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Effect of Lactation and Breed on Reproductive Cyclicity Under Various States of Oxidative Stress in Dairy Cattle Breeds

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A B S T R A C T

This study was carried out to study effects of lactation and breed on reproductive cyclicity under various states of oxidative stress in dairy cattle. Different parameters including body condition score (BCS), daily milk yield (DMY), glucose, malondialdehyde (MDA), cortisol and progesterone were analysed to see the association of these parameters with postpartum days and breeds. Thirty-six periparturient lactating dairy cows, nine each from Sahiwal, Achai, Holstein Frisian (HF) and cross (Sahiwal x HF) breed were selected. Blood sample were collected on post-partum day (PPD) 60, 82 and 105 of the lactation stage during diestrus. DMY varied significantly with PPDs and within breed ($P<0.001$). Interaction between PPDs x breed was highly significant ($P<0.01$) for DMY. Serum glucose and progesterone level significantly increased with increase in PPDs ($P< 0.01$) while MDA and cortisol level decreases with increase in PPDs in all breeds. Moreover breed also significantly affected glucose ($P< 0.001$) and MDA concentrations. BCS was negatively correlated with serum cortisol, glucose and progesterone. Cortisol, DMY and MDA were positively correlated with each other and negative with progesterone and PPDs. The study revealed that HF and cross-bred dairy cows were more prone to oxidative stress than local breeds. Correlation analysis indicates that higher yield is associated with reduced reproductive performance in lactating cows, primarily through delayed ovarian activity as assessed by reduced progesterone concentrations during high milk production phase, suggesting that high milk producers should be provided with extra-managemental support for better productivity and fertility.

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MSQ and SA designed and planned the experiments. HK, G and SA performed the experiments. IK and SA analyzed the data. IK wrote the article.

Key words
Oxidative stress, cross-bred, MDA, milk yield, cortisol, reproduction.

INTRODUCTION

Over the past five decades per cow milk production has increased, however their fertility is reduced. Worldwide metabolic profiles are used for health monitoring, nutritional and reproductive status. Recently, evaluation of oxidative stress has enabled us to understand the basic mechanisms involved in metabolic disorders in dairy cattle, where lactation impose great physiological demands on mechanisms of homeostasis on the body (Castillo *et al*., 2006). The peri-parturient periodis more critical for health and performance in production and reproduction of dairy cows. Mostly the energy demands are higher for milk production than available energy from feed offered. Consequently body energy reserves *i.e.*, lipids are mobilized for compensation of energy shortage and high milk production demands are fulfilled.

Negative energy balance can be defined as less net energy input relative to energy output (Jorritsmaa *et al.*, 2003) which leads to oxidative stress. Mobilization of body energy reserves can promote milk production at the time of negative energy balance, resulting in metabolic problems, poor conception rates and decreased immunity against various infectious diseases (Dechow *et al*., 2004). Several complications like retained placenta, prolapsus uteri and caesarean section etc. due to oxidative stress are noticed in Holstein Friesian post parturition. These lead to decrease in milk production, delayed ovulation, conception failure and prolonged calving intervals leading to low fertility (Ocal, 2002). Cells exposed to environmental or metabolic heat stress may induce oxidative stress due to antioxidant reduction in blood plasma of dairy cattle (Harmon *et al.*, 1997). Oxidative stress decreases feed intake and productivity in terms of milk yield, body weight and reproductive performance (Beatty *et al*., 2006)**.** Oxidative stress can contributes to

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pathological manifestation in dairy cattle by causing per-oxidative damage to cellular membranes of affected tissues (Miller *et al*., 1993).

One of the most commonly used indicators of oxidative stress is the level of lipid peroxidation. Malonyldialdehyde (MDA), formed by peroxidation of unsaturated fatty acids, is frequently used as a marker of peroxidation (DelRio *et al*., 2005). Lipid peroxidation occurs as non-enzymatic chain reaction of unsaturated fatty acid and associated with reactive oxygen species (ROS) presence. Increase production of ROS and cellular oxidation events occur at cellular level (Kim *et al.,* 2005). Biological reactions generate ROS which trap electrons from the bio molecules i.e. fatty acids, proteins and DNA. When cells are exposed to these ROS, it leads disruption in membrane, reduced enzymatic activity and proteins. Living body contain various antioxidant systems to eliminate ROS either directly or enzymatically catalyzed reactions, at the same time other molecules reverse oxidative damage. The intensity of adverse changes in physiological system is caused by ROS increased generation which is directly dependent on the balance between ROS generation and antioxidant systems (Gitto *et al*., 2002). In female reproductive tract ROS and antioxidant presence has been demonstrated by various methodologies. Various types of oxidative stress biomarkers have been investigated including glutathione peroxidase, superoxide dismutase, lipid peroxides, glutaredoxin, conjugated dienes, malonyl aldehyde and thiobarbituric acid.

