Original Research Article

Inflammatory Mediators and Hormonal Changes in Subclinical and Clinically Affected Mastitis Cows

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Abstract

To investigate the changes that occur in inflammatory mediators and reproductive hormones levels in mastitis crossbred cows, the crossbred cows were screened for SCM incidence (24) by mCMT, SCC, electric conductivity of milk and abnormal milk. Based on this, the cows were divided as group I (n=8) - no clinical symptom of mastitis (healthy), group II (n=8) - showing chronic sub-clinical mastitis (SCM), group III (n=8) - showing clinical mastitis symptoms (CM). Blood samples were collected from group I and II the cows at weekly intervals from day 54 to 138 of lactation. A single blood sample was collected from group III before giving antibiotic treatment. Plasma Nitric Oxide (NO), Interleukin-8 (IL-8), Tumor Necrosis Factor-α (TNF-α), cortisol and prostaglandin (PGFM) levels were higher (P<0.001) in clinical mastitis cows followed by low levels in subclinical mastitis cows. Plasma levels of their parameters were lowest (p<0.001) in healthy cows. Plasma progesterone levels were significantly higher (P<0.001) in healthy cows in comparison to group II and III cows. Plasma TNF-α, PGFM and progesterone levels showed significant (P<0.001) variations between days and between groups I and group II cows whereas NO, IL-8 and cortisol levels did not change.

Keywords
NO, TNF-α, IL-8, Cortisol, Progesterone, PGFM, Subclinical mastitis and Clinical mastitis.

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Introduction

Dairy animals encounter mastitis as one of the most important disease causing hindrance in the development of dairy sector. Selective breeding of dairy cattle has led to a dramatic increase in milk yield over recent decades giving India an honour to become the highest milk producer country in the world but simultaneous increase in high incidence of mastitis and huge economic losses have been reported (Heald et al., 2000; Seegers et al., 2003; Oltenacu and Algers, 2005). Researchers agree that the economic impact of subclinical forms of mastitis is larger than clinical mastitis (Singh et al., 2016). The overall prevalence of sub-clinical mastitis has been reported to be 59.43% with quarter level prevalence of 34.78% (Bhat et al., 2016). The annual economic losses due to mastitis have been calculated to be Rs.7165.51 crores both cows and buffaloes almost with rupees 3649.56 and 3515.95 crores, respectively (PDAMAS, 2011). Subclinical mastitis alone causes economic losses of rupees 4151.16 crores (Bogni et al., 2011). This could be minimized by using certain makers in milk and plasma of mastitis animals. It has been found that nitric oxide (NO), interleukins (IL) and tumor necrosis factor (TNF-α) could play a vital role in the pathophysiology of this (Kushibiki et al., 2003; Hansen et al., 2004).
Mastitis not only influence milk production and composition but adversely influence reproductive performance of dairy cows (Schrick et al., 2001; Santos et al., 2004; Hansen et al., 2004) as homeostatic alterations in hormone viz., prostaglandin, progesterone and estrogen affects oocyte maturation, follicular development, luteal life span, resulting the embryonic losses, increased service period and more number of AI per conception and days open. Enhanced cortisol depressed LH and thereby affects ovulation process (Li et al., 1983; Padmanabhan et al., 1983). Considering the economic losses due to mastitis, the present investigation was undertaken to find out plasma inflammatory mediators of infection and hormone levels in mastitis crossbreds.

**Materials and Methods**

**Selection of Animals and management**

The experiment was conducted after getting necessary approval from the Institute’s Animal Ethics Committee. 32 Karan Fries cows immediately after parturition were selected from the experimental herd of the Institute. These were divided into three groups of eight each as healthy, SCM and CM animals. The cows were grouped based on screening by California Mastitis Test (mCMT) and milk SCC. Healthy cows during the experiment served as control while cows suffering from sub clinical mastitis were in SCM group. Eight cows suffering from clinical mastitis were also selected on the basis of clinical symptoms from which milk samples were taken only once.

