HAEMATO-BIOCHEMICAL PROFILE IN DYSTOTIC COWS AND BUFFALOES

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Certificate

This is to certify that the thesis entitled ***"HAEMATO-BIOCHEMICAL PROFILE IN DYSTOTIC COWS AND BUFFALOES"*** submitted by **Mr. AMAR DHURVE., I.D. No. MVNK-1306** in partial fulfillment of the requirements for the award of **MASTER OF VETERINARY SCIENCE** in **VETERINARY GYNAECOLOGY AND OBSTETRICS** of the Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar is a record of bona-fide research work done by him during the period of his study in this University under my guidance and supervision, and the thesis has not previously formed the basis of the award of any degree, diploma, association ship, fellowship or other similar titles.

BIDAR
JULY, 2015

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DEDICATED

TO

MY BELOVED PARENTS

TEACHERS

AND LATE GRAND FATHER

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INTRODUCTION

I. INTRODUCTION

Dystocia is defined as delayed or difficult calving, sometimes requiring significant human assistance. (Lombard *et al*., 2007; Zaborski *et al*., 2009; Uzamy *et al*., 2010). The dystocia has been a long-standing problem in both beef and dairy industry, occurring in 3 to 25% of cattle pregnancies. It is one of the most serious complications of pregnancy in cattle and buffaloes and is associated with numerous factors such as pelvic area of the cow, birth weight of the calf, age of dam, twin pregnancy, presentable disposition, gestation length, sex of the calf and body condition of the cow at calving, hormonal status and nutrition of dam (Noakes, 2001). Calving difficulty can lead to increased post parturient disorders such as retained placenta, uterine infections, increased veterinary costs, reduction of milk production, failure to conceive, long calving intervals and reduced health of cows and survival of calves (Bellows and Lammoglia, 2000).

Amongst all domestic animals, cattle and buffalo are considered the species in which the incidence of dystocia appears to be highest. Although cattle and buffaloes appear to be similar in the parturition process but subtle differences are known to be existent in the anatomy and physiology of the birth canal between cows and buffaloes. (Purohit *et al.,* 2011). The anatomic differences in the pelvis (Kodagali, 2003) and genital structures have been described. The differences in the pelvis between cow and buffalo include more capacious pelvis, larger area of ilium and the free and easily separable fifth sacral vertebra in the buffalo (Kodagali, 2003). The differences in the genital structures include tightly downward curled uterine horns, less conspicuous shorter and narrower cervix, smaller and less tight vagina and elongated and wide apart vulvar lips in the

buffalo (Agarwal and Tomer, 1998). Physiologic differences between cattle and buffalo pregnancy and parturition include a longer gestation period in the buffalo (305 to 320 days for the river and 320 to 340 days for the swamp buffalo (Jainudeen, 1986) compared to 280 days in cattle (Agarwal and Tomer, 1998), lesser time required for completion of first and second stages of labor (Kodagali, 2003; Mody 2002; Ramasamy and Singh , 2002) (70 and 20 minutes in buffalo compared to 2 to 6 and 0.5 - 1 hours in cows), a preponderance for parturition during night hours (Manju and Varma,1985) and an absence of physiological cervical hypertrophy with consecutive calvings in the buffalo (Agarwal and Tomer, 1998). All the above differences between cows and buffaloes point out that, the parturition process is much easier in the river buffalo compared to cows and therefore, Jainudeen (1986), considers that dystocia is not a serious problem in the water buffalo. The incidence of dystocia is considered to be higher in river than in swamp buffalo and also in primipara than in pleuripara (Jainudeen, 1986) however, a few studies consider higher incidence of dystocia in pleuriparous buffaloes (Phogat *et al*., 1992). In cows the incidence of dystocia is higher compared to that in heifers (Berger *et al*., 1992; Mee, 2008 and Zaborski *et al*., 2009). Abnormal calvings in buffalo were found to be between 5.6-12.6% in Murrah, 8.94% in Jaffarabadi and between 4.6 to 5.4% in Surti buffalo (Khan *et al.,* 2009).

The causes of dystocia are generally classified into the maternal and fetal causes (Jackson, 1995 and Arthur *et al.,* 1996). Buffaloes are known to have greater incidence of maternal dystocia (Saxena *et al.,*1989 and Nanda, *et al.*, 2003). The maternal causes of dystocia are considered to be arising either because of the constriction or obstruction of the birth canal or due to a deficiency of the maternal expulsive force (Youngquist, 1997

and Purohit, 2006). The constriction or obstruction of the birth canal can result in maternal dystocia and can be due to pelvic abnormalities, vulvar or vaginal stenosis, neoplasms of the vagina and vulva, vaginal cystocoele, incomplete cervical dilation, uterine torsion and ventral displacement of the uterus. An uncommon cause of constriction of birth canal is carcinoma of urinary bladder (Gupta, 1983) with metastasis in cervix. The most common cause of primary uterine inertia in dairy cows (Jackson, 1995) and buffaloes (Pargaonkar, *et al*., 1993) is considered to be hypocalcaemia, with the animal showing signs of milk fever as calving is about to begin. Secondary uterine inertia occurs due to exhaustion as a result of dystocia (Arthur *et al.*, 1996). Conditions like traumatic reticulitis or pericarditis, painful conditions of diaphragm or chest may cause voluntary inhibition of attempts to strain (Jackson, 1995).

Studies on cattle indicate that the fetus is the major cause of dystocia (Sloss and Johnston, 1967; Majeed *et al*., 1989 and Khammas and Al-Hamedawi, 1994) and abnormal fetal presentations at birth contribute to 1- 5% of total dystocia cases (Nix *et al*., 1998; Bennett and Gregory, 2001). In contrast, fetal origins of dystocia are less frequent in buffalo (Purohit and Mehta, 2006). Broadly speaking, the fetal origins of dystocia in cattle can be divided into those caused by excessive fetal size relative to the maternal pelvis (feto-pelvic disproportion) and those caused by abnormalities of the fetus such as fetal monsters, fetal diseases and fetal maldisposition (Youngquist, 1997 and Zhang *et al*., 1999).

Uterine torsion is the most frequent cause of dystocia in buffalo, followed by incomplete dilatation of cervix and uterine inertia. It is observed commonly in

pluriparous animals at the time of parturition or during the last month of gestation (Roberts, 1986). The occurrence of uterine torsion increases adrenocortical activity and influences blood vascular cellular components as well as the metabolism of liver, kidney and muscular system. Normal parturition in bovines has negligible influence on the blood enzymes (Hussein and Abd Ellah, 2008). Uterine torsion is associated with muscle damage or hepatic dysfunction consequently leading to stressful condition (Manju *et al.,* 1985). The alterations in blood parameters are suggestive of deteriorating condition of the dam and thus help to decide about the institution of various therapies, viz. anti-stress, liver protection and electrolyte therapy (Ghuman, 2010).

The erythrocyte parameters and total and differential leukocyte counts are affected by various physiological determinants (Klinkon, 1992) as well as factors from the environment. However, there is no information available concerning hematological parameters in cattle with dystocia. (Yıldız *et al.,* 2011). RBC count, eosinophil, monocyte and basophils in buffaloes having retention of fetal membranes (Pandey *et al*., 2007), WBC count, eosinophil, monocyte and basophils in cows with retained placenta (Farzaneh *et al*., 2006) and normal parturient animals were non–significantly different. Similarly, RBC, eosinophil and basophils remained unchanged in animals of prolapsed and control groups (Ahmed *et al.*, 2005).

A significant increase in neutrophil count was recorded in dystocia-affected animals as compared to normal control (Yıldız *et al*., 2011). Tarjinder and Singh (1993) and Ahmed *et al.,* (2005) reported an increase in neutrophil count in prolapsed animals. This increase in the neutrophil count may be due to increased level of cortisol because of

stress (Amer *et al.,* 2008). However, neutrophilia has also been reported during excitement, exercise, adrenaline and ACTH release (Rakuljic-Zelov and Zadnik, 2002). It has been shown that hematocrit values in buffaloes with retained placenta (Ahmed *et al*., 2009), prolapse (Ahmed *et al.,* 2005) and uterine torsion (Amer *et al.,* 2008) were lower compared to control animals. In this study, blood hematocrit values were lower in cows suffering from dystocia. This decrease might be due to possible release of antidiuretic hormone as a result of stress, anorexia and toxemia (Kinney, 1967). Similar findings have been reported by Amer *et al.,* (2008) and Ahmed *et al.,* (2005, 2009).

Metabolic profile (Complete haematological and biochemical) test is a pre-symptomatic diagnostic aid capable of giving early warning of certain types of metabolic derangement in dairy animals (Canfield *et al.,* 1984). It has recently been proved that the metabolic profile testing as a best tool for the assessment of dairy herd's nutritional status with simple blood test (Hasanpour *et al.,* 2008). But these indices may vary depending on factors such as origin, climate, management practice, geographical distribution and stage of animals. Therefore, determination of normal haematological and blood biochemical values is important for the clinical interpretation of laboratory data especially in the pregnant animals which require adequate balanced nutrition in the periparturient period to maintain homeostasis for onset of parturition and lactation (Ali, 2008).

Circulating activities of transaminases, phosphatases and lactic dehydrogenase are index of tissue damage and stress (Highman and Atland, 1960). Thus, in uterine torsion biochemical enzyme assay could be useful to predict survival of affected buffaloes and in selection of post operative treatment schedule depending upon the severity and duration

of stress imposed by toxemia consequent to death of fetus (Phogat *et al.,* 1995). Normal parturition in bovines has negligible influence on the blood enzymes (Hussein and Abd Ellah, 2008).

Uterine function is often compromised in cattle by bacterial contamination of the uterine lumen after parturition; pathogenic bacteria frequently persist, causing uterine disease, a key cause of infertility (Sheldon and Dobson, 2004). The presence of pathogenic bacteria in the uterus causes inflammation, histological lesions of the endometrium, delays uterine involution and perturbs embryo survival (Sheldon *et al.,* 2006). In addition, uterine bacterial infection, bacterial products or the associated inflammation, suppress pituitary LH secretion and perturb postpartum ovarian follicular growth and function, which disrupt ovulation in cattle (Sheldon *et al.,* 2002). Thus, uterine disease is associated with lower conception rate, increased intervals from calving to first service or conception and more cattle culled for failure to conceive (Huszenicza *et al.,* 1999; LeBlanc *et al.,* 2001; Sheldon *et al.,* 2006). Postpartum metritis is one of the most important disorders in buffaloes (Rao, 1982; Rao and Sreemannarayana, 1983), causing high economic losses due to prolonged days open and prolonged intercalving intervals, resulting in involuntary culling (Esslemont and Peeler, 1993). Peri-parturient insults, including dystocia, uterine prolapse and retained foetal membranes diminish uterine ability to eliminate contaminated organisms. The exact causes of uterine infections during the postpartum period remain unknown (Lewis, 1997).

In view of this, the present research work was carried out with the following objectives.

1. To study relationship between clinical signs and haematological and serum biochemical values in dystotic cows and buffaloes.

2. To study the uterine microflora in dystotic cows and buffaloes.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

Many authors have investigated various factors affecting the incidence of calving related disorders and their effect on haematological, biochemical parameters and uterine fluid microbiology. The review of literature is divided into following subheadings:

2.1 Incidence of dystocia in cows

Kanakapur (1992) observed in his study that incidence of dystocia was significantly lowest in cows aged eight years and above. Further, the incidence was higher in cows aged four to six years as compared to those aged two to four years. He suggested that this could be due to larger pelvic area of the dam. He also found that the incidence in cows in fourth parity was significantly lower when compared to dams in first parity. He recorded significantly lower incidence of dystocia in cows during cold season than compared to other seasons.

Agarwal and Tomer (1998) found lower incidence of dystocia in buffalo compared to cattle and stated that it could be due to anatomical differences like easily dilatable small sized vaginal canal, elongated and wide apart vulvar lips.

