ANTIOXIDANT STATUS AND METABOLIC PROFILE OF GOATS DURING PREGNANCY AND LACTATION

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Thesis submitted in partial fulfilment of the requirement for the degree of

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DECLARATION

I hereby declare that the thesis, entitled **"ANTIOXIDANT STATUS AND METABOLIC PROFILE OF GOATS DURING PREGNANCY AND LACTATION"** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled **"ANTIOXIDANT STATUS AND METABOLIC PROFILE OF GOATS DURING PREGNANCY AND LACTATION"** is a record of research work done independently by **Dr. Cynthia Jose** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, associateship or fellowship to her.

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EXTERNAL EXAMINER

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Dedicated to Holy Mother

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LIST OF ABBREVIATIONS

ATP	Adenosine 5'-triphosphate
BHBA	Beta hydroxybutyric acid
BUN	Blood urea nitrogen
DNA	Deoxyribonucleic acid
DTNB	5, 5'-dithiobis-2-nitrobenzoic acid
EDTA	Ethylene-diaminetetra-acetic-acid
GSH	Reduced glutathione
GSH-Px	Glutathione peroxidase
GSSG	Oxidized glutathione
Hb	Haemoglobin
K	Potassium
LDL	Low-density lipoprotein
MDA	Malondialdehyde
Na	Sodium
NEB	Negative energy balance
OSF	Oxidative stress factor
PCV	23Packed cell volume
PUFA	Polyunsaturated fatty acids
RNS	Reactive nitrogen species
ROM	Reactive oxygen metabolites
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TBA	Thiobarbituric acid
TCA	Trichloro-acetic-acid
TMP	1,1,3,3 Tetramethoxypropane
VLDL	Very-low-density lipoprotein

Introduction

1. INTRODUCTION

Oxygen, an indispensable element for life has severe deleterious effects on the body under certain conditions. The causes of poisonous property of oxygen were obscure prior to the publication of Gershman's free radical theory of oxygen toxicity in 1954 (Valko *et al.*, 2007). Most of the potentially harmful effects of oxygen are due to the formation and activity of a number of chemical compounds, known as reactive oxygen species (ROS) and reactive nitrogen species (RNS), which have a tendency to donate oxygen to other substances. ROS and RNS are products of normal cellular metabolisms and are well recognized for playing dual role as both deleterious and beneficial species. Many such reactive species are free radicals and have surplus of one or more free floating electrons and therefore unstable and highly reactive.

The science of free radical in living organisms was discovered in less than 50 years ago. Biologically, the most common free radical species are the hydroxyl radical, the superoxide radical, the nitric oxide radical and the lipid peroxide radical (Droge, 2002). Free radicals are derived from normal essential metabolic processes in the body or from external sources such as exposure to x-rays, cigarette smoking, air pollutants and industrial chemicals. In cells, free radical formation occurs continuously as a consequence of both enzymatic and non-enzymatic reactions. Enzymatic reactions which serve as sources of free radicals include those involved in the respiratory chain, phagocytosis, prostaglandin synthesis and in the cytochrome P-450 system (Bagchi and Puri, 1998).

Low levels of ROS may be indispensable in a plethora of processes, including intracellular messaging, leading to proliferation or apoptosis, immunity and defense against microorganisms (Mates and Sanchez-Jimenez, 1999). In contrast, high doses and/or inadequate removal of ROS result in oxidative stress, which may cause severe metabolic malfunctions and damage to biological molecules.

Prime targets for free radical reaction are the unsaturated bonds in membrane lipids. Consequent peroxidation results in a loss of membrane fluidity and receptor alignment and potentially in cellular lysis. Free radical damage to sulfur containing enzymes and other proteins culminates in inactivation, crosslinking and denaturation. The damage to DNA can cause mutation, that could be carcinogenic. Oxidative damage to carbohydrates can alter any of the cellular receptor functions (Machlin and Benedich, 1987). In addition to free radicals, certain aldehydes such as malondialdehyde (MDA) and hydroxynonenal, arising from free radical degradation of poly unsaturated fatty acids (PUFA), can cause cross linkages in lipids, proteins and nucleic acids.