The animals were managed in loose housing with brick floor and asbestos roof shed over the feeding manger. Cows were fed ad lib green fodder (berseem, maize and jowar fodder) and wheat straw and the concentrate mixture was offered based on milk yield. The feed and water was available ad lib all the time to these cows. Blood samples were collected in heparinized vacutainer tubes from healthy and SCM cows at weekly intervals from 54th day to 138 days of lactation. A single blood sample was also collected from clinical mastitis cows before treatment of cows with antibiotic.

Plasma nitric oxide (NO) was determined by method of Shoker et al., (1997). Plasma tumor necrosis factor- α (TNF- α), interleukin-8 (IL-8), progesterone, cortisol and PGFM (prostaglandin F2 -α) were estimated by commercially available analytical ELISA kits.

The data was analyzed statistically by a SYSTAT software package. Mean ± SE was found and the significance was tested by employing two way ANOVA.

**Results and Discussion**

**Inflammatory mediators**

Plasma NO level was higher in CM cows in comparison to SCM and healthy group cows (Table 1 and 2). Significantly higher plasma NO level has been reported earlier Bastan et al., (2011) in cows with mastitis. The concentration tended to increase massively during bacterial infections. The research experiment carried out in vitro and in vivo also suggests that NO concentrations increase during clinical infections being induced with E. coli endotoxin and Staphylococcus spp. (Blum et al., 2000; Boulanger et al., 2001; Blum et al., 2003; Komine et al., 2004). Further bovine PMN have also been considered the source of NO production by (Boulanger et al., 2001; Komine et al., 2004).

Mean plasma TNF-α level was more (p<0.001) in healthy and SCM groups of
cows. Plasma TNF-α levels varied (P<0.001) between the groups. TNF-α is released locally in mammary gland of mastitis cows and its absorption into the circulation elevates plasma concentration (Hoeben et al., 2000). These cytokine induced and mediated neural and endocrine changes play key roles in the induction of systemic symptoms of mastitis, e.g. fever, lethargy, loss of appetite (anorexia) and many catabolic changes in energy (lipid, carbohydrate), protein and mineral metabolism (Huszenicza et al., 2004). Plasma TNF-α concentrations increase within hours after i.v. administration of LPS, in E. coli-induced mastitis and in natural cases of coliform mastitis, in cattle (Hirvonen et al., 1999; Kinsbergen et al., 1994), and TNF-α production initiates immunological and metabolic reactions which could be detrimental locally (Hirvonen et al., 1999). Further large quantities of LPS must be produced continuously to induce and elevated TNF-α concentrations in blood because i.v. administration of LPS causes a transient rise of TNF-α in cattle (Kinsbergen et al., 1994; Kahl et al., 1997). Severe cases of coliform mastitis are accompanied by the highest increase in blood plasma concentrations of both TNF-α and NO (Kahl et al., 1997; Hirvonen et al., 1999; Blum et al., 2000; Komine et al., 2004). Elevated TNF α in blood of mastitis animals (Blum et al., 2000, Hoeben et al., 2000; Ohtuska et al., 2001) can increase PGF2α synthesis (Starzynski et al., 2000) and suppress LH surge leading to inhibition of fertilization and development of embryos (Hansen et al., 2004).

Plasma IL-8 levels varied (p<0.001) between groups. The levels were significantly (P<0.001) higher in CM cows as Compared to the SCM and healthy cows (Table 2). Kim et al., (2011) concluded that significant (P<0.001) IL-8 expression in the serum was not evident for any infected groups of Holstein cows 14 days post infection. Therefore it can be concluded that increased secretion of IL-8 is an important maker of inflammatory processes. Interleukin 8 (IL-8) is a chemokine produced by macrophages and other cell types such as epithelial cells and neutrophils. Several studies have confirmed higher IL-8 level in E. coli infected mastitis cows as compared with healthy glands (Riollet et al., 2000; Lee et al., 2003b; Bannerman et al., 2004c; Vangroenweghe et al., 2004, 2005).

Plasma cortisol levels varied non-significant between the group of cows and between days.