Fourichon *et al.* (2001) recorded that 6.60% of Holstein Friesian and Normande breed cows were dystotic in France.

Meyer *et al.* (2001) showed dystocia rates in Holsteins were 17.80% and 6.10% for heifers and cows respectively in USA and opined that dystocia rate could be up to three times greater in primiparous compared to that in pluripara.

Noakes *et al.* (2001) stated that dystocia was one of the most serious complications of pregnancy in cattle and its incidence was 3 to 25%.

Johanson and Berger (2003) reported that calves born in winter were 15% more likely to need assistance than calves born in summer.

Wehrend and Bostedt (2003) shown the incidence of cervical dystocia 11.1 to 16.7 percent in cows.

Dargatz *et al.* (2004) analyzed 29,375 suckler cows and established 16.7% of dystocia in heifers and 2.8% in other cows.

Clintock (2004) reported that 9.50% and 4.10% of heifers and cows calving respectively had dystocia in Holstein Friesian in Australia

Hansen *et al.* (2004) reported 8.70% of prevalence of dystocia in Holstein Friesian heifers in Denmark.

Rumph and Faust (2006) reported the prevalence of dystocia in Holstein Friesian breed heifers and cows in UK were 6.90% and 2% respectively.

Mee *et al*. (2007) concluded that cows which have experienced dystocia were more likely to experience it again at a subsequent calving.

Mee (2008) concluded that national dystocia rates in dairy cows varied between 2% and 14%

Linden *et al.* (2009) analyzed that dystocia is characterized as abnormal birth that requires assistance and is one of the most serious complications of buffalo and cattle at parturition. Its incidence rate is about 3-25% of all pregnancies in cattle and results in reduced productive performance and economic loss.

Rabbani *et al.* (2010) concluded that the incidence of calving related disorders is high in the buffalo compared to cattle.

Gaafar *et al.* (2011) reported that the overall incidence of dystocia was 6.9%. The percentage of dystocia decreased with increasing live body weight, age, and parity of cows. however, it increased with increasing birth weight of calves. The highest percentage of dystocia was detected in winter season, but the least percentage was in summer season. The percentage of incidence of dystocia was significantly higher with twinning than single calving (15.5% vs. 6.5%), while not significantly affected by the sex of born calves. Incidence of dystocia had adverse effects on reproductive performance and milk yield.

Mee *et al.* (2011) analyzed 1,52,641 calving from Holstein Friesian dams over 4 years in Ireland and reported overall incidence of dystocia as 6.80%. The dystocia incidence in primiparae and pluriparae were 9.30% and 5.80% respectively.

Atashi *et al*. (2012) reported that the average incidence of dystocia was 10.8% and the mean (SD) calf birth weight was 42.13 (5.42) kg. Primiparous cows had calves with lower body weight and were more likely to require assistance at parturition. Female calves had lower body weight and had a lower odds ratio for dystocia than male calves.

Twins had lower birth weight and had a higher odds ratio for dystocia than singletons. Cows which gave birth to a calf with higher weight at birth experienced more calving difficulty.

Purohit *et al*. (2012) stated that in their study on incidence of dystocia in cows, most were presented in their first parity (32.30%), nearly equal proportions (30.20%) of cows were in second parity and significantly lower proportion of cows were presented in their third and subsequent parities.

Jeengari *et al.* (2015) stated that the incidence of bovine dystocia between October, 2012 to September, 2013 was screened. A high incidence of maternal cause of dystocia was found in both cows (78.89%) and buffaloes (80.33%).

2.2 Incidence of dystocia in buffaloes

Jainudeen (1986) stated that buffaloes generally have a lower incidence of dystocia 1 to 2%. However, still it had a considerable impact on buffalo production.

Phogat *et al.* (1992) reported that incidence of narrow pelvis is around 8-9% in bovines.

Sane *et al.* (1994) analyzed the incidence of uterine torsion in Surti buffaloes is relatively high. As a result of torsion, the birth way get narrow and stenosed hindering calving.

Moore and Richardson (1995) stated that the right side uterine torsion was of more than 100 degree incidence (73.33%) than the left side uterine torsion.

Srinivas *et al*. (2007) reported that the incidence of dystocia was less frequent in primiparous (21.13%) compared to pluriparous (78.87%) graded Murrah buffaloes.

Khan *et al*. (2009) analyzed the incidence of dystocia in buffaloes in various breeds and it was 5.60%-12.60% in Murrah, 8.94% in Jaffarabadi and 4.60%-5.40% in Surti buffaloes.

Thiruvenkadan *et al.* (2009) reported that 33.90% of buffaloes with dystocia were presented in their second parity compared to 30.30% which were presented in their first parity and a significantly lower proportion of buffaloes were presented in their subsequent parities.

Purohit *et al.* (2011) stated that in buffaloes, maternal dystocia was common (59.82%) compared to foetal dystocia (40.18%).

Purohit *et al.* (2012) reported that a case analysis of 192 and 112 dystocia in cattle and buffalo, respectively, at referral center revealed that dystocia is significantly higher in first and second parity cows and buffalo and that dystocia of fetal origin is common in cows (65.62%) but less frequent (40.17%) in buffalo.

2.3 Causes of dystocia in cows

Roberts (1971) stated that fetal ascites is seen as an occasional cause of dystocia in many species but occurs most often in the cow.

Noakes *et al.* (2002) reported that incomplete dilatation of cervix is a common cause of dystocia in cattle. Incomplete dilatation in multiparous cows may be associated

with uterine inertia caused by hypocalcaemia. In these animals, the response to calcium therapy is rapid.

Wehrend *et al.* (2002) have observed that incorrect fetal orientation of a dead fetus was the most frequent cause (38.9%) of dystocia in dairy cattle and similar findings were recorded by (Holland *et al*., 1993) in beef cows.

Nanda *et al.* (2003) studied that incomplete dilatation of cervix (50%) in cows.

Uzamy *et al.* (2010) stated that the incidence of dystocia was more frequent in the primiparous heifers and that fetopelvic disproportion was the main cause of dystocia in heifers.

Patil *et al.* (2014) reported that fetal causes of dystocia were common in cows (20.95%).

Jeengari *et al.* (2015) stated that maldisposition of fetus was the commonest cause of fetal dystocia in cows (16.67%). Imperfect dilatation of cervix (50%) the major cause of maternal dystocia in cattle. Other causes of dystocia with low incidence include narrow pelvis, fetal emphysema and fetal monster.

2.4 Causes of dystocia in buffaloes

Deshmukh (1975) reported that incidence of pelvic deformities as a cause of dystocia in buffaloes was 1.2 percent.

Kodagali (2003) stated that incidence of dystocia was lower in buffaloes compared to cattle and this could be due to anatomical differences like, buffalo had a

more capacious pelvis, larger area of ilium, a free and easily separable fifth sacral vertebra.

Kumaresan *et al.* (2003) stated that dystocia due to foetal monster particularly hydrocephalus is rare in buffaloes.

Nanda *et al.* (2003) stated that uterine torsion (55.74%) is the major cause of dystocia in buffaloes.

Purohit and Mehta (2006) stated that there were less frequent fetal dystocia in buffaloes.

Noakes *et al.* (2009) reported that hydrocephalus is one of the rare congenital conditions seen in buffaloes. Hydrocephalus involves dilation of ventricular system and subarachnoid space due to accumulation of fluid in fetus.

Purohit *et al.* (2011) reported that the incidence of uterine torsion is considered to be higher in buffaloes compared to cows.

Naidu *et al.* (2014) considered uterine torsion as the single most important reason for dystocia with an incidence of 76.86%. Fetal dystocia in anterior and posterior presentation were 88.88 and 11.12 percent while those of head and limb maldisposition, fetal emphysema and monsters were 40, 60, 8.88 and 3.34 percent respectively. Highest survival rate of 98.07% was achieved using modified Schaffer's method of detorsion among uterine torsion treatment regimes.

Patil *et al.* (2014) reported that maternal causes of dystocia were common (15.42%) in buffaloes.

Jeengari *et al.* (2015) stated that maldisposition of fetus is the commonest cause of fetal dystocia (18.03%) and uterine torsion (55.74%) in buffaloes.

Sharma *et al.* (2015) reported a case of dystocia due to fetal hydrocephalus in cows.

2.5 Hematology in dystotic cows

Pepper and Lindsay (1960) investigated the alterations in platelets, eosinophil and total leukocyte count during and following caesarean section. Leucocytosis which appeared on the day of surgery, returned to normal level on the fifth post-operative day. The platelet and eosinophil counts revealed a pronounced decline during and immediately following elective operations. After a brief period, these values returned to normal. They suggested that these changes were a consequence of varying reaction of hypophyseal adrenal axis to the imposed surgical stress.

Benysek and Kudlac (1971) made serial observations on hemato biochemical picture of cows during peripartum and reported that during parturition, the values of hemoglobin, packed cell volume and total plasma proteins increased. The changes in the red blood cell picture reached their maximum only after one to six hours following 30 parturition. On the second day post-parturition, packed cell volume and haemoglobin contents had also registered a pronounced drop.

Lee and Kehrli (1998) stated that parturition was followed by the increase of corticosteroids which induced neutrophilia by the increased departure of neutrophil from the bone marrow and demargination from the walls of blood vessels.

Klinkon and Zandnik (1999) observed that total leukocyte count was significantly higher at parturition than compared to before and after parturition levels.

Meglia *et al.* (2001) observed a higher leukocytes count on the day of parturition than before and after calving. They stated that this is result of significant increase in neutrophil and monocyte counts.

Manzoor *et al*. (2008) observed that all hematological indices associated with RBC differed significantly within the respective means except ESR and MCH where no specific trend was observed. Mean hemoglobin values were 09.21±0.30, 10.01±0.22 and 09.21±0.28 g/dL, respectively, in early and late stages differed significantly with mid stage of pregnancy. PCV values recorded were 29.04±0.89%, 31.82±1.06% and 28.50±1.06% for early, mid and late stages of pregnancy, respectively. The ESR values reported were 12.11±0.98mm/24h, 13.11±1.06mm/24h, and 12.46±0.95mm/24h, respectively during the three stages of the pregnancy. The MCV values clearly indicated that the means reported in mid pregnancy were significantly lower when compared with the values reported in early and late stage of pregnancy. The mean MCH values did not differ significantly and were much higher than the values reported in cattle. In contrast to MCH, the mean values of MCHC observed that the values in mid stage differed significantly with values observed in early and late stage of pregnancy. Higher MCV signifies the increase in the size of RBC in advanced pregnancy.

Ambica and Rao (2012) reported moderate decreased levels of Hb, TLC, lymphocytes, MCH and MCV in the post parturient cows suffering from subclinical hypocalcaemia.

2.6 Hematology in dystotic buffaloes

Rakesh Kumar *et al*. (2001) reported that a significant increase in the total erythrocyte count (TEC) and hemoglobin (Hb) concentration after calving. The packed cell volume (PCV) of the pregnant animals was lower and erythrocyte sedimentation rate (ESR) higher which recorded in cows and buffaloes after calving.

Ahmed *et al.* (2005) reported that there was also a significant decrease in PCV, Hb concentration, lymphocytes and monocyte, while an increase in ESR, WBC counts and neutrophil was observed in prolapsed animals as compared to controls. However, there was no difference in haematological and serum macro mineral contents between vaginal prolapsed and uterine prolapsed buffaloes. It was concluded that deficiency of calcium, phosphorus or magnesium might be possible causes of genital prolapse in these buffaloes.