Nature has endowed each cell with adequate protective mechanisms against harmful effects of free radicals. Normally the body is protected against ROS, RNS and their toxic products with a wide range of defense mechanisms. The components of these integrated systems have been classified as antioxidant enzymes, chain breaking antioxidants and transition metal binding proteins (Young and Woodside, 2001). Enzymatic antioxidant defense include superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT). Chain breaking antioxidants are small molecules that can receive an electron from a radical or donate an electron to a radical with the formation of stable byproducts. These include vitamin E, β carotene, flavonoids, ubiquinol-10, vitamin C, reduced glutathione (GSH), uric acid and protein bound thiol groups. Transition metal binding proteins like ferritin, transferrin, lactoferrin and ceruloplasmin act as a crucial component in the antioxidant defense system by sequestering iron and copper so that they are not available to drive the formation of hydroxyl radical.

Pregnancy and lactation periods are considered as physiologically critical stages, characterized by high energy demand for bodily functions. A dramatic increase in energy requirements during this period makes the animal highly susceptible to negative energy balance (NEB) and oxidative stress. Intensified

process of fatty acid oxidation which occur during low energy balance, result in the increased production of ROS, and development of oxidative imbalance.

Transition metals, especially iron, which are abundant in the placenta, are important in the production of free radicals. As the placenta is highly vascular, it is exposed to high maternal oxygen partial pressure. Nitric oxide is also produced by the placenta and together with other RNS contributes to potential oxidative stress in the presence of transition metals. The placenta is also rich in macrophages favoring the local placental production of free radicals (Casanueva and Viteri, 2003). So during the period of pregnancy the body should have sufficient antioxidant reserves to protect the internal organs and fetus against the negative influence of free radicals.

The peripartum and early lactation periods present considerable physiological challenges to homeostasis by imposing significant metabolic stressors that may contribute to the onset of diverse disorders. Investigations of oxidative status in cattle during this period showed significant variations in the oxidative balance (Bernabucci *et al.*, 2005, Castillo *et al.*, 2005, Yokus *et al.*, 2007 and Turk *et al.*, 2008). The literature regarding antioxidant status in goats during pregnancy and lactation is sparse and also little is known about antioxidant protection and adaptation capabilities to oxidative stress in this species. In the last few years, the detection of free radical damage and the body's defenses against it has become increasingly important in clinical medicine as a complementary tool in the evaluation of metabolic status. Hence the present study was undertaken with the following objectives:

1. To evaluate and compare the antioxidant status and metabolic profile of pregnant, lactating and non pregnant dry goats.
2. To evolve any possible relationship between antioxidant markers and other relevant blood parameters.

Review of Literature

2. REVIEW OF LITERATURE

2.1 OXIDATIVE STRESS IN HEALTH AND DISEASE

The peroxidation of PUFA is a feature of many types of cell injury in which free radical intermediates are produced in excess of local defense mechanisms (Slater, 1984). Extensive lipid peroxidation can result in membrane disorganization by peroxidizing mainly the highly unsaturated, PUFA and thereby changing the composition of the PUFA and phospholipid fractions, leading to changes in the ratio of polyunsaturated to other fatty acids.

Machlin and Benedich (1987) reviewed on free radical tissue damage and protective role of antioxidant nutrients and reported that highly reactive molecules called free radicals can cause tissue damage by reacting with polyunsaturated fatty acids in cellular membranes, nucleotides in DNA, and critical sulfhydryl bonds in proteins. Free radical damage contributes to the etiology of many chronic health problems such as emphysema, cardiovascular and inflammatory diseases, cataracts, and cancer. The extent of tissue damage is the result of the balance between the free radicals generated and the antioxidant protective defense system.

Floyd (1990) described the role of oxygen free radicals in carcinogenesis and brain ischemia. He reported that oxygen free radical reactions and oxidative damage are in most cases held in check by antioxidant defense mechanisms, but when an excessive amount of oxygen free radicals are produced or defense mechanisms are impaired, oxidative damage may occur and this appears to be important in contributing to several pathological conditions.