This study was aimed to detect the occurrence of oxidative stress in dairy cattle by determining plasma MDA concentration during the periparturient period and its possible relationship with folliculogenisis modulated through serum progesterone levels.

MATERIALS AND METHODS

The study was conducted at Livestock Research and Development Station Surezai and The University of Agriculture Peshawar, Pakistan.

Selection of animals

The experiment was conducted on thirty-six periparturient dairy cows. Twenty-seven dairy cows (Group-Sh = 09 Sahiwal, Group- Ac= 09 Achai and Group-Xb = 09 cross-bred dairy cows (Sahiwal x HF)) were selected at livestock research and development station Surezai, district Peshawar, Pakistan, while nine pure-exotic (HF) dairy cows (Group-HF = 09) were selected from the University of Agriculture Peshawar dairy farm. The climatic and managemental conditions at both farms are identical as they are located very close to each other. The post-partum days and cyclicity status were determined from the farm record. Animals were kept at these stations under similar managemental conditions with free access to drinking water and feeding of green fodder, wheat straw (bhoosa) and concentrate as basal diet. Milking was practiced twice daily at morning and evening. All the dairy cows were disease free and apparently healthy with normal reproductive cyclicity.

Sampling

Sampling was conducted at early lactation on day-60 postpartum, day-82 and day-105 during the diestrus phase of the estrus cycle. Different physiological (BCS, daily milk yield and serum glucose), stress (Cortisol and MDA) and reproductive (progesterone) parameters were determined.

Body condition scoring

Peters and Ball (1987) method was used for the recording of body condition score (BCS) by the same person throughout the study period. According to this method the thickness of fat over the lumber and tail head area was estimated and was assigned a score from 1 (very weak) to 5 (very fat). 1, spine prominent and transverse processes feel sharp with little fat cover; 2, transverse processes can be felt but are rounded with a thin covering of fat; 3, individual transverse vertebral processes can only be felt by firm pressure; 4, the transverse processes cannot be felt; and 5, the transverse processes covered with a thick layer of fat.

Blood sampling and analysis

Total of 10 ml blood samples, five ml each in separate sterile vacutainer tubes were collected on scheduled dates from jugular veinipuncture. For plasma separation anticoagulant EDTA (1 mg/ml) was added to one tube, while another five ml blood was left as such for clotting and serum separation in other tube. Plasma and serum was separated by centrifugation at 3000rpm for 20 min. Separated plasma and serum was stored at -20°C till further analysis.

Plasma MDA and serum glucose level were determined through spectrophotometry using commercially available kits (MDA Assay Kit BioVision, Inc. USA) and (glucose single reagent kit, MDSS GmbH, Germany) respectively. Progesterone was determined through ELISA test from serum using progesterone enzyme immunoassay kit (Biocheck, Inc). Cortisol was determined through ELISA from serum using kit (Calbiotech, USA).

Statistical analysis

Data was compiled using MS Excel-2007 and was analysed by SPSS package-16 using General Linear Model. Means between four different breeds and three milk yield levels were compared by the method described by the Steel *et al*. (1997). Least significant difference (LSD) test was applied for significant difference between different variables. Correlation of different factors with studied parameters was calculated by Pearson's correlation procedure.

RESULTS

Change in milk yield

Different parameters including BCS, DMY, glucose, MDA, cortisol and progesterone were analyzed in periparteuient dairy cows to see the association of these parameters with PPDs and breeds. DMY decreased significantly with advancing PPDs. Highest DMY was shown by pure-exotic (HF) breed followed by cross-bred, Sahiwal and lowest by Achai during the whole study period (Table I). PPDs and breed showed highly significant effect on DMY (PPD, P<0.001, breed, P<0.001). The interaction (PPDs x breed) effect was also highly significant (P<0.01) for DMY.

A similar changing pattern with more declines from day-60 to day-82 and then little change upto day-105 was observed in DMY (Fig. 2A). The results of least significant difference (LSD) test for PPDs and breed (Table III, IV) revealed that DMY at PPD -60 was significantly (P<0.001) higher from day-82 and 105. However the difference in DMY from day-82 to 105 was not significant (P>0.05), indicating little change in milk yield during these days. Similarly, Sahiwal and Achai breeds yielded almost similar DMY (P>0.05) but were significantly different from cross (P<0.05) and HF (P<0.001) breeds. The cross and HF breeds were also significantly (P<0.001) different in DMY from one another. The BCS was significantly different (P<0.001) among all breeds (Table I). However, no difference was observed in BCS in all breeds indicating that they maintained its BCS throughout the study period.