**Fig.1** Mean plasma cortisol levels in healthy and subclinical mastitis cows during lactation

**Fig.2** Overall mean cortisol levels in different groups of lactating Cows

![Fig.1](image1.png)  
![Fig.2](image2.png)
Fig. 3 Mean plasma progesterone levels in healthy and subclinical mastitis cows during lactation, Fig. 4 Overall mean progesterone levels in different groups of lactating cows

![Graph showing Mean plasma progesterone levels in healthy and subclinical mastitis cows during lactation.](image)

Fig. 5 Mean plasma prostaglandin F<sub>2</sub>-α levels in healthy and subclinical mastitis cows during lactation, Fig. 6 Overall mean prostaglandin F<sub>2</sub>-α levels in different groups of lactating cows

![Graph showing Mean plasma prostaglandin F<sub>2</sub>-α levels in healthy and subclinical mastitis cows during lactation.](image)

Table 1 Mean plasma NO (µmol/L), TNF-α (pg/ml) and IL-8 (pg/ml) levels in healthy and subclinical mastitis cows during lactation

| Postpartum Days | Nitric Oxide (NO) | Tumor Necrosis Factor-α (TNF-α) | Interleukin-8 (IL-8) |
|-----------------|------------------|---------------------------------|----------------------|
|                 | Healthy          | SCM                             | Healthy              | SCM                  |
| 54              | 35.75±1.06       | 38.03±1.6                       | 48.74±4.33           | 197.69±6.58          | 7.43±0.36             | 7.97±0.43             |
| 61              | 35.17±1.08       | 37.3±1.25                       | 50.5±3.51            | 197.01±7.27          | 7.64±0.39             | 8.17±0.46             |
| 68              | 35.62±1.03       | 37.59±1.35                      | 51.54±3.95           | 197.47±4.40          | 7.97±0.36             | 8.36±0.43             |
| 75              | 35.36±1.24       | 37.31±1.24                      | 50.40±3.66           | 196.04±11.59         | 7.49±0.37             | 8.16±0.45             |
| 82              | 34.69±1.24       | 36.56±1.05                      | 54.30±4.94           | 185.19±4.46          | 7.06±0.48             | 7.76±0.30             |
| 89              | 35.47±0.95       | 37.14±0.90                      | 57.04±2.39           | 179.87±4.39          | 6.92±0.88             | 8.08±0.46             |
| 96              | 35.18±1.34       | 37.38±1.31                      | 58.22±4.22           | 177.88±3.96          | 7.21±0.37             | 8.00±0.46             |
| 103             | 35.21±1.66       | 37.00±1.23                      | 54.28±4.24           | 163.92±7.20          | 7.29±0.71             | 7.94±0.43             |
| 110             | 34.24±0.95       | 36.21±1.17                      | 57.17±2.10           | 159.87±7.03          | 7.21±0.59             | 7.81±0.45             |
| 117             | 33.83±1.15       | 36.08±1.20                      | 57.23±2.16           | 157.03±6.37          | 7.34±0.34             | 7.85±0.37             |
| 124             | 33.5±0.61        | 35.5±1.51                       | 56.40±2.81           | 145.01±7.58          | 7.63±0.46             | 8.12±0.38             |
| 131             | 33.29±1.01       | 34.46±0.93                      | 56.25±1.57           | 145.70±6.29          | 7.24±0.31             | 7.82±0.36             |
| 138             | 33.51±0.83       | 34.31±1.29                      | 58.08±2.16           | 135.78±11.5          | 7.20±0.24             | 7.55±0.50             |
| Over all mean± SEM | 34.68±0.24 | 36.75±0.359 | 54.63±0.90 | 171.80±6.32 | 7.35±0.12 | 7.96±0.14 |

Values with different superscripts a and ABCDE differ (p<0.05) in a row and column respectively.
Table 2 Mean plasma NO, TNF-α and IL-8 levels in different group of cows

| Mediators | Groups    | Healthy       | SCM          | CM            |
|-----------|-----------|---------------|--------------|---------------|
| NO(µmol/L)| 34.68±0.24| 36.75±0.359   | 59.96±2.53   |
| TNF-α(pg/ml)| 54.63±0.90 | 171.80±6.32   | 695.50±43.98 |
| IL-8(pg/ml)| 7.35±0.12  | 7.96±0.14     | 24.35±1.19   |