Belch *et al.* (1991) measured the product of lipid peroxidation, MDA and thiols in plasma of 45 patients with congestive heart failure and 45 controls. They found that MDA concentrations were significantly higher and plasma thiols were

significantly lower in the patients with congestive heart failure than in the controls. This study suggests that free radicals may be important in heart failure and the degree of free radical production may be linked to the severity of the disease.

Halliwell and Chirico (1993) explained the mechanism of lipid peroxidation and reported that oxidative stress can damage many biological molecules. Proteins and DNA are often more significant targets of injury than are lipids, and lipid peroxidation often occurs late in the injury process. They also explained the artifacts of many assays available to measure lipid peroxidation.

Miller *et al.* (1993) reported that imbalance between production and safe disposal of reactive oxygen metabolites (ROM) may contribute periparturient disorders in dairy cows like udder edema, milk fever, retained placenta, mastitis, and suboptimal reproduction which reduce profits for dairy producers. They suggested that antioxidant requirements of high producing dairy cows will be higher than generally recognized.

Farber (1994) reviewed on the mechanisms of cell injury by activated oxygen species like superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (.OH). O_2^- and H_2O_2 injure cells as a result of the generation of a more potent oxidizing species. .OH initiates the peroxidative decomposition of the phospholipids of cellular membranes. .OH also damages the inner mitochondrial membrane and due to mitochondrial deenergization, a sequence of events is initiated that similarly leads to the loss of viability of the cell. DNA represents a third cellular target of .OH.

Halliwell and Cross (1994) suggested that mild oxidative stress often induces antioxidant defense enzymes, but severe stress can cause oxidative damage to lipids, proteins and DNA within cells, leading to such events as DNA strand breakage and disruption of calcium ion metabolism.

The ROS are constantly produced during normal aerobic metabolism and are safely removed by a variety of biological antioxidants. Disturbance of the balance between production of ROS such as superoxide, hydrogen peroxide, hypochlorous acid, hydroxyl, alkoxyl, and peroxyl radicals and antioxidant defenses against them produces oxidative stress, which amplifies tissue damage (Gutteridge, 1995).

Sardesai (1995) reviewed that free radicals are produced in the body as byproducts of normal metabolism and as a result of exposure to radiation and some environmental pollutants. They are highly reactive and can damage cellular components leading to a variety of diseases.

The generation of highly reactive oxygen metabolites is an integral feature of normal cellular metabolism like mitochondrial respiratory chain, phagocytosis, arachidonic acid metabolism, ovulation and fertilization; however their production can multiply during pathological circumstances (Roth, 1997).

Sies (1997) reported that oxidative stress in a living organism is a result of an imbalance between ROM production and neutralizing capacity of antioxidant mechanisms. Oxidants are formed as a normal product of aerobic metabolism but can be produced at elevated rates under pathophysiological conditions.

Bagchi and Puri (1998) suggested that the chemical reactivity of inactivated free radicals can damage all cellular macromolecules including proteins, carbohydrates, lipids and nucleic acids. Their destructive effects on protein lead to the causation of cataracts. Free radical damage to DNA is also implicated in the causation of cancer and its effect on low density lipoprotein (LDL) cholesterol is responsible for heart disease.

Bandyopadhyay *et al.* (1999) reviewed the mechanism of ROS mediated oxidative damage of lipids, proteins and DNA. They suggested that the site

specific oxidative damage of some of the susceptible amino acids of proteins is regarded as the major cause of metabolic dysfunction during pathogenesis. The ROS have also been implicated in the regulation of at least two well defined transcription factors which play an important role in the expression of various genes encoding proteins that are responsible for tissue injury.

Hubel (1999) explained the role of oxidative stress in the pathogenesis of preeclampsia. Oxidative stress may be the point at which multiple factors converge, resulting in endothelial cell dysfunction and the consequent clinical manifestations of preeclampsia. Differences in the prevalence of placental versus maternal oxidative stressors could contribute the heterogeneity of preeclampsia.