Amer and Hashem (2008) found a significant decrease in red blood cell count, hemoglobin concentration and packed cell volume with no statistically significant changes in other blood indices, indicating normocytic normochromic anaemia, however total leukocyte count, neutrophil, neutrophil / lymphocyte ratio and monocyte showed significant increase whereas a significant decrease in eosinophil in association with insignificant change in lymphocytic count in buffaloes affected with uterine when compared with the normal buffaloes. The plasma proteins and albumin showed a

significant decrease in all the affected buffaloes with uterine torsion while globulins showed insignificant change when compared with the control except at 24 hours after birth, where they decreased significantly.

Pal and Bhatta (2013) showed that hematologic indices associated with RBC series i.e. Hb, PCV, Platelet counts, MCV, MCH, and MCHC were also found within normal limits. WBC counts along with differential counts were corresponded well within the reference range but, eosinophil and monocyte counts were significantly increased suggesting parasitic infestation.

2.7 Biochemical constituents in dystotic cows

Little (1974) stated that the decrease in total plasma protein levels in dystocia affected animals might be due to increased stress of dystocia leading to decreased liver functions.

Carson *et al.* (1978) conducted a study on a dairy herd because of an increased incidence of dystocia, retention of fetal membranes, and postpartum metritis. The cattle were found to have a mean serum calcium of 8.98 mg% and phosphorus of 8.25 mg%. Feed analysis revealed low Ca and P in the ration. The cattle were supplemented with steamed bone meal for 3 months. At the end of this supplementation, incidence of dystocia reduced from 75% to 10%; the incidence of retention of fetal membranes from 35% to 8%; and the incidence of postpartum metritis from 70% to 10%. The mean serum Ca increased to 10.26 mg% and the mean serum P was 6.72 mg%.

Richardson *et al*. (1981) conducted a study to find out the relationship between serum calcium and phosphorus concentrations and the occurrence of some of the reproductive disorders at the time of parturition in the cows. Samples were obtained from a total of 26 cows with uterine prolapse and 15 with minor dystocia (controls). The serum of animals with uterine prolapse had significantly low calcium concentration, high phosphorus concentration compared with the controls. Mild hypocalcemia (6.9 mg/dL-7.9 mg/dL) was present in 42.3% of the cows with prolapse as compared to only one 6.7% of the controls. Hypophosphatemia was present in 42.3% cows with uterine prolapse and in 66.7% of the controls. Of the uterine prolapse group, 53.8% were two years old, 23.1% were three years old, and 23.1% were four years of age or older. It was concluded that mild hypocalcemia and some degree of dystocia were associated with uterine prolapse.

Curtis *et al*. (1983) reported an association between the parturient Ca concentrations and metabolic disorders in Holstein dairy cows culled during March 1981 through February 1982. Association between low level of parturient calcium with dystocia, retention of fetal membranes, uterine prolapse, ketosis and mastitis was found to be significant.

Risco *et al.* (1984) collected blood samples from 53 dairy cows with uterine prolapse and from 53 cows with normal parturition of controls. Cows with uterine prolapse had significantly low serum calcium compared with controls. Mean serum calcium concentration (mg/dL) for affected cows and controls were 6.08 ± 0.25 and 6.96 ± 0.20, respectively. Hypocalcemia of a severe degree (less than 4 mg/dL) was found in

19% of the affected cows, compared with 1.8% of the controls, 17% of the affected cows and 25% of the controls had normal calcium concentration (greater than 8 mg/dL). The study concluded that hypocalcemia was associated with uterine prolapse in dairy cows.

Rajora and Pachauri (1994) conducted a study to compare the serum macro and micro minerals level in seven crossbred cows at one week pre-partum and one week postpartum at Livestock Research Centre, Pantnagar, India. The mean serum calcium level one week pre-partum was 7.39±0.22 mg/dL while phosphorus and magnesium levels were 4.7±0.12 mg/dL and 2.52±0.12 mg/dL, respectively. Among micro minerals, the mean serum copper level was 116.46±1.07 µg/dL while mean iron and zinc levels were178.60±15.36 µg/dL and 96.1±9.11 µg/dL, respectively.

Singh (1996) stated that total plasma proteins decreased significantly in dystocia affected and caesarean operated animal.

Horst *et al*. (1997) reported factors that predispose cows to milk fever and discussed dietary concepts important in the development of its prevention. Cows with milk fever were susceptible to problems such as dystocia, retention of fetal membranes, uterine prolapse etc. which increased production cost. Age of the cow was also discussed as predisposing factor, incidence of milk fever increased as dairy cows become older.

Kaneko *et al*. (1997) stated that the decrease in total plasma protein levels in dystocia affected animals might be due to inflammation causing increased movement of fluids and proteins into tissues.

Bigras-Poulin and Tremblay (1998) collected data from 1021 calvings of non paretic Holstein cows, in 14 Quebec dairy herds and described calcium metabolism after calving in healthy cows. Serum calcium and phosphorus values were low on the first day postpartum compared to a week later, whereas it was the opposite for magnesium and potassium. No significant difference was observed in albumin values. It was concluded that postpartum hypocalcemia was an event to be expected, especially for the older cow and biochemical profiles near, at and after calving could be used to better assess the cow's health.

Dhindsa *et al*. (2005) stated that injuries, edema and peritonitis in dystocia affected animals might also be a cause for reduction in total plasma proteins concentration.

Dhindsa *et al*. (2008) assessed the effect of delay in dystocia on biochemical alterations in the dam. Concentrations of blood urea nitrogen, plasma creatinine, serum ceruloplasmin and total peritoneal fluid proteins were significantly higher and total plasma proteins were significantly lower in cases where dystocia was for longer duration as compared to shorter duration«12h). Hence, concluded that delay in relieving cases of dystocia may cause detrimental biochemical alterations, which may not be favorable for survivability of the dam.

Turan *et al.* (2008) calving stress appears to affect several blood parameters including cortisol, cholesterol and vitamin A in all the groups and β-carotene and vitamin C in the calves. The analyses of these parameters can be practical to improve the health of dystocia-affected mothers and to increase survival of their newborns.

Pal and Bhatta (2013) showed that biochemical variables were determined using automated analyzers and routine laboratory techniques. Glucose, BUN, creatinine, AST, ALT, total serum protein and albumin level were found very close to standard reference values recorded in the HF cattle. However, mineral profiles particularly calcium (6.76±0.20 mg/dL) and phosphorus (3.03±0.18 mg/dL) levels were found significantly low. This suggests that cross HF cattle were at high risk to calcium metabolic disorders and corrective measures should be employed for better production.

Jeengari *et al.* (2015) showed that there were significant increase in SGOT, SGPT, bilirubin, serum creatinine and BUN in the affected buffaloes, possibly due to high uterine tissue damage. It may be inferred that torsion may lead to imbalance in biochemical profiles that affect the proper functioning of the uterine musculature.

2.8 Biochemical constituents in dystotic buffaloes

Hanif *et al.* (1984) conducted a study to determine the blood mineral profile of Ca, P, Cu, Zn in two hundred Nili-Ravi buffaloes which were divided into two groups (pregnant and non-pregnant). Among the sixteen dry pregnant buffaloes which were in their last trimester of pregnancy, the mean plasma calcium level was 9.85±0.63 mg/dL while mean phosphorus level was 4.33±0.55 mg/dL and the Ca/P ratio was 2.3:1. It was concluded that during the last trimester of pregnancy, the amount of nutrients should be increased in order to meet the extra requirement of fast growing fetus.

Kulkarni *et al*. (1984) reported that the mean serum calcium concentration was 10.18±0.46 mg/dL in dry and 9.98±0.19 mg/dL in lactating buffaloes while serum

inorganic phosphorus concentration was 5.41±0.53 mg/dL in dry and 4.97±0.15 mg/dL in lactating buffaloes.

Bugalia *et al.* (1996) concluded that the pre-caesarean and post-caesarean plasma total protein values in buffaloes affected with uterine torsion and monstrosities did not vary significantly from ante-partum and post-partum values in normal calving respectively and suggested that there was insignificant effect of stress and toxemia on protein metabolism.

Mandal *et al*. (1996) conducted a study to estimate the mineral (Ca, P, Mn, Cu, Fe, Zn) status in serum samples of buffaloes collected from different areas of Mohindergarh District, India. Mean serum calcium concentration ranged from 7.32 to 11.67 mg/dL while that of phosphorus concentration ranged from 2.43 to 9.35 mg/dL in pregnant buffaloes.

Salmanoglu and Salmanoglu (1998) conducted a study to find out relationship between clinical signs of parturient paresis, postpartum reproductive problems and blood calcium concentration. Occurrence of uterine prolapse and retention of fetal membranes was greater in strongly hypocalcemic group compared with slightly hypocalcemic group. No cases of uterine prolapse or retention of fetal membranes were recorded in the control group. The mean blood calcium concentration was significantly low in buffaloes showing strongly hypocalcemic signs compared to animals showing slightly hypocalcemic signs.

Mandali *et al*. (2002) collected blood samples from a total of 131 buffaloes affected with periparturient reproductive and metabolic disorders for the determination of

serum calcium, inorganic phosphorus, magnesium, blood glucose and total proteins. For each disorder 10 control samples were also collected. Serum calcium level was found to be low in cases of retention of fetal membranes, uterine prolapse and milk fever. Mean blood glucose level was significantly low in buffaloes with retention of fetal membranes. Magnesium and total protein concentrations were almost the same compared with controls in the cases of milk fever and retention of fetal membranes. It was concluded that the altered metabolic profiles could be the predisposing factor for most of the periparturient disorders in the buffaloes.

Ahmad *et al.* (2005) conducted a study to determine serum macro mineral concentrations of 30 buffaloes, twenty buffaloes were affected with genital prolapse and ten buffaloes as healthy control. Results revealed significantly low serum concentrations Ca (6.42±1.05 vs 10.96 ± 0.95 mg/dL), P (2.90±0.85 vs 5.50±1.61 mg/dL) and Mg (1.50±0.53 vs 2.40±0.53mg/dL) in prolapsed buffaloes compared with the controls. It was concluded that low levels of Ca, P and Mg might be associated with the incidence of genital prolapse in these buffaloes.

Pandey *et al*. (2007) collected blood samples from a total of 12 buffaloes for determination of serum calcium, inorganic phosphorus and magnesium. Six sampled buffaloes were affected with uterine prolapse and six with normal parturition as control. The mean serum calcium and phosphorus concentrations were significantly low in buffaloes on the day of prolapse. No significant difference was recorded in the mean magnesium concentrations compared with controls.

Ali *et al.* (2011) reported that there were significant increases in the MCHC, monocyte (MON), ALB, AST and CPK, and BUN, and decreases in globulin (GLOB) and P in buffaloes affected with uterine torsion.

2.9 Uterine microflora in dystotic cows

The *E. coli* organisms was needed to damage the endometrium enabling absorption of endotoxins, while other facultative anaerobic bacteria and strictly anaerobic bacteria, which lack the ability to invade intact epithelium, are usually considered facultative pathogens (Dohmen *et al.,* 1995 and Sheldon *et al*., 2004).

Huszenicza *et al*. (1999) reported that *E. coli* dominate the uterus of dairy cows within first few days after calving. This suggests that the bacterial contamination which is mainly *E. Coli* present in the uterus after parturition might favour the development of uterine infection by other highly pathogenic organisms.

Sheldon *et al.* (2006) stated that the development of uterine disease depends on the immune response of the cow, as well as the species and number (load and challenge) of bacteria.

2.10 Uterine microflora in dystotic buffaloes

The high prevalence of bacterial isolation from buffaloes after 6 h of calving in both PPC and NP groups revealed mainly *E. coli.* This suggests that the bacterial contamination which is mainly *E. coli* present in the uterus shortly after parturition in cows (Dohmen *et al.,* 2000; Sheldon *et al.,* 2006).