Values with different superscripts abc differ (p<0.05) in row

However, plasma cortisol level was significantly (P<0.001) higher in clinical mastitis cows than the healthy and subclinical mastitis cows (Figure 2). Kuldeep, (2011) observed three times higher plasma cortisol levels in clinical mastitis cows. Cortisol act as powerful immunosuppressive agent and facilitates the invasion of environmental pathogens leading to increased incidence of mastitis (Goff and Horst, 1997; Kehrli et al., 1991). Higher cortisol level also suppresses the lymphogenic response to mitogens and certain aspects of neutrophil function (Jacob et al., 2001). This could be the reaction of higher cortisol levels in clinical mastitis cows found in this study (Fig. 1).

Endotoxin challenges also cause higher cortisol level (Soliman et al., 2002; Waldron et al., 2003; Lehtolainen et al., 2003).

The PGFM levels varied significantly (P<0.001) between healthy, SCM and CM group cows (Figures 3 and 4). Elevated PGF_2α levels in subclinical and clinical mastitis cows in to comparison to healthy cows, resulted in low progesterone concentration resulting the impaired embryonic development and increased number of services per conception service period and higher oxytocin concentration (Hockett et al., 2000).

Plasma progesterone levels was significantly different (p<0.001) between healthy, SCM and CM group cows (Figures 5 and 6). Higher levels of progesterone after 75 days of lactation in healthy cows indicated pregnancy status. However, SCM cows rise in progesterone levels was less and occur after 124 days. The confirmed pregnancy percent in healthy and SCM cows was 87.5% and 75% respectively. The delay in pregnancy varied significantly (P<0.001) between the groups.

Based on the results of hormonal and biochemical parameters it was concluded that monitoring of inflammatory markers could be used as a biomarker to identify the cows at high-risk of infection. This will facilitate prompt treatment and pro-active management practices in reducing disease incidence and the dairy farms are likely to improve overall productivity of the animal.

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References

Bannerman, D.D., Paape, M.J., Lee, J.W., Zhao, X., Hope, J.C. and Rainard, P. 2004c. Escherichia coli and Staphylococcus aureus elicit differential innate immune responses following intramammary infection. *Clin. Diagn. Lab. Immuno.*, 11:463–472.

Bastan, A., Cengiz, M., Sel, T., Cengiz, S., Akan, M., Akcay, A., Darbaz, I. and
Fidancei, U. 2011. Evaluation of nitric oxide level in prepartum heifer mastitis. *Ankara, univ. Vet. Fak. Derg.*, 58:181-184.

Bhat, A. M., Soodan, J.S. and Tikoo, A. 2016. Study on prevalence of subclinical mastitis in cross bred dairy cattle and its potential risk factors. *J. Anim. Res.*, 6(4): 747-749.

Blum, J.W., Dosogne, H., Hoeben, D., Vangroenweghe, F., Hammon, H.M., Bruckmaier, R.M. and Burvenich, C., 2000. Tumor necrosis factor-b and nitrite/nitrate responses during acute mastitis induced by *Escherichia coli* infection and endotoxin in dairy cows. *Dom. Anim. Endocrinol.*, 19: 223-235.

Bogni C., Odierno L., Raspanti C., Giraudo J., Lasrriestra A., Reinoso E., Lasagno M., Ferrari M., Ducros E., Frigerioc C., Bettera S., Pellegrino M. S., Forla I., Dieser S. and Vissio C. 2011. War against mastitis pathogens. Science against microbial pathogen. Current research and technological advances. A. Mendez-Vilas (Ed.) 483-494

Boulanger, V., Bouchard, L., Zhao, X. and Lacasse, P. 2001. Induction of nitric oxide production by bovine mammary epithelial cells and blood leukocytes. *J. Dairy Sci.*, 84: 1430–1437.