Blood glucose

The glucose concentrations of all four dairy cattle breeds were determined at PPD-60, 82 and 105 as energy level indicator (Table I). The serum glucose concentration was significantly affected by both PPDs (P<0.01) and breed (P<0.001). However, the interaction affect (PPDs x Breed) was not significant. Glucose concentration increased with the increase in PPDs in all breeds (Fig. 2). The LSD analysis of PPDs (Table IV) revealed that glucose concentration at PPD-60 was significantly lower than day-82 (P<0.01) and 105 (P<0.001). However, there was no significant difference in glucose concentration between day-82 and 105. Sahiwal and Achai had almost similar level of glucose concentration at all the study period. The cross and HF breed were different in glucose concentration. The cross and HF breeds showed prominent increase in glucose concentration with increase in PPDs. However, no statistical difference was observed between day-82 and 105 in pure-exotic breed.

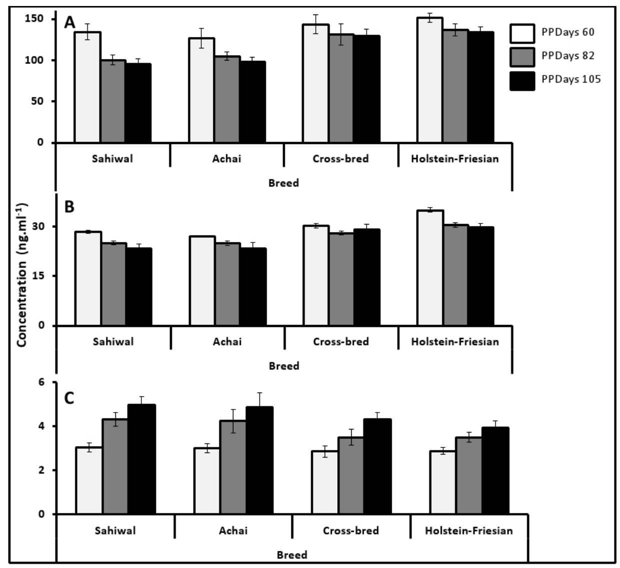

Fig. 1. Concentrations (Mean ± SE) of cortisol (A), MDA (B) and Progesterone (C) in four dairy cattle breeds as affected by PPDs and breed (n= 108).

MDA

MDA was determined as oxidative stress indicator during the periparteuient period for all breeds (Fig. 1). The results showed that the MDA level decreased significantly (P<0.001) with increase in PPDs. Similarly, breed also affected the MDA concentrations significantly (P<0.001). However, the interaction (PPDs x breed) effect was not significant (P>0.05). The LSD results showed that there was significant decrease (P<0.001) in MDA concentration from day-60 to 82 and 105 (Table IV), while no prominent change was observed from day-82 to 105. Mean comparison of MDA for breeds showed that Sahiwal and Achai were not statistically different, while both were significantly different (P<0.001) from cross and HF (Table IV). Similarly, cross and HF breeds were also different significantly (P<0.01). Figure 3 shows the changing pattern of decrease in MDA concentration with increase in PPDs. The figure shows that there was drastic decrease in MDA concentration from day-60 to 82 while little change from day-82 to 105 was observed. The

Table I.- Different physiological parameters (Mean±SEM) from different dairy cattle breeds as affected by post partum days and breed (n=108).

Breed	Post partum days	DMY (Kg)	BCS	Glucose(mg/dl)
Sahiwal	60	6.11±0.43 ^{d}	3.05± 0.09 ^{a}	31.02±2.86 ^{d}
	82	4.27± 0.29 ^{f}	3.05 ± 0.09 ^{a}	37.02 ±2.97 ^{bcd}
	105	4.16± 0.30 ^{f}	3.05 ± 0.09 ^{a}	41.60±2.50 ^{bcd}
Achai	60	4.44± 0.24 ^{ef}	2.77 ± 0.08 ^{b}	33.00±5.42 ^{cd}
	82	4.16± 0.26 ^{f}	2.77 ± 0.08 ^{b}	40.09±2.70 ^{bcd}
	105	4.16± 0.26 ^{f}	2.77 ± 0.08 ^{b}	43.17±1.85 ^{abc}
Cross-bred	60	6.44± 0.37 ^{d}	2.97 ± 0.10 ^{ab}	38.19±5.70 ^{bcd}
	82	5.44± 0.48 ^{de}	2.97± 0.10 ^{ab}	45.60±2.73 ^{ab}
	105	5.44± 0.48 ^{de}	2.97 ± 0.10 ^{ab}	46.67±2.19 ^{ab}
Holstein-Friesian	60	18.77± 0.54 ^{a}	3.00 ± 0.11 ^{ab}	43.05±7.62 ^{abc}
	82	16.61± 0.59 ^{b}	3.00 ± 0.11 ^{ab}	52.59±2.80 ^{a}
	105	15.00± 0.50 ^{c}	3.00 ± 0.11 ^{ab}	52.83±2.90 ^{a}
P- Value	Breed	< 0.001	< 0.001	< 0.001
	P.P Days	< 0.001	1.00	0.001
	Interaction	0.001	1.00	0.998

$^{a, b, c, d}$ means with different superscript with in the column are different significantly at p= 0.05
BCS, body condition score; DMY, daily milk yield.