According to Mates and Sanchez-Jimenez (1999), low concentrations of reactive oxygen intermediates may be beneficial or even indispensable in processes such as intracellular messaging and defense against micro-organisms, but higher amounts of active oxygen may be harmful to cells and organisms. They described the existence of wide variety of non-enzymatic and enzymatic antioxidant defenses including SOD, GSH-Px and CAT.

A study conducted by Trevisan *et al.* (2001) revealed that age, plasma glucose level, insulin resistance and postmenopausal status in women were associated with increased oxidative stress and reduced antioxidant potentials. Oxidative status and antioxidant potentials also appear to be significantly associated with a number of cardiovascular disease risk factors.

Young and Woodside (2001) explained the basic chemistry of free radical formation in the body, the consequences of free radical induced tissue damage and the function of antioxidant defense system, particularly in the development of atherosclerosis. Oxidative stress, arising as a result of an imbalance between free radical production and antioxidant defenses, is associated with damage to a wide range of molecular species including lipids, proteins, and nucleic acids.

At high concentrations, free radicals and radical-derived, non radical reactive species are hazardous for living organisms and damage all major cellular constituents. An excessive or sustained increase in ROS production has been implicated in the pathogenesis of cancer, diabetes mellitus, atherosclerosis, neurodegenerative diseases, rheumatoid arthritis, ischemia/reperfusion injury, obstructive sleep apnea, and other diseases (Droge, 2002).

Bisla *et al.* (2003) determined the oxidative stress factor (OSF) from the values of MDA, GSH and packed cell volume (PCV) during the study of therapeutic effect of α-tocopheryl acetate and sodium selenite combination in the treatment schedule of trans-abdominal diaghragmatic herniorrhaphy in buffaloes. They observed a reduction in OSF in the treatment group compared to diseased control group which indicated a reduction of oxidative stress during this therapy.

Devasagayam *et al.* (2004) reviewed the possible role of free radicals in disease and antioxidants in its prevention. They opined that free radicals can adversely alter lipids, proteins and DNA and have been implicated in aging and a number of human diseases. Lipids are highly prone to free radical damage resulting in lipid peroxidation that can lead to adverse alterations. Free radical damage to protein can result in loss of enzyme activity. Damage caused to DNA can result in mutagenesis and carcinogenesis.

Poston and Raijmakers (2004) described the methodology to determine oxidative stress by measurement of a decrease in total antioxidant capacity, through depletion of individual antioxidants such as vitamin E, vitamin C or GSH-PX, or more often, by estimation of the products of oxidative damage to lipids, proteins and DNA.

Jackson *et al.* (2005) evaluated the association between oxidative stress and endometriosis in women. They found a weak association between thiobarbituric acid reactive substances, a measure of overall oxidative stress, and

endometriosis and suggested that oxidative stress might play a role in the development and progression of endometriosis.

Juranek and Bezek (2005) reviewed on the involvement of ROS in tissue injury and reported that ROS easily react *in vitro* with most biological molecules, causing their degradation and destruction and when excessively produced *in vivo*, ROS are deleterious to integral components of the cell and cause their dysfunctions. ROS mediated lipid peroxidation, protein oxidation and oxidative alterations to nucleic acids are crucial events of unfavorable actions of ROS.

Valko *et al.* (2007) reviewed on the free radicals and antioxidants in normal physiological functions and human disease and suggested that ROS and RNS are well recognized for playing a dual role as both deleterious and beneficial species. Overproduction of ROS results in oxidative stress, a deleterious process that can be an important mediator of damage to cell structures, including lipids and membranes, proteins, and DNA.

Cindrova-Davies (2009) described the important role of placental oxidative stress in the pathophysiology of preeclampsia. Placental oxidative stress and ROS production can potentiate downstream stress and inflammatory signaling pathways, culminating in the release of inflammatory mediators, apoptotic debris, anti-angiogenic factors and other mediators, which then stimulate the maternal inflammatory reaction that manifests in endothelial dysfunction and the symptoms of preeclampsia.