Goff, J.P. and Horst, R.L. 1997. Physiological changes at parturition and their relationship to metabolic disorders. *J. Dairy Sci.*, 80: 1260–1268.

Hansen, P.J., Paolete S. and Natzke R.P. 2004. Mastitis and fertility in cattle–possible involvement of inflammation or immune activation in embryonic mortality. *American Journal of Reproductive Immunology.*, 51: 294-301.

Heald, C.W., Kim, T., Sischo, W.M., Coopper, J.B. and Wolfgang, D.R. 2000. A computerized mastitis decision aid using farm-based records: an artificial neural network approach. *J. Dairy. Sci.* 83 (4):711-722.

Hirvonen, J., Eklund, K., Teppo, A.M., Huszenicza, G., Kulcsar, M., Saloniemi, H., and Pyorala, S. 1999. Acute phase response in dairy cows with experimentally induced Escherichia coli mastitis. *Acta. Vet. Scand.*, 40: 35–46.

Hockett, M.E., Hopkins, F.M., Lewis, M.J., Saxton, A.M., Dowlen, H.H., Oliver, S.P. and Schrick, F.N. 2000. Endocrine profiles of dairy cows following experimentally induced clinical mastitis during early lactation. *Anim. Reprod. Sci.*, 58: 241–251.

Hoeben, D., Burvenich, C., Tervisi, E., Bertoni, G., Hamann, J., Bruckmaier, R.M. and Blum, J.W., 2000. Role of endotoxin and TNF-α in the pathogenesis and experimentally induced colliform mastitis in periparturient cows. *J. Dairy Res.*, 67: 503-514.

Huszenicza, G., Janosi, S., Gaspardy, A. and Kulcsar, M. 2004. Endocrine aspects in pathogenesis of mastitis in postpartum dairy cows. Animal Reproduction Science, 82:389-400

Jacob, S.K., Ramnath, V., Philomina, P.T., Raghunandhanan, K.V. and Kannan, A. 2001. Assessment of Physiological Stress in Periparturient Cows and Neonatal Calves. *Indian J. Physiol. Pharmacol.*, 45 (2): 233-238.

Kahl, S., Elsasser, T.H. and Blum, J.W. 1997. Nutritional regulation of plasma TNF-α and plasma and urinary nitrite nitrate responses to endotoxin in cattle. *Proc. Soc. Exp. Biol. Med.*, 215: 370–376.

Kehrli, M.E., Weigel, K.A., Freeman, A.E., Thurston, J.R. and Kelley, D.H. 1991. Bovine sire effects on daughter’s *in vitro* blood neutrophil functions, lymphocyte blastogenesis, serum complement and conglutinin levels. *Vet. Immunol. Immunopathol.*, 27: 303–319.
Kim, Y., Atalla, H., Mallard, B., Robert, C. and Karrow, N. 2011. Changes in Holstein cow milk and serum proteins during intramammary infection with three different strains of Staphylococcus aureus. BMC Vet. Res. 7:51.

Kinsbergen, M., Bruckmaier, R.M. and Blum, J.W. 1994. Metabolic, endocrine and hematological responses to E. coli endotoxin administration in one-week old calves. J. Vet. Med., 41: 530–547.

Kumine, K., Kuroishi, T., Komine, Y., Watanabe, K., Kobayashi, J., Yamaguchi, T., Kamata, S. and Kumagai, K. 2004. Induction of nitric oxide production mediated by tumor necrosis factor alpha on staphylococcal enterotoxin c-stimulated bovine mammary gland cells. Clinical and Diagnostic Laboratory Immunology. 11: 203–210.

Kuldeep. 2011. Studies on immunological variables and nitric oxide synthase (iNOS) expression during different stages of lactation and mastitis in crossbred cattle. M.V.Sc. Thesis, NDRI (deemed University), Karnal, India.

Kushibiki, S., Hodate, K., Shingu, H., Obara, Y., Touno, E., Shinoda, M. and Yokomizo, M., 2003. Metabolic and lactational responses during recombinant bovine tumor necrosis factor-a. J. Dairy Sci., 86: 819–827.

