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A STUDY ON CERTAIN BLOOD BIOMARKERS OF PREGNANT CHOLISTANI COWS

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ABSTRACT

The study investigates the alterations in hematological and cortisol levels in three trimesters of pregnancy in the cows of Cholistani breed being reared under nomadic pastoralism production system at Cholistan desert, Pakistan. Pregnant cows (n=12) ranging between 3 to 5 years of age were incorporated in the study and were grouped as (n= 04 each) *viz.* belonging to 1st, 2nd and 3rd trimesters of pregnancy. Blood was collected and analyzed for salient hematological parameters through manual methods, and for cortisol assessment through commercial ELISA kit. Each cow was bled thrice in a month hence making a total of 36 blood samples. The mean values for Total Erythrocytic Count and hemoglobin were higher ($P<0.05$) in the first as compared to the second and third trimesters, respectively. There was no difference ($P> 0.05$) in the Packed Cell Volume during the three trimesters of pregnancy. Regarding the erythrocytic indices, an increase ($P<0.05$) was observed for Mean Corpuscular Volume in the three trimesters of pregnancy. Whereas, a decrease ($P<0.05$) was noted for Mean Corpuscular Hemoglobin Concentration. There was no difference ($P>0.05$) for Mean Corpuscular Hemoglobin across the length of pregnancy. There was an increase ($P<0.05$) in the Total Leukocytic Count in the second and third trimesters of pregnancy, however,there was no difference ($P>0.05$) in the Differential Leukocytic Count during the three trimester of pregnancy. Cortisol tended to increase ($P<0.05$) with advancing stage of pregnancy. The alterations in hematological parameters and cortisol level of pregnant Cholistani cows are marked in each trimester. Hemodilution, an enhanced TLC and cortisol level are the main features of pregnancy in pregnant Cholistani cows. However, in general, they tend to maintain a harmonious pattern which may be an indication of their innate adaptive stress-tolerant ability.

Key words: Cortisol, Cholistani cows, hematology, stress indicators.

INTRODUCTION

Hematological investigation is considered a vital management and diagnostic tool in animals all over the world. It provides dependable diagnostic information to assess the health status and level of stress in animals (Farooq *et al*., 2011; Fadare *et al.*, 2012). Furthermore, it offers authentic information about the nutritional, physiological and pathological status of a living organism (Doyle, 2006). It is also helpful in distinguishing between normal state and any kind of stress, which could be due to nutrition or harsh environmental conditions (Aderemi, 2004). The assessment of these hematological indices at various physiological states of an animal is helpful in diagnosing different pathological/metabolic disturbances that may have a negative impact on productive/reproductive status of cows resulting in huge economic losses (Sattar and Mirza, 2009). Pregnancy is a physiological condition in which hematological profile keeps on changing during the gestation period in all animal species (Mir *et al.*, 2008). Review of literature reveals that studies on hematological profile in pregnant cross bred and *Bos taurus* cattle breeds have been documented (Sattar and Mirza, 2009; Mir *et al*., 2008; Mirzadeh *et al*., 2010). However, to the best of our knowledge, no such experimentation has been reported for Cholistani breed of cattle – a *Bos indicus* breed- being reared in Cholistan desert, Pakistan under the nomadic pastoralism.

Cholistani is a humped or Zebu breed of cattle of Indian origin. This neglected breed has gained substantial attention in the last decade or so, and extensive work has started pouring in regarding its productive and reproductive parameters (Mahmood *et al*., 2013, 2014; Farooq *et al*., 2010, 2012, 2013a, 2013b, 2015, 2017) from all over Pakistan. This indigenous heat-tolerant breed of cattle is being reared by the nomads of the Cholistan desert, Pakistan, under nomadic pastoralism. It is well adapted to harsh conditions in the Cholistan desert, serving as a dual purpose animal (milk and draught), and playing a pivotal role in the socio-economic development of Cholistani nomads (Farooq *et al*., 2012, 2013a, 2017). The present study was devised to investigate alterations in hematological and cortisol levels in the three trimesters of pregnancy in the cows of Cholistani breed being reared under nomadic pastoralism.

MATERIALS AND METHODS

Study Area: The present study was conducted in the Cholistan Desert, Pakistan. Sprawling over an area of 26,000 Km^2, this desert is located at latitudes 27º42′and 29º45′ North and longitudes 69º52′ and 75º24′East and at an altitude of about 112m above sea level (Farooq *et al*., 2017). The detail of its climate and geomorphology have extensively been reviewed by one of the co-authors (Farooq *et al*., 2010, 2017).

