ 0.03	0.78 <sup>fx</sup> $\pm$ 0.10	0.72 <sup>efx</sup> $\pm$ 0.16
28	1.37 <sup>dx</sup> $\pm$ 0.17	1.30 <sup>dex</sup> $\pm$ 0.14	0.99 <sup>efx</sup> $\pm$ 0.11	1.03 <sup>dex</sup> $\pm$ 0.17
35	1.64 <sup>cdx</sup> $\pm$ 0.20	2.10 <sup>cdx</sup> $\pm$ 0.28	1.24 <sup>dex</sup> $\pm$ 0.17	1.41 <sup>dx</sup> $\pm$ 0.18
42	1.05 <sup>dex</sup> $\pm$ 0.18	1.37 <sup>dexy</sup> $\pm$ 0.18	1.68 <sup>dexy</sup> $\pm$ 0.24	1.96 <sup>cdy</sup> $\pm$ 0.27
49	0.66 <sup>fx</sup> $\pm$ 0.07	1.03 <sup>ey</sup> $\pm$ 0.32	1.34 <sup>efy</sup> $\pm$ 0.40	1.63 <sup>cdy</sup> $\pm$ 0.63
56	1.25 <sup>dx</sup> $\pm$ 0.20	0.93 <sup>cx</sup> $\pm$ 0.15	0.88 <sup>efy</sup> $\pm$ 0.20	1.24 <sup>dx</sup> $\pm$ 0.43
63	0.74 <sup>efx</sup> $\pm$ 0.24	1.44 <sup>dex</sup> $\pm$ 0.26	1.33 <sup>dex</sup> $\pm$ 0.26	1.22 <sup>dx</sup> $\pm$ 0.43
70	2.47 <sup>bcx</sup> $\pm$ 0.30	2.36 <sup>cx</sup> $\pm$ 0.26	2.09 <sup>cx</sup> $\pm$ 0.40	2.19 <sup>cx</sup> $\pm$ 0.53
77	1.98 <sup>cdx</sup> $\pm$ 0.27	1.79 <sup>dex</sup> $\pm$ 0.20	1.62 <sup>dx</sup> $\pm$ 0.34	2.46 <sup>cx</sup> $\pm$ 0.30
84	1.36 <sup>dx</sup> $\pm$ 0.32	1.08 <sup>ex</sup> $\pm$ 0.22	0.99 <sup>efx</sup> $\pm$ 0.15	1.38 <sup>dx</sup> $\pm$ 0.41
90	1.11 <sup>dex</sup> $\pm$ 0.14	1.32 <sup>dexy</sup> $\pm$ 0.18	1.40 <sup>ex</sup> $\pm$ 0.23	0.95 <sup>efx</sup> $\pm$ 0.26

Values within a row having different superscripts (x,y) are significantly (P<0.05) different from each other.

Values within a row having different superscripts (a,b,c,d,e,f and g) are significantly (P<0.05) different from each other.

But the first estrus was observed at about 84<sup>th</sup> to 86<sup>th</sup> day in all groups as represented in Table 3. Therefore, there is progesterone secretion prior to the first visible postpartum estrus. Similar findings have been reported by several researchers (Montgomery *et al.*, 1985). There were non-significant variations recorded among healthy and mastitis groups. This shows, occurrence of SCM at any time during early lactation period does not affect the onset of first cyclicity. The levels remained elevated for almost 12-14 days in first cycle. The peak progesterone levels were obtained between 35-42 days in different groups.

There were non-significant variations recorded among groups. A similar trend was observed in the 2<sup>nd</sup> cycle, but the peak progesterone levels were significantly (P<0.05) higher than 1<sup>st</sup> cycle. These results are in agreement with those of other studies which have reported progesterone cycles of short length (Bloomfield *et al.*, 1986) before first postpartum estrus.

**Table 2:** Mean ( $\pm$ SE) values for appearance of increased plasma progesterone concentrations duration of elevated plasma progesterone, peak concentration of plasma progesterone and first and second cycle length

Cycle	Elevation in plasma Progesterone concentration (days)	Duration of elevated levels (Days)	Peak concentration (ng/ml)	Cycle length (days)	
1 <sup>st</sup>	Group I	22.10 $\pm$ 2.30	13.70 $\pm$ 1.25	2.05 $\pm$ 0.15	27.22 $\pm$ 1.82
	Group II	23.37 $\pm$ 1.28	12.30 $\pm$ 1.82	2.45 $\pm$ 0.19	21.00* $\pm$ 2.06
	Group III	27.38 $\pm$ 2.26	13.98 $\pm$ 3.26	2.27 $\pm$ 0.26	27.13 $\pm$ 3.08
	Group IV	26.50 $\pm$ 2.62	12.90 $\pm$ 2.61	2.34 $\pm$ 0.25	28.00 $\pm$ 3.13
2 <sup>nd</sup>	Group I	63.45 $\pm$ 2.27	16.34 $\pm$ 1.71	3.29* $\pm$ 0.34	27.23 $\pm$ 2.32
	Group II	64.49 $\pm$ 2.34	16.91 $\pm$ 3.18	3.42* $\pm$ 0.28	29.89 $\pm$ 2.17
	Group III	63.45 $\pm$ 1.89	17.84 $\pm$ 2.12	3.14* $\pm$ 0.21	29.67 $\pm$ 2.26
	Group IV	66.78 $\pm$ 2.03	17.91 $\pm$ 2.19	3.09* $\pm$ 0.29	28.98 $\pm$ 3.78

Values within a column having superscript (\*) are significantly (P<0.05) different from others in the column.