Table II.- Pearson Correlation between different studied parameters of different dairy cattle breeds (Pearson's correlation coefficient, n=108).

Variable	BCS	Cortisol	DMY	Glucose	MDA	P_4
Cortisol	-0.005					
P-value	0.958					
DMY	0.111	0.392**				
P-value	0.250	0.000				
Glucose	-0.041	-0.034	-0.307**			
P-value	0.669	0.727	0.001			
MDA	0.013	0.471**	0.609**	0.032		
P-value	0.892	0.000	0.000	0.735		
P_4	-0.132	-0.358**	-0.206	0.007	-0.186	
P-value	0.170	0.000	0.031	0.937	0.053	
PPDs	0.000	-0.336**	-0.133	0.310	-0.361**	0.520**
P-value	1.000	0.000	0.169	0.001	0.000	0.000

$^{*, **}$ Correlation is significant at the 0.05 and 0.01 level respectively.

overall results revealed that MDA concentration was higher during the early PPDs than the late lactation days. The pure-exotic breed showed highest level of MDA followed by cross-bred and the two local breeds, indicating that the pure and cross-bred dairy cows feel more oxidative stress than the two local breeds of Sahiwal and Achai.

Blood cortisol

Cortisol as a general stress indicator was determined in this experiment (Fig. 1). Both PPDs and breed significantly affected the serum cortisol concentration (P<0.001). The interaction (PPDs x breed) effect was however, not significant (P<0.05). Decrease in cortisol concentration was observed with increase in PPDs in all breeds. The mean comparison of PPDs showed that the cortisol concentration significantly decreased from postpartum day-60 to 82 and 105 (Table IV). The decrease was not significant from day-82 to 105. Mean comparison of breeds for cortisol concentrations showed that all breeds were significantly different from one another except Sahiwal and Achai (Table IV). Cross-bred and pure-exotic HF were highest in cortisol concentration followed by the two local breeds. Sahiwal and Achai

Table III.- Least Significant Difference (LSD) test for comparing postpartum days of different dairy breeds for various studied parameters.

Dependent variable	PPDs (I)	PPDs (J)	Mean difference (I-J)	Std. Error	Sig.
DMY	60	82	1.319*	0.316	0.000
		105	1.750*	0.316	0.000
	82	105	0.431	0.316	0.176
MDA	60	82	3.061*	0.688	0.000
		105	3.753*	0.688	0.000
	82	105	0.693	0.688	0.317
Progesterone	60	82	-0.944*	0.245	0.000
		105	-1.574*	0.245	0.000
	82	105	-0.631*	0.245	0.011
Cortisol	60	82	20.696*	5.871	0.001
		105	24.747*	5.871	0.000
	82	105	4.051	5.871	0.492
Glucose	60	82	-7.509*	2.683	0.006
		105	-9.755*	2.683	0.000
	82	105	-2.245	2.683	0.405

* The mean difference is significant at the 0.05 level.

Table IV.- Least Significant Difference (LSD) test for comparing breeds for various studied parameters.

Dependent variable	Breed (I)	Breed (J)	Mean Difference (I-J)	Std. Error	Sig.
DMY	Sahiwal	Achai	0.593	0.365	0.107
		Cross	-0.926*	0.365	0.013
		HF	-11.944*	0.365	0.000
	Achai	Cross	-1.519*	0.365	0.000
		HF	-12.537*	0.365	0.000
	Cross	HF	-11.019*	0.365	0.000
MDA	Sahiwal	Achai	0.473	0.795	0.553
		Cross	-3.500*	0.795	0.000
		HF	-6.106*	0.795	0.000
	Achai	Cross	-3.973*	0.795	0.000
		HF	-6.579*	0.795	0.000
	Cross	HF	-2.606*	0.795	0.001
Progesterone	Sahiwal	Achai	0.064	0.283	0.822
		Cross	0.549	0.283	0.055
		HF	0.669*	0.283	0.020
	Achai	Cross	0.486	0.283	0.089
		HF	0.606*	0.283	0.034
	Cross	HF	0.120	0.283	0.672
Cortisol	Sahiwal	Achai	-0.181	6.779	0.979
		Cross	-24.714*	6.779	0.000
		HF	-30.995*	6.779	0.000
	Achai	Cross	-24.533*	6.779	0.000
		HF	-30.814*	6.779	0.000
	Cross	HF	-6.281	6.779	0.356
Glucose	Sahiwal	Achai	-2.207	3.098	0.478
		Cross	-6.942*	3.098	0.027
		HF	-12.945*	3.098	0.000
	Achai	Cross	-4.734	3.098	0.130
		HF	-10.737*	3.098	0.001
	Cross	HF	-6.003	3.098	0.055

* The mean difference is significant at the 0.05 level.

showed almost similar pattern of cortisol concentrations (Fig. 2D). It is clear from the Figure 2D that there was prominent decrease in cortisol concentration from day-60 to 82 in all breeds. However, there was little change in HF and cross-bred dairy cows from day-82 to 105, while the two local breeds showed further decline in cortisol concentration. The results revealed that the pure-exotic and cross-bred dairy breeds were more stressed than the two local breeds of Sahiwal and Achai in early postpartum days than the late lactation.