Lewis (1997) concluded that bacterial isolation from RP buffaloes in both NP and PPC groups after 48 of calving included mainly *A. pyogenes, E. coli, P. melaninogenicus, S. aureus, Pseudomonas aeruginosa, Bacillus licheniformis, haemolytic Streptococci and F. necrophorum.* Furthermore, the data obtained from bacteriological studies of RP buffaloes cases indicated that these bacterial isolates were in high density growth rate and might be concluded that these non-specific bacteria were the most pathogenic bacteria causing a severe uterine infection. They are called non-specific bacteria because the initial colonizing bacterium is not known and the specific bacteria causing the signs of infection are not known. Periparturent insults, including dystocia, uterine prolapse and retained fetal membranes diminish uterine ability to eliminate contaminated organisms. The exact causes of uterine infections during the postpartum period remain unknown.

Bondurant (1999) reported that presence of *Lactobacillus sp.* in the uterus indicated a healthy uterus. From bacteriological studies of buffalo cows included in this study, it can be suggested that uterine inflammation occurs as a result of postpartum ascending contamination by non-specific environmental organisms. Over 90% of uteri are contaminated in the first days postpartum.

Azawi (2006) stated that in PPC group, the most prevalent bacteria after 6 h of calving were *Escherichia coli, b- haemolytic Streptococci and Lactobacillus acidophilus.* Total bacterial isolates in the uterus of buffaloes with RP in PPC group after 24 and 48 h were 129 and 183 respectively. Among the isolates, *Archanobacterium pyogenes, Fusobacterium necrophorum, Prevotella melaninogenicus and Staphylococcus aureus* were the most prevalent isolates after 48 h of RP buffaloes in PPC group.

MATERIALS AND METHODS

III. MATERIALS AND METHODS

3.1 Experimental dystotic cows and buffaloes

The present research investigation entitled "Hemato-biochemical profile in dystotic cows and buffaloes" was carried out from August 2014 to June 2015. The study was carried out in 42 dystotic cows and buffaloes presented to various Veterinary Dispensaries of Bidar district, and VGO-OPD, Veterinary College, Bidar. The objectives of the research investigation were to study relationship between clinical signs and haematological and serum biochemical values in dystotic cows and buffaloes and also to study the uterine microflora in dystotic cows and buffaloes. The dystotic cows and buffaloes (42) were grouped as: Uterine torsion in buffalo (n=10), Prepartum cervical prolapse in buffalo (n=6), Anterior presentation of calf in buffalo (n=11), Anterior presentation of calf in cow (n=9) and Posterior presentation of calf in cow (n=6).

3.2.1 Haematological analysis

Two blood samples were collected aseptically from the Jugular vein in the sterile vial coated with EDTA as an anticoagulant for haematological studies, first blood sample before the obstetrical manipulation and second sample collection was done after 48 hours after relieving dystocia. Haematological parameters such as Total Erythrocyte Count (TEC), Hemoglobin (Hb), Packed Cell Volume (PCV), Total leukocytes count (TLC), Differential leukocytes count (DLC), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were estimated by methods described by Jain,1986 using automated haeme analyzer (SYSMEXKX-21; Sysmex® XS-Series (XS-1000)).

3.2.2 Blood biochemical analysis

Serum was separated from blood by centrifugation at 3000 rpm for 5 to 6 minutes and collected in sterile plastic screw cap vials. Aspartate aminotransferase (AST/SGOT) and Alanine aminotransferase (ALT/SGPT), were estimated immediately and remaining of the serum was stored at -20^{0}C until used for estimation of other serum biochemical parameters. The serum biochemical parameters. The serum samples were subjected for Glucose, Total protein, Albumin, Calcium, Phosphorus, Blood Urea Nitrogen, Creatinine and Cholesterol analysis as per the assay procedures mentioned in kits (Swemed Diagnostics, Bangalore) using Auto chemistry blood Analyzer (Artos Elita, Swemed Biomedicals, Pvt Ltd, Bangalore).

3.2.2.1 Estimation of serum glucose

Serum samples were analyzed using Glucose kit (M/s Swemed Diagnostics, Bangalore) by GOD/ POD method (Trinder, 1969) and the kit contained glucose reagent and glucose standard.

3.2.2.2. Estimation of serum total protein

Serum samples were analyzed using total protein kit (M/s Swemed Diagnostics, Bangalore) (Gornall, 1981) and the kit contained total protein reagent and total protein standard.

3.2.2.3 Estimation of serum albumin

Serum samples were analyzed using Albumin kit (M/s Swemed Diagnostics, Bangalore) by Bromo cresol green (BCG) dye method (Tietz *et al.*, 1976) and the kit contained albumin reagent and albumin standard.

3.2.2.4 Estimation of serum calcium

Serum samples were analyzed using Calcium kit (M/s Swemed Diagnostics, Bangalore) by Arsenazo III method (Faulker *et al.,* 1982) and the kit composed of Arsenazo III reagent and calcium standard.

3.2.2.5 Estimation of serum phosphorous

Serum samples were analyzed using phosphorous kit (M/s Swemed Diagnostics, Bangalore) by UV method (Tietz, 1976) and the kit contained reagent R1 and phosphorous standard.

3.2.2.6 Estimation of serum blood urea nitrogen

Serum samples were analyzed using Urea Berthelot kit (M/s Swemed Diagnostics, Bangalore) (Fawcett, 1960) and the kit contained reagent R1, reagent R2, reagent R3 and standard.

3.2.2.7 Estimation of serum creatinine

Serum samples were analyzed using creatinine kit-SR (M/s Swemed Diagnostics, Bangalore) by Jaffe's kinetic method (Tietz *et al.,* 1976) and the kit contained creatinine reagent and creatinine standard.

3.2.2.8 Estimation of serum cholesterol

Serum samples were analyzed using Cholesterol kit (M/s Swemed Diagnostics, Bangalore) by CHOD/ POD method (Natio, 1988) and the kit contained cholesterol reagent and cholesterol standard.

3.2.2.9 Estimation of SGPT

Serum samples were analyzed using SGPT kit (M/s Swemed Diagnostics, Bangalore) by modified IFCC method (Henley and Pollard, 1955) and the kit contained reagent R1 and reagent R2.

3.2.2.10 Estimation of SGOT

Serum samples were analyzed using SGOT kit (M/s Swemed Diagnostics, Bangalore) by modified IFCC method (Bergmeyer, 1986) and the kit contained reagent R1and reagent R2.

3.3.1 Nutrient broth preparation:

Nutrient broth powder (13.0 g) (Hi Media, Mumbai) was suspended in 1000 ml distilled water and heated to dissolve the medium completely and later sterilized by autoclave at 15 lbs pressure (121ºC) for 15 minutes.

3.3.2 Nutrient agar 1.5 % preparation:

Nutrient agar powder (31.0 g) (Hi Media, Mumbai) was suspended in 1000 ml distilled water and heated to boiling to dissolve the medium completely and later sterilized by autoclave at 15 lbs pressure (121ºC) for 15 minutes. After mixing, it was poured into sterile Petri plates.

3.3.3 Uterine microbial flora

Uterine fluid samples were collected immediately after relieving of dystocia in cows and buffaloes and were transferred to nutrient broth. These samples were further

cultured for isolation and identification of microbial flora as per the standard procedure. Identification of the bacteria was based on the characteristics of colony, Gram's staining pattern and morphology under a trinocular microscope.

3.3.4 Microbial culturing

The uterine samples were inoculated into 10 ml of nutrient broth tubes and were incubated at 37˚C for 18 hours. Following which the materials from the tubes were streaked on to the nutrient agar plates and again incubated for 18 hours at 37˚C. After incubation, the microbial colonies were picked up based on their characteristics and were grown further in the nutrient broth for pure culture of the isolated microbes. The broth cultures were subjected to Gram's staining so as to find out the type of organisms as per standard procedures. The isolates were further processed by streaking on to enriched media like nutrient agar. The streaked agar plates were incubated at 37˚C for 18 hours, then colonies from agar plate were smeared on glass with one drop of distilled water and then was heat fixed and subjected for Gram's staining for identification of organism as per standard procedure.

3.3.4 Statistical analysis

The data collected with regard to all the above parameters was analyzed using standard statistical procedure as per Snedecor and Cochran (1967).

Plate 1: Relieving of right sided uterine torsion in a buffalo

Plate 2: Prepartum cervical prolapse in a buffalo

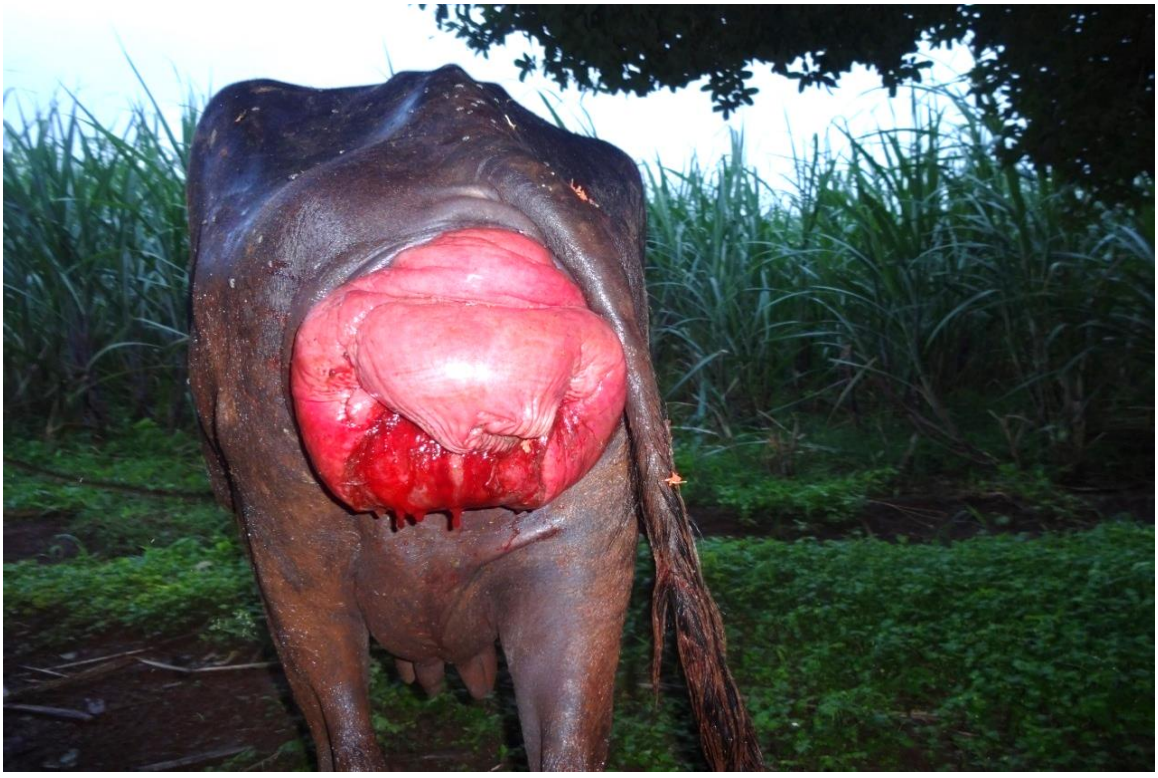

Plate 3: Removal of fetus in anterior presentation in a buffalo

Plate 4: Removal of fetus in anterior presentation in a cow

Plate 5: Removal of fetus in posterior presentation in a cow

RESULTS

IV. RESULTS

The present research work entitled ‘’Hemato-biochemical profile in dystotic cows and buffaloes’’ was carried out with the objectives to study relationship between clinical signs and haematological and serum biochemical values and to study the uterine micro flora in dystotic cows and buffaloes. The dystotic cows and buffaloes (42) were grouped as: Uterine torsion in buffalo (n=10), Prepartum cervical prolapse in buffalo (n=6), Anterior presentation of calf in buffalo (n=11), Anterior presentation of calf in cow (n=9) and Posterior presentation of calf in cow (n=6). The dystotic cows and buffaloes were relieved of dystocia as per standard therapeutic management procedure.