2.2 ANTIOXIDANT DEFENSE SYSTEM

Ascorbate completely protects plasma lipids against detectable peroxidative damage induced by aqueous peroxyl radicals and it is the only plasma antioxidant that can do so. Plasma devoid of ascorbate, but no other endogenous antioxidant, is extremely vulnerable to oxidant stress and susceptible

to peroxidative damage to lipids. The plasma proteins' thiols, although they become oxidized immediately upon exposure to aqueous peroxyl radicals, are inefficient radical scavengers and appear to be consumed mainly by autoxidation (Frei *et al.*, 1989).

Halliwell and Gutteridge (1989) have defined antioxidants as substances that are able, at relatively low concentrations, to compete with other oxidizable substrates and significantly delay or inhibit the oxidation of these substrates. This definition includes the enzymes SOD, GSH-Px, and CAT, as well as nonenzymic compounds such as alpha-tocopherol (vitamin E), β-carotene, ascorbate (vitamin C) and glutathione.

Mascio *et al.* (1991) reviewed on the role of carotenoids, tocopherols and thiols in antioxidant defense systems and reported that glutathione, the most important cellular thiol, is acting as a substrate for several transferases, peroxidases and other enzymes that prevent or mitigate the deleterious effects of oxygen free radicals. Thiol groups act as intracellular antioxidants by scavenging free radicals and through enzymatic reactions. The protection of biological membranes against lipid peroxidation is an interesting aspect of its function, since this water-soluble thiol prevents damage in a lipid environment.

Niki (1991) reviewed on the action of ascorbic acid as a scavenger of active and stable oxygen radicals and reported that ascorbic acid reduces stable oxygen, nitrogen and thyl radical and acts as a primary defense against aqueous radicals in the blood. When radicals were formed in aqueous suspensions of erythrocytes or LDL, ascorbic acid scavenge the radicals before they reach membranes and LDL. Although ascorbic acid cannot scavenge lipophilic radicals within the lipid compartment by itself, it acts as a synergist with tocopherol for the reduction of lipid peroxyl radicals within the lipid compartment by reacting with tocopheroxyl radical and regenerating active tocopherol.

Vitamin E, the primary lipid soluble small molecule antioxidant, and vitamin C, the terminal water soluble small molecule antioxidant, cooperate to protect lipids and lipid structures against peroxidation. Eventhough vitamin E is located in membranes and vitamin C is located in aqueous phases, vitamin C is able to recycle vitamin E, thereby permitting it to function again as a free radical chain breaking antioxidant (Buettner, 1993).

Beyer (1994) reviewed on the role of ascorbate in antioxidant protection of biomembranes and its interactions with vitamin E and coenzyme Q. He reported that ascorbic acid protects the aqueous compartments of the cell, extracellular matrix and circulatory system in which it resides and hydrophobic phases such as circulatory system lipoproteins and membrane systems. Ascorbic acid can perform its antioxidant function either by quenching various free radical species directly or by reducing membrane-bound oxidized vitamin E at the membrane surface.

The most important H_2O_2 removing enzymes in human cells are GSH-Px, which require selenium for their action and they remove H_2O_2 by using it to oxidize GSH to oxidized glutathione (GSSG). Glutathione reductase, a flavoprotein enzyme, regenerates GSH from GSSG, with NADPH as a source of reducing power (Halliwell and Cross, 1994).

Free radicals are normally neutralized by efficient systems in the body that include the antioxidant enzymes like SOD, CAT, and GSH-Px and the nutrient derived antioxidant small molecules such as vitamin E, vitamin C, carotenes, flavonoids, glutathione, uric acid, and taurine. In healthy individuals, a delicate balance exists between free radicals and antioxidants and in some pathologic conditions, oxidative stress causes the level of antioxidants to fall below normal (Sardesai, 1995).