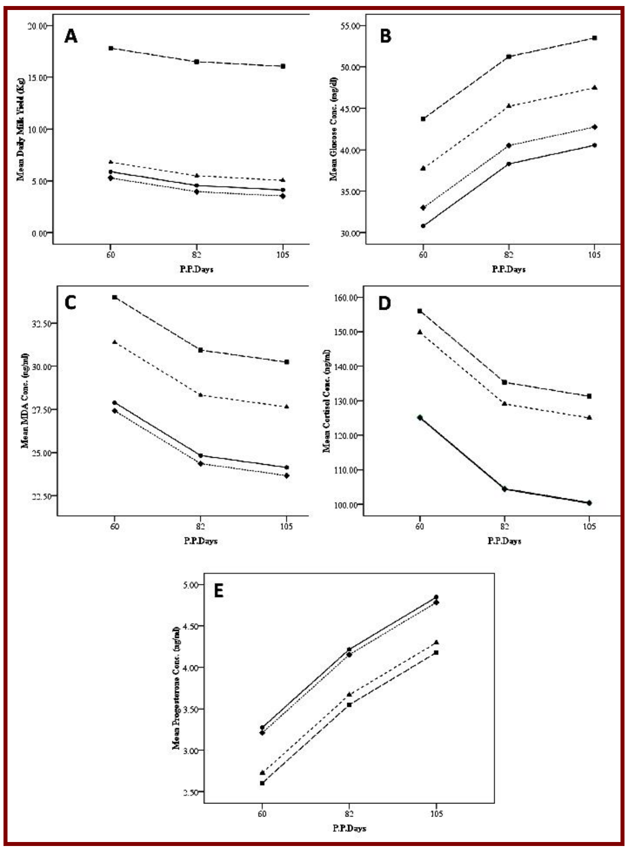

Fig. 2. Mean DMY (A), glucose (B), MDA (C), cortisol (D) and progesterone (E) of four studied dairy cattle breeds as affected by post partum period.(● Sahiwal, ♦ Achai, ▲ Cross-bred and ■ HF).

Progesterone profiles

Progesterone was determined in this experiment as reproductive parameter because of its importance in folliculogenisis. Figure 1 shows the mean concentrations of progesterone concentrations of all breeds at PPD-60, 82 and 105. The results revealed that the individual effect of both PPDs ($P<0.001$) and breed ($P<0.05$) on progesterone concentrations was significant, while the interaction (PPDs x breed) effect was not significant ($P>0.05$). The mean comparison (Tables III, IV) through LSD for PPDs and breed showed that progesterone concentration increase significantly ($P<0.001$) with increase in PPDs from day-60 to 82 and the increase continued upto day-105 ($P<0.01$). Similarly, Sahiwal and Achai had almost similar level of progesterone, higher than cross and HF breeds. The overall results revealed that progesterone concentration increased with increase in PPDs in all the four studied breeds (Fig. 2E). The concentration of progesterone was higher in Sahiwal and Achai in comparison to the two exotic blood breeds. This reveals that HF and cross-bred dairy breeds are more susceptible to oxidative stress than the local breeds.

Relationship among various parameters

The Pearson's correlation test was applied to find out the relationship between various studied parameters (Table III). The results revealed that BCS was negatively correlated with cortisol, glucose and serum progesterone level. However, the correlation was not significant. Cortisol was positively correlated with DMY ($r= 0.392$, $P<0.001$), and MDA ($r= 0.471$, $P<0.001$) and negatively correlated with progesterone ($r= -0.358$, $P<0.001$) and PPDs ($r= -0.336$, $P<0.001$) indicating that cortisol level of cows having good BCS increases with increase in DMY and MDA and lowers with increase in progesterone concentration and early PPDs. DMY showed positive correlation with glucose ($r= 0.307$, $P<0.001$) and MDA ($r= 0.609$, $P<0.001$) and negative correlation with progesterone ($r= -0.206$, $P<0.031$). These results indicated that the level of MDA and glucose increased with higher DMY, however, the progesterone level decreased with increase in DMY. The progesterone concentration has negative correlation with MDA ($r= -0.186$, $P= 0.053$). It reveals that as the MDA level increases, the progesterone level decreases and vice versa.