4.1 Hematological analysis in dystotic buffaloes

The buffaloes with uterine torsion and pre-partum cervical prolapse have shown variable mean Hb, TEC, PCV, MCH, MCHC, TLC, Neutrophil, Lymphocytes and Eosinophil before and after relieving dystocia and the difference was non-significant (Table 1 and 2).

The buffaloes suffering with dystocia in anterior presentation have shown variable mean Hb, TEC, PCV, MCH, MCHC, TLC, Neutrophil and Eosinophil with non-significant difference. However, the lymphocyte mean values were increased from 39.7±0.04% to 52.7±4.71% before and after relieving dystocia with significant difference (Table 3).

Table 1: Hematology in dystotic buffaloes with uterine torsion

Parameters	Before (Mean±SE)	After (Mean±SE)	Df	t-value	p-value	Remarks
Hb (g%)	10.7±0.87	9.83±0.79	9	1.03	0.326	NS
TEC (10^6/µL)	5.23±0.57	5.1±0.34	9	0.32	0.751	NS
PCV (%)	32.06±3.5	30.41±2.7	9	0.47	0.644	NS
MCH (pg)	21.5±1.28	19.5±0.77	9	1.57	0.149	NS
MCHC (%)	35.27±2.04	32.6±0.97	9	1.41	0.190	NS
TLC (1000/µL)	10.1±1.6	7.87±1.10	9	1.98	0.079	NS
Neutrophil (%)	54±3.33	52.6±4.9	9	0.20	0.839	NS
Lymphocyte (%)	45.3±3.3	41.5±6.9	9	0.43	0.671	NS
Eosinophil (%)	0.3±0.26	0.7±0.3	9	1.5	0.167	NS

NS= Non-significant, Before = Before relieving dystocia, After=After relieving dystocia

Table 2: Hematology in buffaloes with prepartum cervical prolapse

Parameters	Before (Mean±SE)	After (Mean±SE)	Df	t-value	p-value	Remarks
Hb (g%)	10.7±0.8	9.81±0.64	5	1.93	0.111	NS
TEC (10^6/µL)	4.5±0.5	4.8±0.42	5	1.14	0.305	NS
PCV (%)	29.8±2.92	29±1.83	5	0.29	0.776	NS
MCH (pg)	22±0.95	20.85±0.8	5	1.30	0.247	NS
MCHC (%)	37.41±2.9	33.8±0.30	5	0.94	0.387	NS
TLC (1000/µL)	8.8±0.73	8.75±1.0	5	0.02	0.981	NS
Neutrophil (%)	48.6±7.14	50.16±6.5	5	0.12	0.907	NS
Lymphocyte (%)	51.3±7.14	49.5±6.35	5	0.15	0.886	NS
Eosinophil (%)	0	0.33±0.25	5	1	0.363	NS

NS= Non-significant, Before = Before relieving dystocia, After=After relieving dystocia

Table 3: Hematology in dystotic buffaloes with anterior presentation of calf

Parameters	Before (Mean±SE)	After (Mean±SE)	Df	t-value	p-value	Remarks
Hb (g%)	12±0.42	11.1±0.65	10	1.66	0.128	NS
TEC (10^6/µL)	6.07±0.31	5.56±0.41	10	1.90	0.085	NS
PCV (%)	35.8±1.80	32.6±2.61	10	1.85	0.093	NS
MCH (pg)	20.01±0.6	20.3±0.68	10	1.01	0.336	NS
MCHC (%)	34±1.51	34.1±2.20	10	1.21	0.252	NS
TLC (1000/µL)	11.39±0.8	9.54±0.66	10	1.92	0.083	NS
Neutrophil (%)	59.7±3.83	46.9±4.68	10	2.21	0.051	NS
Lymphocyte (%)	39.7±3.90	52.7±4.71	10	2.25	0.047	S
Eosinophil (%)	0.36±0.04	0.36±0.15	10	0	1	NS

NS= Non-significant, S= Significant, Before = Before relieving dystocia, After=After relieving dystocia

4.2 Hematological analysis in dystotic cows

The mean Hb, TEC, PCV, MCH, MCHC, TLC, Neutrophils Lymphocytes and Eosinophils values varied in dystotic cows suffering with anterior and posterior presentation of calf without any significant difference (Table 4 and 5).

4.3 Blood biochemical analysis in dystotic buffaloes

The mean serum SGOT (84±11.6 vs 39±7.04 Units/L), Total proteins (11.3±0.6 vs 9.1±0.7 g/dL), Albumin (4.2±0.17 vs 3.6±0.17 g/dL) values reduced in dystotic buffaloes significantly with uterine torsion however values of SGPT, Glucose, Calcium, Phosphorus, BUN, Creatinine, Cholesterol values varied without any significant difference before and after relieving dystocia (Table 6).

The mean serum SGPT (14.5±15.2 to 9.46±1.32 Units/L), Total proteins (11±0.5 to 8.93±0.31 g/dL), Albumin (4.86±0.5 to 3.59±0.15 g/dL) Calcium (5.04±0.84 to 2.41±0.8 mg/dL) and BUN (65.8±0.95 to 198±16.1 mg/dL) values varied with significant difference and SGOT, Glucose, Phosphorus, Creatinine and Cholesterol values also varied before and after correction in buffaloes with pre-partum cervical prolapse (Table 7).

The mean serum SGOT (29.6±3.10 vs 15.5±4.49 Units/L), Glucose (60.4±7.60 vs 25.31±8.2 mg/dL), Total proteins (10.81±0.4 vs 9.45± 0.30 g/dL), Albumin (3.76±0.22 vs 3.26±0.19 g/dL), Calcium (5.3±0.94 vs 3.45±0.67 mg/dL), BUN (69.9±2.22 vs 150±17.7 mg/dL) varied in dystotic buffaloes with anterior presentation of fetus before vs after relieving dystocia with significant difference whereas the mean values of SGPT, Phosphorus, Creatinine and Cholesterol also varied without any significance (Table 8).

Table 4: Hematology in dystotic cows with anterior presentation of calf

Parameters	Before (Mean±SE)	After (Mean±SE)	Df	t-value	p-value	Remarks
Hb (g%)	11.6±0.76	10.4±0.5	8	2.26	0.053	NS
TEC (10^6/µL)	6.81±0.52	6.31±0.5	8	0.93	0.376	NS
PCV (%)	39.5±2.97	32.4±4.10	8	1.60	0.147	NS
MCH (pg)	17.2±0.3	17.5±0.30	8	1.59	0.150	NS
MCHC (%)	29.7±0.6	31±0.74	8	1.80	0.109	NS
TLC (1000/µL)	14.7±2.95	10.96±1.2	8	1.15	0.283	NS
Neutrophil (%)	41.7±3.70	43.33±6.3	8	0.25	0.806	NS
Lymphocyte (%)	58±3.5	56.55±6.4	8	0.23	0.818	NS
Eosinophil (%)	0.22±0.21	0	8	1	0.346	NS

NS= Non-significant, Before = Before relieving dystocia, After=After relieving dystocia

Table 5: Hematology in dystotic cows with posterior presentation of calf

Parameters	Before (Mean±SE)	After (Mean±SE)	Df	t-value	p-value	Remarks
Hb (g%)	11.9±0.48	10.9±0.85	5	0.97	0.375	NS
TEC (10^6/µL)	6.3±0.6	6.06±0.41	5	0.57	0.588	NS
PCV (%)	37.3±1.63	33.45±2.5	5	1.65	0.159	NS
MCH (pg)	20.2±2.23	17.9±0.42	5	0.76	0.476	NS
MCHC (%)	31.6±0.25	32.7±0.82	5	1.10	0.320	NS
TLC (1000/µL)	11.5±1.53	8.16±0.94	5	1.81	0.129	NS
Neutrophil (%)	53±5.08	44.8±5.58	5	1.07	0.332	NS
Lymphocyte (%)	46.8±5.13	55.16±5.5	5	1.10	0.321	NS
Eosinophil (%)	0.16±0.12	0	5	1	0.363	NS

NS= Non-significant, Before = Before relieving dystocia, After=After relieving dystocia

Table 6: Serum biochemical constituents in dystotic buffaloes with uterine torsion

Parameters	Before (Mean±SE)	After (Mean±SE)	Df	t-value	p-value	Remarks
SGOT (units/L)	84±11.6	39±7.04	9	3.27	0.009	S
SGPT (units/L)	43±3.28	43±7.30	9	0.06	0.952	NS
Glucose (mg/dL)	73±21.8	28±8.5	9	1.76	0.112	NS
TP (g/dL)	11.3±0.6	9.1±0.7	9	2.91	0.017	S
Albumin (g/dL)	4.2±0.17	3.6±0.17	9	3.72	0.004	S
Calcium (mg/dL)	2.9±0.7	2.3±0.53	9	0.88	0.399	NS
Phosphorous (mg/dL)	5.26±0.7	8.25±1.3	9	2.04	0.071	NS
BUN (mg/dL)	91±15.6	80.7±12	9	0.51	0.621	NS
Creatinine (mg/dL)	2.30±0.4	2.85±0.5	9	1.23	0.248	NS
Cholesterol (mg/dL)	87.4±11	64±4.40	9	1.79	0.106	NS

Note: S= Significant, NS= Non-significant, Before = Before relieving dystocia, After = After relieving dystocia

Table 7: Serum biochemical constituents in buffaloes with prepartum cervical prolapse

Parameters	Before (Mean±SE)	After (Mean±SE)	Df	t-value	p-value	Remarks
SGOT (units/L)	63±15.2	41.8±14.1	5	2.51	0.053	NS
SGPT (units/L)	14.5±1.52	9.46±1.32	5	2.75	0.040	S
Glucose (mg/dL)	81.7±12.6	54.8±16.3	5	1.65	0.157	NS
TP (g/dL)	11±0.5	8.93±0.31	5	5.25	0.003	S
Albumin (g/dL)	4.86±0.5	3.59±0.15	5	2.68	0.043	S
Calcium (mg/dL)	5.04±0.84	2.41±0.8	5	5.69	0.002	S
Phosphorous (mg/dL)	5.9±1.02	4.5±0.54	5	1.13	0.308	NS
BUN (mg/dL)	65.8±0.95	198±16.1	5	6.23	0.001	S
Creatinine (mg/dL)	2.14±0.25	2.27±0.40	5	0.35	0.735	NS
Cholesterol (mg/dL)	58.54±5.3	105±16.1	5	2.20	0.078	NS

Note: NS= Non-significant, S= Significant, Before = Before relieving dystocia, After = After relieving dystocia

Table 8: Serum biochemical constituents in dystotic buffaloes with anterior presentation of calf

Parameters	Before (Mean±SE)	After (Mean±SE)	Df	t-value	p-value	Remarks
SGOT (units/L)	29.6±3.10	15.5±4.49	10	2.33	0.041	S
SGPT (units/L)	26.6±3.26	19.8±2.43	10	1.40	0.190	NS
Glucose (mg/dL)	60.4±7.60	25.31±8.2	10	3.17	0.009	S
TP (g/dL)	10.81±0.4	9.45±0.30	10	2.48	0.032	S
Albumin (g/dL)	3.76±0.22	3.26±0.19	10	4.46	0.001	S
Calcium (mg/dL)	5.3±0.94	3.45±0.67	10	2.62	0.025	S
Phosphorous (mg/dL)	7.06±1.32	5.30±0.8	10	1.48	0.167	NS
BUN (mg/dL)	69.9±2.22	150±17.7	10	4.67	0.0008	S
Creatinine (mg/dL)	2.30±0.34	1.61±0.24	10	1.72	0.114	NS
Cholesterol (mg/dL)	71.6±3.66	81.03±8.3	10	1.13	0.283	NS

Note: S = Significant, NS = Non-significant, Before = Before relieving dystocia, After = After relieving dystocia

4.4 Blood biochemical analysis in dystotic cows

The mean serum SGPT (36.72±1.5 vs 61.8±1.78 Units/L), Albumin (3.53±0.14 vs 3.15±0.22 g/dl) and Phosphorus (6.85±0.80 vs 5.53±0.48 mg/dL) varied before and after relieving dystocia in cows with anterior presentation of calf however, the SGOT, Glucose, Total proteins, Calcium, BUN, Creatinine, Cholesterol values were also differed without any significance (Table 9).