Study Animals: The primary source of water in the Cholistan Desert is rain water, which is stored in natural depressions or manmade ponds (called 'Tobas' in the local dialect) for use by humans and their animals (Farooq *et al*., 2010, 2017). One such Toba, namely 'Maujgarh; was selected in the desert, with Cholistani cows settled near by. A total of twelve pregnant Cholistani cows ranging between 3 to 5 years of age were included in the study and assigned to three groups (n= 04 each) *viz.* belonging to 1st, 2nd and 3rd trimesters of pregnancy. There was no clinical abnormality in any of the animals included in the study and they were all free of endo/ectoparasites. All the livestock in Cholistan desert are reared under nomadic pastoralism production system (Ali *et al*., 2009; Farooq *et al*., 2010, 2015, 2017). A written consent was taken on an appropriate proforma from the Cholistani pastoralists involved in our study. The study was approved in full by the "Ethical Review Committee for the Use of Animals" which comes under the administrative control of the 'Office of the Research, Innovation and Commercialization' of the Islamia University of Bahawalpur, Pakistan.

Blood Collection: The present study was conducted in the month of April-June. About 7mL of blood was collected from the jugular vein of the animals under appropriate restraining and stored as two aliquots: clotted for harvesting serum and un-clotted (0.5M EDTA) for hematological analysis. Collection was made aseptically from the jugular vein with the help of a disposable syringe and was transferred into screw capped tubes containing 0.5 mL of 1% EDTA solution as an anticoagulant. For the sake of standardization of collection technique, all samples were collected by the same person from the animals restrained by the same technique. Each cow was bled thrice a month hence making a total of 36 blood samples. Transportation of samples was made in an ice box to the Physiology Laboratory, University College of Veterinary and Animal Sciences, the Islamia University of Bahawalpur, Pakistan, refrigerated and analyzed within 12h for the assessment of hematological parameters and serum cortisol level.

Hematological Analysis: The hematological parameters were analysed through manual methods. Hemoglobin (Hb) was detected by cyanmethemoglobin method, Packed Cell Volume (PCV) by microhematocrit (Apel, HC 702, Japan), and Total Erythrocyte Count (TEC) and Total Leukocyte Count (TLC) by Neubauer's haemocytometer. Jain's formula (1998) was used to deduce erythrocytic indices *viz.* Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC). The Differential Leukocytic Count (DLC) was carried out through blood slides stained with Wright's Stain.

Serum Cortisol Assay: In-vitro quantitative measurement of cortisol was made from the extracted serum. The ELISA kit for cortisol (Cloud-Clone Corp. Houston, TX, USA; CAT No. CEA462Ge; Detection Range 12.35-1000ng/mL; Minimum Detection Limit 4.52ng/mL) was used to determine the estimated value(s) through reading at 450nm with ELISA reader.

Statistical Analysis: Statistical analysis was conducted through Statistical Package for Social Science (SPSS for Windows version 12, SPSS Inc., Chicago, IL, USA). The mean values (± SEM) for the hematological parameters and cortisol concentration were calculated. Variation in these parameters was deducted through one-way ANOVA and the difference between the mean values for three trimesters was attained through the Duncan's Multiple Range Test. Significance was attributed at $P < 0.05$.

RESULTS

The results of salient red blood cell parameters are presented in Table 1. The mean values for TEC were higher ($P < 0.05$) in the first as compared to the second and third trimesters, respectively. Hemoglobin concentration showed a similar pattern of variation in accordance with that recorded for TEC. There were no differences in PCV ($P> 0.05$) among the three trimesters of pregnancy for the cows in the present study. Regarding the erythrocytic indices, an increase ($P<0.05$) was observed for MCV in the three trimesters of pregnancy for the cows. Whereas, a decrease ($P<0.05$) was noticed for MCHC. No variation ($P>0.05$) was noted for MCH across the length of pregnancy. The results for white blood cell parameters are given in Table 2. The TLC showed an increase ($P < 0.05$) in the second and third trimesters of pregnancy in the present study as compared to the first one. None of the parameters of DLC showed a significant ($P > 0.05$) variation in the present study during three trimesters for pregnant Cholistani cows. A statistical non-significant increase ($P > 0.05$) in neutrophil count and decrease ($P > 0.05$) in lymphocytes was, however, observed in the last trimester. In the present study, cortisol levels were studied which revealed a significant ($P < 0.05$) increase in all the three trimesters of pregnancy for the cows (Fig. 1).