Simple linear regression was applied for investigating and modeling the relationship between different variables studied in this experiment. Cortisol, MDA, glucose and progesterone were regressed with DMY (Fig. 3A) to find out the effect of high milk production on these parameters. Cortisol and MDA increases with increase in DMY, while glucose and progesterone levels regress inversely with DMY. This increase in stress indicators showed that dairy cattle during high milk production are more prone to stress as compare to low milk production which ultimately affect the energy level badly resulting into low progesterone concentrations. MDA as oxidative stress indicator was regressed with different studied parameters (Fig. 3B).

Cortisol regressed directly with MDA while glucose and progesterone regressed inversely. The strength of relationship of MDA was strong with cortisol and progesterone than glucose as shown by the R^2 value in the figure.

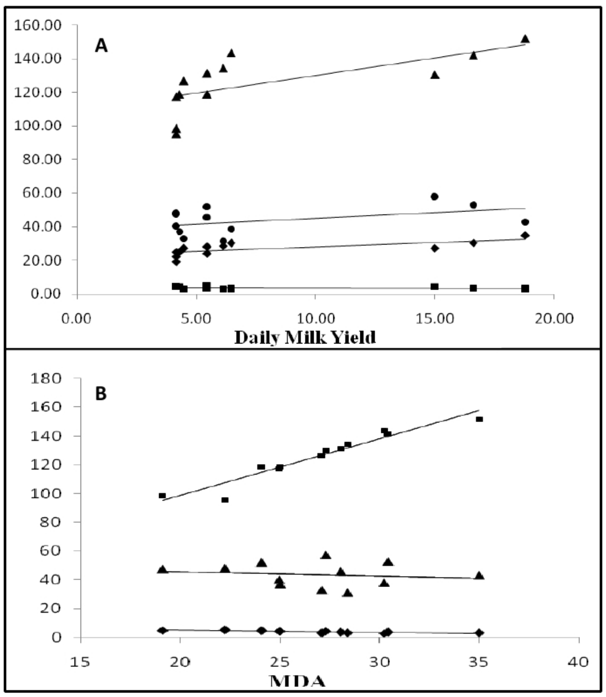

Fig. 3. **(A)** Linear regression graph between DMY and plasma MDA, serum cortisol, glucose and progesterone concentrations. (■ Progesterone $y = -0.0502x + 4.2466$; ▲ cortisol $y = 2.0874x + 109.13$ $R^2 = 0.4463$; ♦ MDA $y = 0.5405x + 22.545$ $R^2 = 0.5065$; ● glucose $y = 0.6711x + 38.427$, $R^2 = 0.2022$). **(B)** Linear regression graph between plasma MDA and serum cortisol, glucose and progesterone concentration. (■ Cortisol, $y = 3.9481x + 19.751$ $R^2 = 0.9208$; ▲ glucose $y = -0.2901x + 51.525$ $R^2 = 0.0218$; ♦ progesterone $y = -0.1627x + 8.213$ $R^2 = 0.7216$).

DISCUSSION

There is strong evidence that increase in genetic merit will reduce fertility (Pryce and Veerkamp, 2001). Intensive milk production in cattle focused on the profitability and maximum utilization of production traits presents a metabolic load for the dairy cattle, which results into various disorders of health and reproduction. Profitability of dairy herds depends on both production and fertility (Lopez-Gatius, 2003). This experiment was conducted to find out oxidative stress due to metabolic burden of lactation and its effect on various physiological and reproductive parameters in dairy cattle breeds under the tropical environment.

Blood glucose supports lactation initially

The glucose concentrations of all the dairy cows decreased in this experiment with increase in milk production. The pure-exotic HF and cross-bred dairy cows showed more prominent changes in glucose concentration than the two local dairy cattle breeds. Higher concentration of glucose is needed for synthesis of more milk production. The milk production of HF and cross-bred dairy cows was higher than the Sahiwal and Achai breeds. Resultantly, glucose concentration of HF and cross-bred dairy cows was lower than the Sahiwal and Achai breeds. A strong negative correlation (r = -0.307, P= 0.001) was observed between DMY and glucose concentration. These results are in agreement with the works of several other researchers, mentioned below.

Glucose availability is a precondition for milk production in dairy cows, and low serum glucose concentration may lead to low milk yield and vice versa (Lohrenz *et al*., 2010). Harrison *et al*. (1990) and Bines *et al*. (2008) has also reported similar results. However, some authors reported no significant change in glucose concentrations during different lactation stages (Regina and Paul, 2013). Previous studies suggest no significant difference between high and low genetic merit cattle with regard to glucose concentration and decrease with ongoing selection for milk. It is debatable if either glucose concentration in high genetic merit is the significant cause of reduced fertility or not. However, the availability of glucose may have important role in reproduction (Veerkamp *et al*., 2003).