Lee, J.W., Paape, M. J., Elsasser, T.H. and Zhao, X. 2003b. Recombinant soluble CD14 reduces severity of intramammary infection by Escherichia coli. Infect. Immun. 71:4034–4039.

Lehtolainen, T., Suominen, S., Kutila, T. and Pyorala, S. 2003. Effect of intramammary Escherichia coli endotoxin in early- vs. late-lactating dairy cows. J. Dairy Sci., 86: 2327–2333.

Li, P.S. and Wagne, W.C. 1983. In vivo and in vitro studies on the effect of adrenocorticotropic hormone or cortisol on the pituitary response to gonadotropin releasing hormone. Biol. Reprod., 29: 25.

Ohitsuuka, H., Kudo, K., Mori, K., Nagai, F., Hatsugay, A., Tajima, M., Tamura, K., Hoshi, F., Ko, M. and Kawamura, S. 2001. Acute phase response in naturally occurring coliform mastitis, J. Vet. Med. Sci., 63: 675–678.

Oltenacu, P.A. and Algers, B. 2005. Selection for increased production 349 and the welfare of dairy cows; are new breeding goals needed. Ambio., 34: 308312.

Padmanabhan, V., Keech, C. and Convey, E.M., 1983. Cortisol inhibits and adrenocorticotropic has no effect on luteinizng hormone-releasing hormone-induced release of luteinizing hormone from bovine pituitary cells in vitro. Endocrinology, 112: 1782.

PDADMAS (2011) PD-ADMAS news. Project directorate on animal disease monitoring and surveillance, January – June, 1(1):8.

Riollet, C., Rainard, P. and Poutrel, B., 2000. Differential induction of complement fragment C5a and inflammatory cytokines during intramammary infections with Escherichia coli and Staphylococcus aureus. Clin. Diagn. Lab. Immunol., 7(2): 161–167.

Santos, J.E.P., Cerri, R.L.A., Ballou, M.A., Higginbotham, G.E., Kirk, J.H., 2004. Effect of timing of first clinical mastitis occurrence on lactational and reproductive performance of Holstein dairy cows. Anim. Repro. Sci., 80: 31–45.

Seegers, H., Fourichon, C. and Beaudeau, F.
2003. Production effects related to mastitis and mastitis economics in dairy cattle herds. *Vet. Res.*, 34: 475–491.
Shoker A.S., Yang, H. and Murabit, M.A. (1997). Analysis of the in vitro effect of exogenous nitric oxide on human lymphocytes. *Mol. Cell Biochem.*, 171: 75–83.
Singh, M., Sharma, A., Mittal D., Yadav P. and Charaya, G. 2016. Assessment of lactate dehydrogenase enzyme activity in milk as a marker for detection of subclinical mastitis [online]. *J. Anim. Res.*, 6(2): 113-116.
Soliman, M., Ishioka, K., Kimura, K., Kushibiki, S. and Saito, M. 2002. Plasma leptin responses to lipopolysaccharide and tumor necrosis factor-α in cows. *Jpn. J. Vet. Res.*, 50: 107–114.
Starzynski, D.J., Miyamoto, Y., Okuda K., 2000. Production of PGF2α by cultured bovine endometrial cells in response to tumor necrosis factor α: cell type specificity and intracellular mechanisms. *Biol. Repord.*, 62: 1160
Vangroenweghe, F., Lamote, I. and Burvenich, C. 2005. Physiology of the periparturient period and its relation to severity of clinical mastitis. *Domest. Anim. Endocrinol.*, 29: 283–293.
Vangroenweghe, F., Rainard, P., Paape, M., Duchateau, L. and Burvenich, C. 2004. Increase of Escherichia coli inoculum doses induces faster innate immune response in primiparous cows. *J. Dairy Sci.*, 87: 4132–4144.
Waldron, M. R., Nishida, T., Nonnecke, B. J. and Overton, T. R. (2003). Effect of lipopolysaccharide on indices of peripheral and hepatic metabolism in lactating cows. *J. Dairy Sci.* 86: 3447–3459.

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