The mean serum SGPT (27.4±2.5 vs 19.1±2.1 Units/L), Phosphorus (7.02±0.3 vs 5.4±0.09 mg/dL) and Cholesterol (89.8±3.7 vs 138±11.8 mg/dL) varied significantly in dystotic cows with posterior presentation of calf whereas, the values of SGOT, Glucose, Total proteins, Albumin, Calcium, BUN and Creatinine also varied without any significant difference (Table 10).

4.5 Uterine micro flora in buffaloes

The various bacteria isolated from the uterine fluid collected from 27 buffaloes after relieving of dystocia were *Bacillus* (51.85 %), *Streptococcus* (18.51 %), *Escherichia coli* (14.83 %), *Staphylococcus* (11.11 %) and *Clostridium species* (3.70 %) respectively.

4.6 Uterine micro flora in cows

The various bacteria isolated from the uterine fluid collected from 15 cows after relieving of dystocia were *Bacillus* (40.00 %), S*treptococcus* (20.00 %), *Escherichia coli* (20.00 %), *Staphylococcus* (13.34 %), and *Clostridium species* (6.66 %) respectively.

Table 9: Serum biochemical constituents in dystotic cows with anterior presentation of calf

Parameters	Before (Mean±SE)	After (Mean±SE)	Df	t-value	p-value	Remarks
SGOT (units/L)	38.78±8.3	38.2±21.3	8	0.03	0.974	NS
SGPT (units/L)	36.72±1.5	61.8±1.78	8	16.7	0.000	S
Glucose (mg/dL)	70.3±27.2	32.8±9.63	8	1.60	0.147	NS
TP (g/dL)	10.5±0.60	9.13±0.59	8	1.95	0.086	NS
Albumin (g/dL)	3.53±0.14	3.15±0.22	8	3.15	0.013	S
Calcium (mg/dL)	6.41±0.46	6.32±0.63	8	0.27	0.792	NS
Phosphorous (mg/dL)	6.85±0.80	5.53±0.48	8	2.51	0.036	S
BUN (mg/dL)	57.9±3.59	92.3±15.3	8	2.25	0.053	NS
Creatinine (mg/dL)	2.36±0.44	2.29±0.32	8	0.09	0.930	NS
Cholesterol (mg/dL)	89.9±6.94	108±12.27	8	1.29	0.232	NS

Note: NS = Non-significant, S = Significant, Before = Before relieving dystocia, After = After relieving dystocia

Table 10: Serum biochemical constituents in dystotic cows with posterior presentation of calf

Parameters	Before (Mean±SE)	After (Mean±SE)	Df	t-value	p-value	Remarks
SGOT (units/L)	21.5±2.6	15.6±1.2	5	2.56	0.050	NS
SGPT (units/L)	27.4±2.5	19.1±2.1	5	2.66	0.044	S
Glucose (mg/dL)	84.2±17	58.7±7.8	5	1.74	0.140	NS
TP (g/dL)	11±0.65	8.43±0.4	5	2.24	0.075	NS
Albumin (g/dL)	3.65±0.2	3.2±0.21	5	2	0.100	NS
Calcium (mg/dL)	6.6±1.01	8.22±1.0	5	2.16	0.082	NS
Phosphorous (mg/dL)	7.02±0.3	5.4±0.09	5	5.18	0.003	S
BUN (mg/dL)	87.4±15	111±20	5	1.25	0.263	NS
Creatinine (mg/dL)	2.7±0.69	1.29±0.3	5	1.34	0.235	NS
Cholesterol (mg/dL)	89.8±3.7	138±11.8	5	3.07	0.027	S

Note: NS = Non-significant, S = Significant, Before = Before relieving dystocia, After = After relieving dystocia

DISCUSSION

V. DISCUSSION

The present research work entitled ‘’Hemato-biochemical profile in dystotic cows and buffaloes’’ was carried out with the objectives to study relationship between clinical signs and haematological and serum biochemical values in dystotic cows and buffaloes and to study the uterine micro flora in dystotic cows and buffaloes. The dystotic cows and buffaloes (42) were grouped as: Uterine torsion in buffalo (n=10), Prepartum cervical prolapse in buffalo (n=6), Anterior presentation of calf in buffalo (n=11), Anterior presentation of calf in cow (n=9) and Posterior presentation of calf in cow (n=6).The dystotic cows and buffaloes were relieved of dystocia as per standard therapeutic management procedure.

The discussion pertaining to the results is mentioned below.

5.1 Hematological analysis in dystotic buffaloes

The buffaloes with uterine torsion and pre-partum cervical prolapse have shown variable mean Hb, TEC, PCV, MCH, MCHC, TLC, Neutrophil, Lymphocytes and Eosinophil before and after relieving dystocia and the difference were non-significant.

The buffaloes suffering with dystocia in anterior presentation have shown variable mean Hb, TEC, PCV, MCH, MCHC, TLC, Neutrophil and Eosinophil with non-significant difference. However, the lymphocyte mean values were increased from 39.7±0.04% to 52.7±4.71% before and after relieving dystocia with significant difference.

According to Rakesh Kumar *et al*. (2001) reported a significant increase in the total erythrocyte count (TEC) and hemoglobin (Hb) concentration after calving. The packed cell volume (PCV) of the pregnant animals was lower and erythrocyte sedimentation rate (ESR) higher which recorded in cows and buffaloes after calving.

On contrary, Ahmed *et al.* (2005) reported that there was also a significant decrease in PCV, Hb concentration, lymphocytes and monocyte, while an increase in ESR, WBC counts and neutrophil was observed in prolapsed animals as compared to controls. However, there was no difference in haematological and serum macro mineral contents between vaginal prolapsed and uterine prolapsed buffaloes. It was concluded that deficiency of calcium, phosphorus or magnesium might be possible causes of genital prolapse in these buffaloes.

Similarly, Amer and Hashem (2008) found a significant decrease in red blood cell count, hemoglobin concentration and packed cell volume with no statistically significant changes in other blood indices, indicating normocytic normochromic anaemia, however total leukocyte count, neutrophil, neutrophil / lymphocyte ratio and monocyte showed significant increase whereas a significant decrease in eosinophil in association with insignificant change in lymphocytic count in buffaloes affected with uterine torsion when compared with the normal buffaloes. The plasma proteins and albumin showed a significant decrease in all the affected buffaloes with uterine torsion while globulins showed insignificant change when compared with the control except at 24 hours after birth, where they decreased significantly.

Pal and Bhatta (2013) showed that hematologic indices associated with RBC series i.e. Hb, PCV, Platelet counts, MCV, MCH, and MCHC were also found within normal limits. WBC counts along with differential counts were corresponded well within the reference range but, eosinophil and monocyte counts were significantly increased suggesting parasitic infestation.

5.2 Hematological analysis in dystotic cows

The mean Hb, TEC, PCV, MCH, MCHC, TLC, Neutrophil and Eosinophil values varied in dystotic cows suffering with anterior and posterior presentation without any significant difference.

Similarly, Benysek and Kudlac (1971) made serial observations on hemato-biochemical picture of cows during peri-partum and reported that during parturition, the values of hemoglobin, packed cell volume and total plasma proteins increased. The changes in the red blood cell picture reached their maximum only after one to six hours following parturition. On the second day post-parturition, packed cell volume and hemoglobin contents had also registered a pronounced drop.

Klinkon and Zandnik (1999) observed that total leukocyte count was significantly higher at parturition than compared to before and after parturition levels.

Meglia *et al.* (2001) observed a higher leukocytes count on the day of parturition than before and after calving. They stated that this is result of significant increase in neutrophil and monocyte counts.

Ambica and Rao (2012) reported moderate decreased levels of Hb, TLC, lymphocytes, MCH and MCV in the post parturient cows suffering from subclinical hypocalcaemia.

5.3 Blood biochemical analysis in dystotic buffaloes

The mean serum SGOT (84±11.6 vs 39±7.04 Units/L), Total proteins (11.3±0.6 vs 9.1±0.7 g/dL), Albumin (4.2±0.17 vs 3.6± 0.17 g/dL) values reduced in dystotic buffaloes significantly with uterine torsion however values of SGPT, Glucose, Calcium, Phosphorus, BUN, Creatinine, Cholesterol values varied without any significant difference before and after relieving dystocia.

The mean serum SGPT (14.5±15.2 to 9.46±1.32 Units/L) Total proteins (11±0.5 to 8.93±0.31 g/dL), Albumin (4.86±0.5 to 3.59±0.15 g/dL) Calcium (5.04±0.84 to 2.41±0.8 mg/dL) and BUN (65.8±0.95 to 198±16.1 mg/dL) values varied with significant difference and SGOT, Glucose, Phosphorus, Creatinine and Cholesterol values also varied before and after relieving dystocia in buffaloes with pre-partum cervical prolapse.

The mean serum SGOT (29.6±3.10 vs 15.5±4.49 Units/L), Glucose (60.4±7.60 vs 25.31±8.2 mg/dL), Total proteins (10.81±0.4 vs 9.45± 0.30 g/dL), Albumin (3.76±0.22 vs 3.26±0.19 g/dL), Calcium (5.3±0.94 vs 3.45±0.67 mg/dL), BUN (69.9±2.22 vs 150±17.7 mg/dL) varied in dystotic buffaloes with anterior presentation of fetus before vs after relieving dystocia with significant difference whereas the values of SGPT, Phosphorus, Creatinine and Cholesterol also varied without any significance.

Similarly, Jeengari *et al.* (2015) showed that there were significant increase in SGOT, SGPT, bilirubin, serum Creatinine and BUN in the affected buffaloes, possibly due to high uterine tissue damage. It may be inferred that torsion may lead to imbalance in biochemical profiles that affect the proper functioning of the uterine musculature.

Hanif *et al.* (1984) reported the mean plasma calcium level as 9.85±0.63 mg/dl while mean phosphorus level 4.33±0.55 mg/dL in pregnant buffaloes and the Ca/P ratio 2.3:1. It was concluded that during the last trimester of pregnancy, the amount of nutrients should be increased in order to meet the extra requirement of fast growing fetus.

Kulkarni *et al*. (1984) reported that the mean serum calcium concentration 10.18±0.46 mg/dL in dry and 9.98±0.19 mg/dL in lactating buffaloes while serum inorganic phosphorus concentration was 5.41±0.53 mg/dL in dry and 4.97±0.15 mg/dL in lactating buffaloes.

Bugalia *et al.* (1996) concluded that the pre-caesarean and post-caesarean plasma total protein values in buffaloes affected with uterine torsion and monstrosities did not vary significantly from ante-partum and post-partum values in normal calving respectively and suggested that there was insignificant effect of stress and toxemia on protein metabolism.

Mandal *et al*. (1996) concluded that the mean serum calcium concentration range from 7.32 to 11.67 mg/dl while that of phosphorus concentration range from 2.43 to 9.35 mg/dL in pregnant buffaloes.

Salmanoglu and Salmanoglu (1998) found the occurrence of uterine prolapse and retention of fetal membranes greater in strongly hypocalcaemia group compared with slightly hypocalcaemia group. No cases of uterine prolapse or retention of fetal membranes were recorded in the control group. The mean blood calcium concentration was significantly low in buffaloes showing strongly hypocalcaemia signs compared to animals showing slightly hypocalcaemia signs.