Therefore, it can be suggested from the present study that the appearance of first postpartum estrus generally follows the formation of a poorly developed but functional corpus luteum (CL). The significantly (P<0.05) higher peak progesterone levels of 2<sup>nd</sup> cycle over 1<sup>st</sup> cycle indicates that the animals were recovered from parturition stress and reproductive organs gained their optimum potential for next conception. The first cycle length was less in group II cows which were compensated later on in second cycle. The results pertaining to influence of mastitis on reproductive parameters (first service interval, Inter-estrus



interval, services per conception, days open and calving interval) are depicted in table 3. The results revealed that first service interval and inter-estrus intervals were non-significantly higher in all mastitic groups as compared to non-mastitic cows, whereas days open, services per conception and calving interval were significantly ( $P<0.05$ ) higher in group IV cows compared to group I. Cows having CM in early lactation/ after AI (group IV) had increased services per conception, increased days open as well as increased calving interval. But at the same time cows having SCM immediately after calving (group II) or in early postpartum period (group III) did not have any significant effect on different reproductive parameters studied (first service interval, inter estrus interval, services per conception, days open and calving interval).

**Table 3:** Reproductive performances of different groups of cows in postpartum period

Parameters	Group I	Group II	Group III	Group IV
Days of first AI (days)	84.4 <sup>a</sup> ± 5.4(30)	84 <sup>a</sup> ± 2.3(6)	86 <sup>a</sup> ± 1.9(4)	86 <sup>a</sup> ± 2.1(5)
Inter-estrus interval (days)	29 <sup>a</sup> ± 4	31 <sup>a</sup> ± 1	34 <sup>ab</sup> ± 3	38 <sup>ab</sup> ± 3
Days open (days)	137.1 <sup>a</sup> ± 7.1	141.3 <sup>a</sup> ± 4.3	154.3 <sup>a</sup> ± 7.7	190.98 <sup>b</sup> ± 4.8
Services per conception (no.)	1.9 <sup>a</sup> ± 0.4	1.9 <sup>a</sup> ± 0.1	2.2 <sup>a</sup> ± 0.2	2.8 <sup>b</sup> ± 0.2
Calving interval	423.8 <sup>a</sup> ± 10.1	435 <sup>a</sup> ± 7.3	440 <sup>a</sup> ± 9.5	482 <sup>b</sup> ± 8.3

Values within a row having different superscripts (a,b) are significantly ( $P<0.05$ ) different from each other.

There were several contrasting reports regarding mastitis and reproductive performances. Our results are in agreement with Barker *et al.*, (1998) about CM and reproductive performances where they concluded that clinical mastitis during early lactation markedly influence reproductive performances of lactating Jersey cows and this phenomenon is not limited to the type of pathogen involved. Lavon and co-worker, (2011) explained that in chronic SCM and acute CM the follicular function is directly affected due to the direct depression of estradiol production which subsequently resulted in depression of GnRH and LH production. According to their study, the occurrence of chronic subclinical mastitis during early

lactation resulted detrimental effects on reproductive performance similar to those for cows exhibiting clinical mastitis. Up to 30% of cows with clinical mastitis fail to ovulate while infected. In our study, out of 8 cows of group III, 5 had increased services per conception that accounts 37% of cows that had SCM in early lactation. Gunay and Gunay, (2008) demonstrated that the occurrence of acute mastitis before AI can delay the calving to first service interval, increases the calving to conception interval and elevated the number of services per conception.

## CONCLUSION

The results of present study, provide evidence that the consequences of mastitis resulted in suppression of reproductive performance. For optimal reproductive efficiency, a cow should calve at 12 month intervals. For that the animals need to be pregnant by 80 days of calving. Therefore, it is desirable that ovarian activity must initiated as early as possible postpartum and the animal should conceive with 1-2 AI. As clinical mastitis affects the conception rate, therefore by reducing its incidence we can improve the profit of a dairy farmer. Clinical mastitis has a negative effect on reproductive performance of crossbred cows. The relationship of mastitis on reproduction, often ignored by dairy producers, but it is an important managemental factor for successful production and reproduction programs due to its stringent prognosis like culling.

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## ETHICAL APPROVAL

The experimental protocol was duly cleared by the Institute Animal Ethic Committee.

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