Table 1. Values for red blood cell parameters in Cholistani cows at different trimesters of pregnancy.

Parameter	1st Trimester	2nd Trimester	3rd Trimester
TEC (10^{12}/L)	7.58 ± 0.5[a]	6.63 ± 1.0[b]	6.11 ± 0.7[b]
Hb (g/L)	95.8 ± 0.3[a]	87.0±0.7[b]	78.0 ± 0.2[b]
PCV (L/L)	0.302 ± 0.2	0.299 ± 0.2	0.304 ± 0.2
MCV (fL)	40.9 ± 1.3[a]	45.84±1.4[b]	50.84±1.5[c]
MCH (pg)	32.2 ± 0.4	31.1 ± 0.4	32.1 ± 0.5
MCHC (g/L)	319 ± 0.7[a]	292±0.2[a]	257 ± 0.1[b]

Data shown are mean±SE (n=4).
[a,b,c]Denote difference (P<0.05) within rows.

Table 2. Values for white blood cell parameters in Cholistani cows at different trimesters of pregnancy.

Parameter	1st Trimester	2nd Trimester	3rd Trimester
TLC (10^9/L)	6.92 ± 0.5[a]	7.49 ± 0.4[b]	7.62 ± 0.2[b]
Neutrophils (%)	35.58 ± 0.3	34.08 ± 0.5	37.75±0.9
Lymphocytes (%)	60.16±1.1	63.33±1.9	58.92±1.1
Eosinophils (%)	1.58±0.6	1.33±0.4	1.33±0.4
Basophils (%)	1.33±0.5	1.33±0.4	1.33±0.5
Monocytes (%)	0.58±0.5	0.5±0.5	0.66±0.4

Data shown are mean±SE (n=4).
[a,b]Denote difference (P<0.05) within rows.

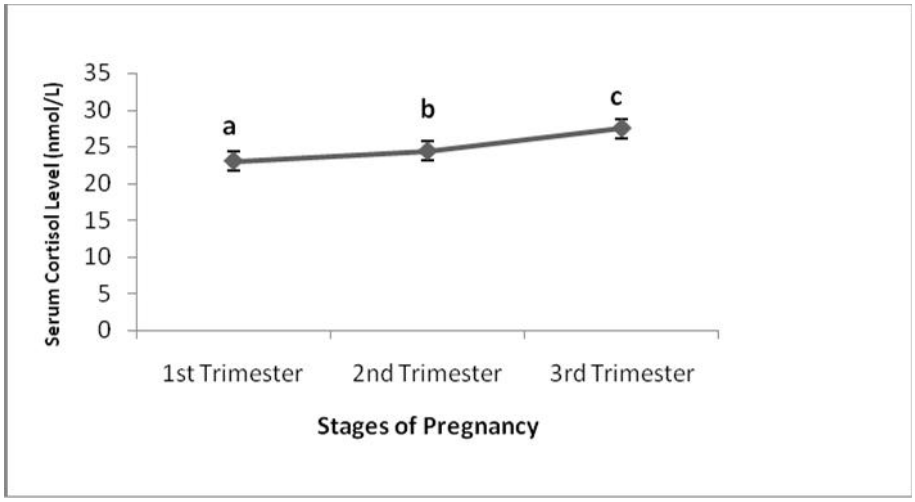

Fig 1. Serum cortisol concentrations in Cholistani cows during different trimesters of pregnancy. Data are mean ±SE (n=4). [a,b,c]Denote difference (P<0.05) within timesters.

DISCUSSION

The present preliminary study is the first of its kind which was designed to ascertain variation in salient hematological parameters and cortisol level in Cholistani cows during the three trimesters of pregnancy.

The mean values for TEC were significantly higher in the first as compared to the second and third trimesters, respectively. These results are in line with previous reports for various species of pregnant animals such as mares, sows, sheep, bitches and goats (Azab and Abdel-Maksoud, 1999; Bozdogan *et al*., 2003). Similar results have also been reported for cross bred (Mir *et al.*, 2008), *Bos taurus* (Kupczyński and Chudoba-Drozdowska, 2002; Piccione *et al*., 2012) and *Bos indicus* pregnant cows (Ahmad, 1995). This decrease in TEC in the last trimester has been attributed to the hemodilution effect because of an increased plasma volume (Guyton and Hall, 1996). This might have a physiological significance as it reduces blood viscosity thereby, enhancing blood flow to the micro-circulation. A plausible significance is enhanced blood flow through the placenta which increases diffusion of materials across it which in turn is responsible for appropriate fetal growth (Azab and Abdel-Maksoud, 1999; Bozdogan *et al*., 2003; Mir *et al*., 2008).