Higher milk yield reduces blood glucose concentration

Our study shows a decline in blood glucose concentration with the increase in milk yield beyond 15 kg/day. Milk production has significant effect on glucose drain as energy source. It has been found that lactating cow had relatively low level of glucose (Harrison *et al*., 1990; Jonsson *et al*., 1999; Snijders *et al*., 2001) as shown in our studies, however some studies disagree (Michel *et al*., 1991; Westwood *et al*., 2000). Others found that glucose concentration was low during parturition (Lukes *et al*., 1989) or the glucose drain can occur in late lactation and last longer in non defined animals (Westwood *et al*., 2000).

Glucose-lactation-ovarian cyclicity interaction

In this study it is clearly indicated that post-partum glucose concentrations are lower in superior genetic merit cattle than lower one, same as earlier reported earlier by (Jonsson *et al*., 1999; Harrison *et al*., 1990; Sanjiders *et al*., 2001). Glucose is major source for ovarian functionality (Rabiee *et al*., 1999) and its administration may mask the effects of recombinant GH on growth of follicles (Oldick *et al*., 1997). It is also considered that hypogylcemia may be the primary biochemical change resulting in infertility in dairy cows (McClure *et al*., 1978).

The major gluconegenic substance in ruminants *i.e.*, propionate is suggesting the positive effects of glucose on fertility (Trenkle, 1981) increasing GnRH- induced LH release (Randel and Rhodes., 1980), LH surge due to estrogen (Bushmich *et al*., 1980). Moreover, 2-deoxy-D-glucose inhibits glycolysis, which may block cyclicity and ovulation in cattle (Mc Clure *et al*., 1978). It is considered that influence of glucose on fertility is related as metabolic fuel, primarily (Wahab *et al*., 2016).

MDA as an oxidative stress marker

In the present study lipid peroxidation (plasma MDA concentration) was significantly ($P<0.001$) higher in early lactation than the other two lactation stages in the studied dairy cattle breeds. The production of MDA was higher in HF and cross-bred dairy cows than the local breeds. A positive relationship between oxidative stress (lipid peroxidation) and DMY was found.

The energy requirements for milk production during early lactation often exceed the available energy from feed intake and the cow goes into negative energy balance. As a result, the deficiency in energy needed for milk production demand is compensated by the mobilization of body lipid reserves. This net shortage in energy leads to oxidative stress (Jorritsmaa *et al*., 2003) as demonstrated in our results by increased concentration of MDA. Mobility of body Fat reserves can increase production of milk, although as a consequence increased metabolic complications reduced fertility and increased susceptibility to various infectious and non-infectious diseases (Dechow *et al*., 2004). Several complications like retained placenta, prolapsus uteri and caesarian section etc. due to oxidative stress are commonly observed in Holstein cattle after parturition. Oxidative stress also causes reduction in milk yield, delay in first estrus signs, conception failure and prolonged calving intervals leading to a low fertility (Ocal, 2002). Similar findings are reported by (Beatty *et al*., 2006).

Our findings fully corroborate with the findings of Saleh *et al.* (2007) as they used thiobarbituric acid reactive substances (TBARS) values for lipid peroxidation finding markers in cows. Oxidative stress contributes in disease pathogenesis in dairy cows (Miller *et al*., 1993) and increases its susceptibility to diseases (Sordillo, 2005). Since metabolic demands are linked with lactation initiation, can expectedly increase ROS production leading to oxidative stress. It has been reported that depletion of antioxidant activity and elevated oxidative stress in periparturient period simulated results (Saleh *et al*., 2007). Imbalance between ROS production and antioxidants increases oxidative stress, which may contribute to periparturient disabilities in milch cattle (Waller, 2000; Gitto *et al*., 2002). During periparturient period disease incidence is increased due to numerous genetic, environmental and physiological factors that affect the cow's immunity (Sordillo, 2005). Therefore during parturition oxidative stress may be the major cause of inflammation and compromised immunity in milking cows (Sordillo *et al*., 2009). The performance of high yielding dairy cattle can be optimized to a certain extent by supplementing with diets having optimal levels of micronutrients along with antioxidant (Sordillo *et al*., 2009).