Sies and Stahl (1995) reviewed on antioxidant functions of vitamins E and C, β-carotene, and other carotenoids and suggested that tocopherols and tocotrienols (vitamin E), ascorbic acid and the carotenoids react with free radicals, notably peroxyl radicals and with singlet molecular oxygen, which is the basis for their function as antioxidants.

Ascorbic acid provides *in vivo* antioxidant protection primarily as an aqueous phase peroxyl and oxygen radical scavenger and increased ascorbate nutriture is associated with reduced risk of some degenerative diseases, especially cancer and eye cataracts. The oxidized form of the vitamin (dehydroascorbic acid) is readily converted back to the reduced form by reduced glutathione, NADPH, or both (Jacob and Burri, 1996).

The fundamental defense of the organism against ROS include scavenger enzymes such as, SOD, CAT, GSH-Px and lipid and water soluble antioxidant compounds like ascorbic acid, glutathione, albumin, transferrin, etc. (Roth, 1997).

Glutathione serves as a substrate for GSH-Px, an enzyme that functions to remove H_2O_2. The GSH is acted on by GSH-Px to produce the GSSG. The mineral selenium is an essential component of GSH-Px. Along with other antioxidant enzymes like SOD, CAT, and glutathione reductase, GSH-Px function to reduce lipid peroxidation (Clarkson and Thompson, 2000).

Finkel and Holbrook (2000) reviewed on oxidants, oxidative stress and the biology of ageing and suggested that the burden of ROS production is largely counteracted by an intricate antioxidant defense system that includes the enzymatic scavengers SOD, CAT and GSH-Px. The enzyme SOD speeds the conversion of superoxide to H_2O_2, whereas CAT and GSH-Px convert hydrogen peroxide to water. The balance between ROS production and antioxidant defenses determines the degree of oxidative stress and consequences of this stress include modification to cellular proteins, lipids and DNA.

According to Young and Woodside (2001), antioxidants prevent free radical induced tissue damage by preventing the formation of radicals, scavenging them, or by promoting their decomposition.

Fang *et al.* (2002) reviewed recent advances in free radical biology and antioxidant nutrients, with emphasis on oxidative defense systems against radiation-induced radical damage and concluded that dietary antioxidants and other nutrients play an important role in preventing cells from radical induced cytotoxicity.

Antioxidant effects of vitamin C have been studied *in vitro* by Padayatty *et al.* (2003) and they found that vitamin C either has no effect or produces modest reductions in the concentrations of biomarkers of oxidation but combinations of many antioxidants are more effective.

Spears and Weiss (2008) demonstrated important role of a number of antioxidants and trace minerals in immune function and health status of dairy cows. They described that vitamin E and β-carotene are important cellular antioxidants. Selenium is involved in the antioxidant system via its role in the enzyme GSH-Px. Supplementation of vitamin E and/or Selenium had reduced the incidence of mastitis and retained placenta, and reduced duration of clinical symptoms of mastitis. β-carotene supplementation may enhance immunity and reduce the incidence of retained placenta and metritis in dairy cows.

Antioxidants protect cells against the effects of harmful free radicals. They function by one or more of the mechanisms such as, reducing activity, free radical scavenging, forming complexes of pro-oxidant metals and quenching of singlet oxygen to protect living systems against lipid peroxidation and other anomalous molecular modifications of oxidative stress (Mathew and Nair, 2009).

2.3 OXIDATIVE STATUS DURING PREGNANCY AND LACTATION

Hubel *et al.* (1989) studied on the occurrence of lipid peroxidation in normal and preeclamptic pregnancy and reported that vascular endothelial dysfunction may be caused by uncontrolled lipid peroxidation and this may play an important role in the pathophysiology of preeclampsia.

Davidge *et al.* (1992) studied the sera antioxidant protective mechanisms in women with preeclampsia and concluded that in women with preeclampsia, antioxidant activity was markedly reduced by late gestation and this may result in a greater potential for endothelial oxidative damage.