Hemoglobin concentration showed a similar pattern of variation in accordance with that recorded for TEC *i.e*. being significantly higher in the first trimester as compared to the second and third trimesters. This is not in accordance with previous reports which have established that during advanced pregnancy, the hemodilution occurs without any reduction in Hb concentration (Mir *et al.,* 2008; Piccione *et al*., 2012). This phenomenon is vital for maintaining O_2 level in blood for unhindered fetal growth. On the contrary, Bozdogan *et al.*, (2003) have reported similar results for pregnant crossbred cows. The decrease in Hb level of Cholistani cows in the second and third trimester of our study could be an innate ability of this breed as an adaptive mechanism to pregnancy, or it could be attributed to variation in breed and lab tests implied for Hb estimation (Jain, 1998; Wassmuth *et al.*, 2011).

The PCV did not vary significantly during the three trimesters of pregnancy for the Cholistani cows in the present study. This is not in accordance to the previous work reported for various species of animals (Azab and Abdel-Maksoud, 1999; Piccione *et al.*, 2012) in which a significant ($P<0.05$) decrease was noticed with the advancing pregnancy which has been related to decreased TEC. The variable results of our study could be correlated to the variation in breed, an inherited predisposition or geographical variation.

Regarding the erythrocytic indices, a significant increase was noticed for MCV in the three trimesters of pregnancy for Cholistani cows, whereas, a statistically significant decrease was noticed for MCHC. No significant variation was noted for MCH across the length of pregnancy. These results are in line with various earlier published reports for various species of farm animals (Balikci and Yildiz, 2005; Opara *et al.*, 2006; Mir *et al.*, 2008; Piccione *et al.*, 2012). A higher MCV with advancement of pregnancy has been correlated to an increased size of RBCs during pregnancy (Mir *et al.*, 2008).

The TLC showed a significant increase in the second and third trimesters of pregnancy in the present study as compared to the first one. The present results are in line with earlier published reports which have observed marked leukocytosis with advancement in pregnancy and at birth (Azab and Abdel-Maksoud, 1999; Quiroz-Rocha *et al.*, 2009; Piccione *et al.*, 2012). This has been attributed to pregnancy associated stress in pregnant animals.

None of the parameters of DLC showed a significant variation in the present study during the three trimesters for pregnant Cholistani cows. A statistically non-significant increase in neutrophil count and decrease in lymphocytes was, however, observed in the last trimester. Previous reports have mentioned a significant variation in these parameters (Quiroz-Rocha *et al.*, 2009; Piccione *et al.*, 2012). However, similar results have been reported by Mir *et al.,* (2008). A higher TLC in advanced stages of pregnancy in farm animals is, in fact, because of neutrophilia (Jain, 1998; Quiroz-Rocha *et al.*, 2009; Piccione *et al.*, 2012). A decreased lymphocytes count in the last trimester has been justified owing to decreased lymphocytic proliferation (Sattar and Mirza, 2009). In general, a harmonious pattern of white blood cells during the three trimesters in the present study could be attributed to the innate adaptive ability of this breed as reported earlier (Farooq *et al.*, 2010, 2012, 2017).

Hormones are the major stabilizing factors in pregnancy. Tracking hormonal changes is an essential part of a pregnancy study to define their normal values, exceed or decrease from which can be dangerous (Alameen and Abdelatif, 2012). In the present study, cortisol levels were studied which revealed a significant increase in all the three trimesters of pregnancy for Cholistani cows. The present results clearly follow those of Higashiyama *et al.,* (2005), Meinlschmidt *et al.,* (2010), and Alameen and Abdelatif (2012) which have shown that both the season and pregnancy are responsible for a rise in cortisol levels. Cortisol plays a central role in biochemical changes that occur in pregnancy. Its increase is an indicator of emotional stress and physiological challenges in pregnancy and also a possible risk signal (Sandman *et al.*, 2006).

In conclusion, the alterations in hematological parameters and cortisol level of pregnant Cholistani cows are marked in each trimester. Hemodilution, an enhanced TLC and cortisol level are the main features of pregnancy in pregnant Cholistani cows. However, in general, they tend to maintain a harmonious pattern which may be an indicative of their innate adaptive stress-tolerant ability. A detailed approach with a larger population, larger sample size allied with biochemical, behavioral and physiological parameters is needed for future scenarios.

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