Cortisol and ovarian cyclicity

Cortisol is a main stress hormone and was used as general stress indicator in this experiment. Concentration of cortisol was significantly affected ($P<0.001$) by breed and PPDs. Higher milk yielders (HF and cross-bred) showed higher concentration of cortisol in early PPDs as compare to low milk yielders (Sahiwal, Achai). Several other researchers (Noakes, 2001; Wise *et al*., 1988) also reported higher concentration of cortisol under various stress conditions. Cortisol showed strong positive correlation with both MDA ($r= 0.471$, $P<0.001$) and DMY ($r= 0.392$, $P<0.001$) in this experiment. Literature cited revealed that high milk production needs more energy which in early lactation leads into negative energy balance (NEB) due to non-availability of energy from feed intake. This condition increase lipid peroxidation (MDA) which ultimately results into oxidative stress (Jorritsmaa *et al*., 2003). Similarly, stress of any origin is capable of depleting the body's antioxidant resources (Sconberg *et al*., 1993). It is well documented that hypothalamo-pituitary-gonadal (HPG) axis is activated under normal conditions. However, certain stressors (intrinsic or extrinsic) have the potential to activate the hypothalamo-pituitary-adrenal cortical axis (HPA) (Minton, 1994). Both cortisol and progesterone are synthesized from cholesterol (Staples *et al*., 1998). Body favors HPA axis in stress which ultimately results in the increased production of cortisol.

The progesterone profiles

Progesterone was determined in this study as reproductive parameter because of its role in folliculogenisis and reproductive cyclicity. Significant effects of PPDs and breed was observed on progesterone concentration in this experiment. The correlation analysis (Table IV) showed that progesterone was negatively correlated with cortisol (r= -0.358, P<0.001), DMY (r= -0.20, P= 0.031) and MDA (r= -0.186, P=0.053) and positively correlated to PPDs (r= 0.520, P<0.001) and glucose; however, the correlation with glucose was not significant. the intensity of changes was greater in high genetic merit breeds (HF and cross-bred) dairy cows as compared to local low genetic merit breeds (Sahiwal and Achai) dairy cows, indicating that high genetic merit breeds are more sensitive to oxidative stress as compared to local low genetic merit breeds.

Milk yield and reproductive traits correlations indicate that higher milk yield is associated with reproductive performance in milking cows. Different studies recently reported that through delayed ovulation and reduced conception rates compromised the reproductive performance of dairy cattle as the milk yield is achieved. Selection for higher milk production increases blood somatotropin and prolactin concentration, and decrease lactation antagonist hormone named as insulin, however important for normal follicles development. Alteration in concentration of hormones increase milk production but detrimental for normal physiological function *i.e.,* fertility especially when metabolic demands for lactation are not fulfilled by proper management. Negative energy intensity and timing determine the extent to which GnRH secretion from hypothalamus and **its** effects on the gonadotropins secretions. Therefore, progesterone from ovaries affects the estrus expression and support early pregnancy (Nebel and McGilliard, 1993).

Progesterone is one of steroids from ovary that modulate and effect the functioning of hypothalamus, ovary and pituitary gland. The luteal tissue activity is reflected by the hormone progesterone for ovarian activity, having both anti and pro-ovulatory functions. Similarly if progesterone is administered at the beginning of cyclicity it will exert negative feedback on the gonadotropin cells. On the other hand inside the graffian follicle, progesterone may seep to circulation and further sensitize the pituitary gland for GnRH induced gonadotropins surge if preceded by estrogen priming (Zalanyi, 2001). Only few studies have suggested ovarian steroid production and secretion for gonadotropins. In Jersey cattle plasma progesterone level in the luteal phase of first post-partum was lower than that of control (Lucy and Crooker, 2001). In jersey cattle the higher rate of progesterone can be excluded, it is concluded that corpus luteum produce less progesterone either due to smaller size or lower synthetic ability (Lucy and Crooker, 2001). In milk progesterone level differences among various genetic merit cattle were also observed (Veerkamp *et al*., 2000). Milk progesterone follows differences among production groups with less concentration in higher genetic potential than lower one. This tendency of comparatively lower progesterone in higher genetic merit cattle is in line with earlier findings of Eley *et al*. (1981a, b) and Lucy and Crooker (2001).

Decreasing post-partum blood progesterone level may explain at least partly the decreasing fertility in lactating cattle (Lucy and Crooker, 2001). The negative energy balance may play vital role in this regard. Earlier studies by Villa Godoy *et al*. (1988, 1990) investigated the effect of early lactation energy on progesterone secretion, indicated that feed intake in early postpartum influences more the progesterone concentration than milk production in breeding season. Since the mature follicle that ovulates in breeding are in initial stage of development, therefore due to lactation they become sensitive to negative energy balance. Exact time and intensity of negative energy balance obviously interacts to find the extent to which hypothalamic secretion of GnRH and gonadotropins are altered by negative energy balance.

CONCLUSIONS AND RECOMMENDATIONS

Correlations between reproductive traits and milk yield indicate that higher milk yield is associated with reduced reproductive performance in lactating cows, primarily through delayed ovarian activity as assessed by reduced progesterone concentrations during high milk production phase, suggesting that higher genetic merit (HF and cross-bred) dairy breeds should give extra managemental support during periparturient period for their better productivity and fertility.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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