Brzezinska-Slebodzinska *et al.* (1994) investigated the possible relationships among dietary antioxidants, oxidative status, and placental retention in periparturient dairy cows and suggested that inadequate dietary antioxidants may increase oxidative stress, production of lipid peroxides, and incidence of retained fetal membranes.

In normal pregnancy lipid peroxides increase, but antioxidants also increase to offset their toxic actions. But in women with preeclampsia, circulating levels of lipid peroxides were found increased, but net antioxidant activity was decreased as compared to normally pregnant women (Walsh, 1994).

Poranena *et al.* (1996) studied the lipid peroxidation and antioxidants in normal and pre-eclamptic pregnancies. They found that lipid peroxidation was increased and the activity of antioxidant enzymes SOD and glucose 6-phosphate dehydrogenase were decreased in preeclamptic placenta. The peroxyl radical trapping capacity was high in the serum of preeclamptic patients.

Mutlu-Turkoglu *et al.* (1998) investigated lipid peroxidation and the antioxidant system of normal and preeclamptic pregnant women. They observed a

increase of ROS on days 40 and 200 of lactation and during dry period compared to values obtained on 1st d of lactation. This shows high oxidative processes which occur during lactation in ewes. Low values of ROS at the start of experimental period could be due to

significant increase in thiobarbituric acid reactive substances, significant decreases in total thiol content and SOD activity, and unchanged vitamin C levels and GSH-Px activity in the plasma of preeclamptic women compared to women with normal pregnancies. They concluded that preeclampsia is associated with an imbalance between lipid peroxides and the antioxidant system.

Madazli *et al.* (1999) determined the changes in plasma levels of lipid peroxide, vitamin E and vitamin C in women with preeclampsia and found that MDA, a lipid peroxidation product, was significantly increased, while vitamins E and C were significantly decreased in the preeclamptic group compared to healthy pregnant women. They concluded that lipid peroxidation is an important factor in the pathogenesis of preeclampsia and in preeclampsia, antioxidant nutrients are excessively utilised to counteract the cellular changes mediated by free radicals.

Jenkins *et al.* (2000) examined the role of antioxidants within the normal menstrual cycle, in healthy pregnancy, and in women suffering first trimester miscarriage and concluded that miscarriage and pregnancy appeared to be associated with increased oxidative stress. In a successful pregnancy, however, changes occurred within the peripheral blood that offered protection from oxidant attack.

Sainz *et al.* (2000) studied the changes in lipid peroxidation during pregnancy and after delivery in rats and effect of pinealectomy on it. They concluded that during pregnancy high levels of oxidative stress induced an increase in oxidative damage to lipids, which in some cases is inhibited by the antioxidative actions of pineal indoles.

Gorecka *et al.* (2002) investigated the changes in antioxidant components in the blood of mares during pregnancy and after foaling by analyzing the activity of SOD, GSH-Px and total antioxidant status. They found that greater changes in antioxidant system were encountered especially during perinatal period.

Ilhan *et al.* (2002) assessed and compared antioxidant enzyme and trace metal status between the pregnant women with or without preeclampsia and healthy controls. They found that the MDA and copper levels were significantly increased in preeclamptic group, while zinc and super oxide dismutase levels were significantly decreased compared to normal control group and healthy pregnant women.

Casanueva and Viteri (2003) correlated the iron concentration and oxidative stress in pregnancy and reported that, iron deficiency and excess iron supplementation result in free radical mitochondrial damage.

Llurba *et al.* (2004) analyzed the potential role of oxidative stress as a mechanism underlying endothelial damage in preeclampsia and the susceptibility of pregnant woman to the disease. They found significantly enhanced antioxidant enzyme SOD and GSH-Px activities in erythrocytes and significantly decreased plasma vitamin C and protein thiol levels, in women with preeclampsia when compared to women with normal pregnancy. Erythrocyte GSH content, total plasma antioxidant capacity and whole plasma oxidizability values were similar in women with preeclampsia and normal pregnancy. Significantly elevated plasma total lipid hydroperoxides; the major initial reaction products of lipid peroxidation were found in severe preeclampsia as compared to women with normal pregnancy. There were no intracellular or extracellular increases in any of the secondary end-products of lipid peroxidation, malondialdehyde or lipoperoxides in preeclampsia.