Mandali *et al*. (2002) found low level serum calcium in cases of retention of fetal membranes, uterine prolapse and milk fever. Mean blood glucose level was significantly low in buffaloes with retention of fetal membranes. Magnesium and total protein concentrations were almost the same compared with controls in the cases of milk fever and retention of fetal membranes. It was concluded that the altered metabolic profiles could be the predisposing factor for most of the peri-parturient disorders in the buffaloes.

Ahmad *et al.* (2005) revealed significantly low serum concentrations Ca (6.42±1.05 vs 10.96 ± 0.95 mg/dL), P (2.90±0.85 vs 5.50±1.61 mg/dL) and Mg (1.50±0.53 vs 2.40±0.53 mg/dL) in prolapsed buffaloes compared with the controls. It was concluded that low levels of Ca, P and Mg might be associated with the incidence of genital prolapse in these buffaloes.

Pandey *et al*. (2007) reported the mean serum calcium and phosphorus concentrations significantly low in buffaloes on the day of prolapse. No significant difference was recorded in the mean magnesium concentrations compared with controls.

Ali *et al.* (2011) reported that there were significant increases in the MCHC, monocyte (MON), ALB, AST and CPK and BUN, and decreases in globulin (GLOB) and P in buffaloes affected with uterine torsion.

Jeengari *et al.* (2015) showed that there were significant increase in SGOT, SGPT, bilirubin, serum Creatinine and BUN in the affected buffaloes, possibly due to high uterine tissue damage. It may be inferred that torsion may lead to imbalance in biochemical profiles that affect the proper functioning of the uterine musculature.

5.4 Blood biochemical analysis in dystotic cows

The mean serum SGPT (36.72±1.5 vs 61.8±1.78 Units/L), Albumin (3.53±0.14 vs 3.15±0.22 g/dL) and Phosphorus (6.85±0.80 vs 5.53±0.48 mg/dL) varied before and after relieving dystocia in cows with anterior presentation of fetus however, the SGOT, Glucose, Total proteins, Calcium, BUN, Creatinine, Cholesterol values were also differed without any significance (Table 9).

The mean serum SGPT (27.4±2.5 vs 19.1±2.1 Units/L), Phosphorus (7.02±0.3 vs 5.4±0.09 mg/dL) and Cholesterol (89.8±3.7 vs 138±11.8 mg/dL) varied significantly in dystotic cows with posterior presentation of calf whereas, the values of SGOT, Glucose, Total proteins, Albumin, Calcium, BUN and Creatinine also varied without any significant difference.

Little (1974) stated that the decrease in total plasma protein levels in dystocia affected animals might be due to increased stress of dystocia leading to decreased liver functions.

Carson *et al.* (1978) found mean serum calcium of 8.98 mg/dL and phosphorus of 8.25 mg/dL in cattle. Feed analysis revealed low Ca and P in the ration. The cattle were supplemented with steamed bone meal for 3 months. At the end of this supplementation, incidence of dystocia reduced from 75% to 10%; the incidence of retention of fetal membranes from 35% to 8%; and the incidence of postpartum metritis from 70% to 10%. The mean serum Ca increased to 10.26 mg/dL and the mean serum P was 6.72 mg/dL

Richardson *et al*. (1981) found that the serum of animals with uterine prolapse had significantly low calcium concentration, high phosphorus concentration compared with the controls. Mild hypocalcaemia (6.9 mg/dL-7.9 mg/dL) was present in 42.3% of the cows with prolapse as compared to only one 6.7% of the controls. Hypophosphatemia was present in 42.3% cows with uterine prolapse and in 66.7% of the controls. Of the uterine prolapse group, 53.8% were two years old, 23.1% were three years old, and 23.1% were four years of age or older. It was concluded that mild hypocalcaemia and some degree of dystocia were associated with uterine prolapse.

Curtis *et al*. (1983) reported an association between the parturient Ca concentrations and metabolic disorders in Holstein dairy cows. Association between low level of parturient calcium with dystocia, retention of fetal membranes, uterine prolapse, ketosis and mastitis was found to be significant.

Risco *et al.* (1984) reported that cows with uterine prolapse had significantly low serum calcium compared with controls. Mean serum calcium concentration (mg/dL) for affected cows and controls were 6.08 ± 0.25 and 6.96 ± 0.20, respectively. Hypocalcaemia of a severe degree (less than 4 mg/dL) was found in 19% of the affected

cows, compared with 1.8% of the controls, 17% of the affected cows and 25% of the controls had normal calcium concentration (greater than 8 mg/dL). The study concluded that hypocalcaemia was associated with uterine prolapse in dairy cows.

Rajora and Pachauri (1994) stated that the mean serum calcium level one week pre-partum was 7.39±0.22 mg/dL while phosphorus and magnesium levels were 4.7±0.12 mg/dL and 2.52±0.12 mg/dL, respectively. Among micro minerals, the mean serum copper level was 116.46±1.07 µg/dL while mean iron and zinc levels were 178.60±15.36 µg/dL and 96.1±9.11 µg/dL, respectively.

Singh (1996) stated that total plasma proteins decreased significantly in dystocia affected and caesarean operated animal.

Horst *et al*. (1997) reported factors that predispose cows to milk fever and discussed dietary concepts important in the development of its prevention. Cows with milk fever were susceptible to problems such as dystocia, retention of fetal membranes, uterine prolapse etc. which increased production cost. Age of the cow was also discussed as predisposing factor, incidence of milk fever increased as dairy cows become older.

Kaneko *et al*. (1997) stated that the decrease in total plasma protein levels in dystocia affected animals might be due to inflammation causing increased movement of fluids and proteins into tissues.

Bigras-Poulin and Tremblay (1998) found that the serum calcium and phosphorus values were low on the first day postpartum compared to a week later, whereas it was the opposite for magnesium and potassium. No significant difference was observed in

albumin values. It was concluded that postpartum hypocalcaemia was an event to be expected, especially for the older cow and biochemical profiles near, at and after calving could be used to better assess the cow's health.

Dhindsa *et al*. (2005) stated that injuries, edema and peritonitis in dystocia affected animals might also be a cause for reduction in total plasma proteins concentration.

Dhindsa *et al*. (2008) reported concentrations of blood urea nitrogen, plasma Creatinine, serum ceruloplasmin and total peritoneal fluid proteins were significantly higher and total plasma proteins were significantly lower in cases where dystocia was for longer duration as compared to shorter duration «12 h). Hence, concluded that delay in relieving cases of dystocia may cause detrimental biochemical alterations, which may not be favorable for survivability of the dam.

Turan *et al.* (2008) calving stress appears to affect several blood parameters including cortisol, cholesterol and vitamin A in all the groups and β-carotene and vitamin C in the calves. The analyses of these parameters can be practical to improve the health of dystocia-affected mothers and to increase survival of their newborns.

Pal and Bhatta (2013) showed that biochemical variables were determined using automated analyzers and routine laboratory techniques. Glucose, BUN, Creatinine, AST, ALT, total serum protein and albumin level were found very close to standard reference values recorded in the HF cattle. However, mineral profiles particularly calcium (6.76±0.20 mg/dL) and phosphorus (3.03±0.18 mg/dL) levels were found significantly

low. This suggests that cross HF cattle were at high risk to calcium metabolic disorders and corrective measures should be employed for better production.

5.5 Uterine micro flora in buffaloes

The various bacteria isolated from the uterine fluid collected from 27 buffaloes after relieving of dystocia were B*acillus* (51.85 %), S*treptococcus* (18.51 %), *Escherichia coli* (14.83%), *Staphylococcus* (11.11 %) and *Clostridium species* (3.70 %) respectively.

Similarly, the high prevalence of bacterial isolation from buffaloes after 6 h of calving in both PPC and NP groups revealed mainly *E. coli.* This suggests that the bacterial contamination which is mainly *E. coli* present in the uterus shortly after parturition in cows (Dohmen *et al.,* 2000; Sheldon *et al.,* 2006).

Lewis (1997) concluded that bacterial isolation from RP buffaloes in both NP and PPC groups after 48 of calving included mainly *A. pyogenes, E. coli, P. melaninogenicus, S. aureus, Pseudomonas aeruginosa, Bacillus licheniformis, haemolytic Streptococci and F. necrophorum.* Furthermore, the data obtained from bacteriological studies of RP buffalo cases indicated that these bacterial isolates were in high density growth rate and might be concluded that these non-specific bacteria were the most pathogenic bacteria causing a severe uterine infection. They are called non-specific bacteria because the initial colonizing bacterium is not known and the specific bacteria causing the signs of infection are not known. Peri-parturient insults, including dystocia, uterine prolapse and retained fetal membranes diminish uterine ability to eliminate contaminated organisms. The exact causes of uterine infections during the postpartum period remain unknown.

Bondurant (1999) reported that presence of *Lactobacillus sp.* in the uterus indicated a healthy uterus. From bacteriological studies of buffalo cows included in this study, it can be suggested that uterine inflammation occurs as a result of postpartum ascending contamination by non-specific environmental organisms. Over 90% of uteri are contaminated in the first days postpartum.

Azawi (2006) stated that in PPC group, the most prevalent bacteria after 6 h of calving were *Escherichia coli, B. haemolytic Streptococci and Lactobacillus acidophilus.* Among the isolates, *Archanobacterium pyogenes, Fusobacterium necrophorum, Prevotella melaninogenicus and Staphylococcus aureus* were the most prevalent isolates after 48 h of RP buffaloes in PPC group.

5.6 Uterine micro flora in cows

The various bacteria isolated from the uterine fluid collected from 15 cows after relieving of dystocia were *Bacillus* (40.00 %), S*treptococcus* (20.00 %), *Escherichia coli* (20.00 %), *Staphylococcus* (13.34 %) and *Clostridium species* (6.66 %) respectively.

Similarly, *E. coli* organisms was needed to damage the endometrium enabling absorption of endotoxins, while other facultative anaerobic bacteria and strictly anaerobic bacteria, which lack the ability to invade intact epithelium, are usually considered facultative pathogens (Dohmen *et al.*, 1995; Sheldon *et al*., 2004).

Huszenicza *et al*. (1999) reported that *E. coli* dominate the uterus of dairy cows within first few days after calving. This suggests that the bacterial contamination which is

mainly *E. Coli* present in the uterus after parturition might favour the development of uterine infection by other highly pathogenic organisms.

Sheldon *et al.* (2006) stated that the development of uterine disease depends on the immune response of the cow as well as the species and number (load and challenge) of bacteria.

SUMMARY

VI. SUMMARY

The present research work was carried out with the objectives to study relationship between clinical signs and haematological and serum biochemical values in dystotic cows and buffaloes and to study the uterine micro flora in dystotic cows and buffaloes. The dystotic cows and buffaloes (42) were grouped as: Uterine torsion in buffalo (n=10), Prepartum cervical prolapse in buffalo (n=6), Anterior presentation of calf in buffalo (n=11), Anterior presentation of calf in cow (n=9) and Posterior presentation of calf in cow (n=6). The dystotic cows and buffaloes were relieved of dystocia as per standard therapeutic management procedure. The dystotic cows and buffaloes were relieved of dystocia as per standard therapeutic management procedure.

Two blood samples from each dystotic cow and buffalo were collected aseptically from the Jugular vein in the sterile vial coated with EDTA as an anticoagulant for haematological studies; first blood sample before the obstetrical manipulation and second sample collection was done after 48 hours after relieving dystocia. Haematological parameters such as Total Erythrocyte Count (TEC), Hemoglobin (Hb), Packed Cell Volume (PCV), Total leukocytes count (TLC), Differential leukocytes count (DLC), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were estimated by methods described by Jain, (1986) using automated haeme analyzer (SYSMEXKX-21; Sysmex® XS-Series (XS-1000)).

The serum was separated from blood by centrifugation at 3000 rpm for 5 to 6 minutes and collected in sterile plastic screw cap vials. Aspartate aminotransferase (AST/SGOT) and Alanine aminotransferase (ALT/SGPT), were estimated immediately

and remaining of the serum was stored at -20^{0}C until used for estimation of other serum biochemical parameters. The serum samples were subjected for Glucose, Total protein, Albumin, Calcium, Phosphorus, Blood Urea Nitrogen, Creatinine and Cholesterol analysis as per the assay procedures mentioned in kits (Swemed Diagnostics, Bangalore) using Auto chemistry blood Analyzer (Artos Elita, Swemed Biomedicals, Pvt Ltd, Bangalore).

Serum samples were analyzed using Glucose kit (M/s Swemed Diagnostics, Bangalore) by GOD/ POD method (Trinder, 1969) and the kit contained glucose reagent and glucose standard.

Serum samples were analyzed using total protein kit (M/s Swemed Diagnostics, Bangalore) (Gornall, 1981) and the kit contained total protein reagent and total protein standard.

Serum samples were analyzed using Albumin kit (M/s Swemed Diagnostics, Bangalore) by Bromo cresol green (BCG) dye method (Tietz *et al.,* 1976) and the kit contained albumin reagent and albumin standard.

Serum samples were analyzed using Calcium kit (M/s Swemed Diagnostics, Bangalore) by Arsenazo III method (Faulker *et al.,* 1982) and the kit composed of Arsenazo III reagent and calcium standard.

Serum samples were analyzed using phosphorous kit (M/s Swemed Diagnostics, Bangalore) by UV method (Tietz, 1976) and the kit contained reagent R1 and phosphorous standard.

Serum samples were analyzed using Urea Berthelot kit (M/s Swemed Diagnostics, Bangalore) (Fawcett, 1960) and the kit contained reagent R1, reagent R2, reagent R3 and standard.

Serum samples were analyzed using creatinine kit-SR (M/s Swemed Diagnostics, Bangalore) by Jaffe's kinetic method (Tietz *et al.*, 1976) and the kit contained creatinine reagent and creatinine standard.

Serum samples were analyzed using Cholesterol kit (M/s Swemed Diagnostics, Bangalore) by CHOD/ POD method (Natio, 1988) and the kit contained cholesterol reagent and cholesterol standard.

Serum samples were analyzed using SGPT kit (M/s Swemed Diagnostics, Bangalore) by modified IFCC method (Henley and Pollard, 1955) and the kit contained reagent R1 and reagent R2.

Serum samples were analyzed using SGOT kit (M/s Swemed Diagnostics, Bangalore) by modified IFCC method (Bergmeyer, 1986) and the kit contained reagent R1and reagent R2.

Nutrient broth powder (13.0 g) (Hi Media, Mumbai) was suspended in 1000 ml distilled water and heated to dissolve the medium completely and later sterilized by autoclave at 15 lbs pressure (121˚C) for 15 minutes.

Nutrient agar powder (31.0 g) (Hi Media, Mumbai) was suspended in 1000 ml distilled water and heated to boiling to dissolve the medium completely and later

sterilized by autoclave at 15 lbs pressure (121˚C) for 15 minutes. After mixing, it was poured into sterile Petri plates.

Uterine fluid samples were collected immediately after relieving of dystocia in cows and buffaloes and were transferred to nutrient broth. These samples were further cultured for isolation and identification of microbial flora as per the standard procedure. Identification of the bacteria was based on the characteristics of colony; Gram's staining pattern and morphology under a trinocular microscope.

The uterine samples were inoculated into 10 ml of nutrient broth tubes and were incubated at 37ºC for 18 hours. Following which the materials from the tubes were streaked on to the nutrient agar plates and again incubated for 18 hours at 37ºC. After incubation, the microbial colonies were picked up based on their characteristics and were grown further in the nutrient broth for pure culture of the isolated microbes. The broth cultures were subjected to Gram's staining so as to find out the type of organisms as per standard procedures. The isolates were further processed by streaking on to enriched media like nutrient agar. The streaked agar plates were incubated at 37ºC for 18 hours, then colonies from agar plate were smeared on glass with one drop of distilled water and then was heat fixed and subjected for Gram's staining for identification of organism as per standard procedure.

The buffaloes with uterine torsion and pre-partum cervical prolapse have shown variable mean Hb, TEC, PCV, MCH, MCHC, TLC, Neutrophil, Lymphocytes and Eosinophil before and after relieving dystocia and the difference were non-significant.

The buffaloes suffering with dystocia in anterior presentation have shown variable mean Hb, TEC, PCV, MCH, MCHC, TLC, Neutrophil and Eosinophil with non-significant difference. However, the lymphocyte mean values were increased from 39.7±0.04% to 52.7±4.71% before and after relieving dystocia with significant difference.

The mean Hb, TEC, PCV, MCH, MCHC, TLC, Neutrophil and Eosinophil values varied in dystotic cows suffering with anterior and posterior presentation without any significant difference.

The serum SGOT (84±11.6 vs 39±7.04 Units/L), Total proteins (11.3±0.6 vs 9.1±0.7 g/dL), Albumin (4.2±0.17 vs 3.6± 0.17 g/dL) values reduced in dystotic buffaloes significantly with uterine torsion however values of SGPT, Glucose, Calcium, Phosphorus, BUN, Creatinine, Cholesterol, values varied without any significant difference before and after relieving dystocia.

The serum SGPT (14.5±15.2 to 9.46±1.32 Units/L) Total proteins (11±0.5 to 8.93±0.31 g/dL), Albumin (4.86±0.5 to 3.59±0.15 g/dL) Calcium (5.04±0.84 to 2.41±0.8 mg/dL) and BUN (65.8±0.95 to 198±16.1 mg/dL) values varied with significant difference and SGOT, Glucose, Phosphorus Creatinine and Cholesterol values also varied before and after relieving dystocia in buffaloes with pre-partum cervical prolapse.

The serum SGOT (29.6±3.10 vs 15.5±4.49 Units/L), Glucose (60.4±7.60 vs 25.31±8.2 mg/dL), Total proteins (10.81±0.4 vs 9.45± 0.30 g/dL), Albumin (3.76±0.22 vs 3.26±0.19 g/dL), Calcium (5.3±0.94 vs 3.45±0.67 mg/dL), BUN (69.9±2.22 vs 150±17.7

mg/dL) varied in dystotic buffaloes with anterior presentation of fetus before vs after relieving dystocia with significant difference whereas the values of SGPT, Phosphorus, Creatinine and Cholesterol also varied without any significance.

The serum SGPT (36.72±1.5 vs 61.8±1.78 units/L), Albumin (3.53±0.14 vs 3.15±0.22 g/dL) and Phosphorus (6.85±0.80 vs 5.53±0.48 mg/dL) varied before and after relieving dystocia in cows with anterior presentation of fetus however, the SGOT, Glucose, Total proteins, Calcium, BUN, Creatinine, Cholesterol values were also differed without any significance.

The serum SGPT (27.4±2.5 vs 19.1±2.1 Units/L), Phosphorus (7.02±0.3 vs 5.4±0.09 mg/dL) and Cholesterol (89.8±3.7 vs 138±11.8 mg/dL) varied significantly in dystotic cows with posterior presentation of calf whereas, the values of SGOT, Glucose, Total proteins, Albumin, Calcium, BUN and Creatinine also varied without any significant difference.

The various bacteria isolated from the uterine fluid collected from 27 buffaloes after relieving of dystocia were B*acillus* (51.85 %), S*treptococcus* (18.51 %), *Escherichia coli* (14.83 %), *Staphylococcus* (11.11 %), and *Clostridium species* (3.70 %) respectively.

The various bacteria isolated from the uterine fluid collected from 15 cows after relieving of dystocia were *Bacillus* (40.00 %), S*treptococcus* (20.00 %), *Escherichia coli* (20.00 %), *Staphylococcus* (13.34 %), and *Clostridium species* (6.66 %) respectively.

Based on the present findings, the following conclusions were drawn.

1. Dystocia stress appears to affect several blood hematological values in cows and buffaloes. The analysis of these parameters can be of practical value to improve the health of dystocia affected dams and to increase survival of their calves.

2. The elevated plasma glucose levels in dystocia may be related stress to meet the increased energy demand.

3. Severe alterations in various biochemical parameters with the increase in duration between the occurrence and relieving of the dystocia in bovines were indicative of onset of severe inflammation, protein catabolism and stress.

4. All the estimated value of hematologic indices and serum biochemical components are recommended as a baseline values except which are significantly outside the common normal range.

5. The levels of SGOT, Albumin, SGPT, Calcium, Glucose and Total proteins were reduced 48 hours postpartum in dystocia affected buffaloes whereas the levels of BUN increased significantly.

6. The levels of Albumin, Phosphorus were reduced 48 hours postpartum whereas the levels of Cholesterol increased significantly in dystotic cows.

7. The various bacteria isolated from the uterine fluid collected from 27 buffaloes after relieving of dystocia were *Bacillus* (51.85 %), S*treptococcus* (18.51 %), *Escherichia*

coli (14.83 %), *Staphylococcus* (11.11 %) and *Clostridium species* (3.70 %) respectively.

8. The various bacteria isolated from the uterine fluid collected from 15 cows after relieving of dystocia were *Bacillus* (40.00 %), S*treptococcus* (20.00 %), *Escherichia coli* (20.00 %), *Staphylococcus* (13.34 %) and *Clostridium species* (6.66 %) respectively.

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ABSTRACT

HEMATO-BIOCHEMICAL PROFILE IN DYSTOTIC COWS AND BUFFALOES

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ABSTRACT

The lymphocyte mean values were increased from 39.7±0.04% to 52.7±4.71% before and after relieving dystocia with significant difference. The mean Hb, TEC, PCV, MCH, MCHC, TLC, Neutrophil and Eosinophil values varied in dystotic cows suffering with anterior and posterior presentation without any significant difference. The serum SGOT (84±11.6 vs 39±7.04 Units/L), Total proteins (11.3±0.6 vs 9.1±0.7 g/dL), Albumin (4.2±0.17 vs 3.6± 0.17 g/dL) values reduced in dystotic buffaloes significantly with uterine torsion. The serum SGPT (14.5±15.2 to 9.46±1.32 Units/L) Total proteins (11±0.5 to 8.93±0.31 g/dL), Albumin (4.86±0.5 to 3.59±0.15 g/dL) Calcium (5.04±0.84 to 2.41±0.8 mg/dL) and BUN (65.8±0.95 to 198±16.1 mg/dL) values varied with significant difference. The serum SGOT (29.6±3.10 vs 15.5±4.49 Units/L), Glucose (60.4±7.60 vs 25.31±8.2 mg/dL), Total proteins (10.81±0.4 vs 9.45± 0.30 g/dL), Albumin (3.76±0.22 vs 3.26±0.19 g/dL), Calcium (5.3±0.94 vs 3.45±0.67 mg/dL), BUN (69.9±2.22 vs 150±17.7 mg/dL) varied in dystotic buffaloes with anterior presentation of fetus before vs after relieving dystocia with significant difference. The serum SGPT (36.72±1.5 vs 61.8±1.78), Albumin (3.53±0.14 vs 3.15±0.22) and Phosphorus (6.85±0.80 vs 5.53±0.48) varied before and after relieving dystocia in cows with anterior presentation of fetus. The serum SGPT (27.4±2.5 vs 19.1±2.1 Units/L), Phosphorus (7.02±0.3 vs 5.4±0.09 mg/dL) and Cholesterol (89.8±3.7 vs 138±11.8 mg/dL) varied significantly in dystotic cows with posterior presentation of calf. The various bacteria isolated from the uterine fluid collected from buffaloes after relieving of dystocia were *Bacillus*, S*treptococcus*, *Escherichia coli*, *Staphylococcus* and *Clostridium* and *Bacillus*, S*treptococcus*, *Escherichia coli*, *Staphylococcus* and *Clostridium* from cows respectively.