Raijmakers *et al.* (2004) reviewed on recent investigations into oxidative stress and its relevance to the cause and prevention of preeclampsia and reported a combination of vitamins C and E, which acts in synergy to prevent lipid peroxidation, may be effective in the prevention of preeclampsia.

Agarwal *et al.* (2005) reviewed that oxidative stress plays a role during pregnancy and normal parturition and in initiation of preterm labor. The ROS serves as key signal molecules in physiological processes but also have a role in pathological processes involving the female reproductive tract. The pathological effects are exerted by various mechanisms including lipid damage, inhibition of protein synthesis, and depletion of ATP. They emphasized that free radicals have important physiological functions in the female reproductive tract as well as excessive free radicals precipitate female reproductive tract pathologies.

Bernabucci *et al.* (2005) studied the influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows and reported that, after calving, cows showed a decrease of plasma and erythrocyte thiol groups and SOD, and an increase of ROM, thiobarbituric acid-reactive substances, and plasma GSH-Px. They concluded that oxidative status of dairy cows was related to energy status and cows can experience oxidative stress during the peripartum period.

Castillo *et al.* (2005) evaluated the antioxidant status of healthy Holstein cows during late pregnancy and lactation onset under field conditions using plasma levels of MDA; a degradation product of lipid peroxidation and total antioxidant status. They observed an increased rate of lipid peroxidation around parturition and proposed the relationship between antioxidant status markers and other relevant blood parameters.

Sordillo (2005) reviewed on the factors affecting mammary gland immunity and mastitis susceptibility and suggested that the physiological stresses associated with rapid differentiation of secretory parenchyma, intense mammary gland growth, and the onset of milk synthesis and secretion during lactation period are accompanied by a high energy demand and an increased oxygen requirement. This augments the production of ROS that plays a central role in mediating uncontrolled inflammatory responses and causing tissue injury.

Borisenkov *et al.* (2006) studied the antioxidant status of ewe's blood during pregnancy and lactation and revealed that the activities of antioxidant enzymes were increased at the end of pregnancy, when the level of progesterone in blood was maximum.

Castillo *et al.* (2006) investigated the antioxidant status and metabolic parameters during lactation in dairy cows and suggested that the measurement of oxidant-antioxidant balance could provide complementary information about the homeostasis of animals than the conventional metabolic parameters alone.

Di Trana *et al.* (2006) evaluated the effects of the hot season and nutrition on the oxidative status and metabolic profile of lactating goats during mid lactation and reported that seasonal rather than nutritional factors have a more pronounced effect on oxidative status markers.

Piccione *et al.* (2006) investigated the effects of the antioxidant mechanisms during different phases of milking and dry period in ewes and found oxidative processes increased at the end of milking period, characterized by low values of ROS at the beginning of lactation and by a significant increase at the mid-point of lactation.

Mohamed (2007) assessed antioxidant status, ROS and RNS in healthy and camels with endometritis. A significant increased MDA as well as a decreased total antioxidant status, ascorbic acid, alpha tocopherol, copper and zinc was observed in camels with endometritis compared to healthy. They suggested that free radical overproduction may be a contributing factor in reduction of antioxidant status and increase in the degree of lipid peroxidation.

Mohamed (2007) assessed antioxidant status and the degree of oxidative stress in mastitic and healthy camels. He found a reduction in both individual and

total antioxidant status and increase in lipid peroxidation, manifested by an increase in MDA in mastitic dairy camels compared with healthy ones.

Yokus *et al.* (2007) investigated pre-partum and post-partum changes in the total anti-oxidant capacity, total peroxide and oxidative stress index of cows with dystocia compared with healthy animals. They assessed the possible relationships between these markers and relevant blood parameters and reported that there were no changes in oxidative stress either in dystocia or during normal parturition eutoci. The decreased serum total protein concentration observed, might be a useful parameter in diagnosis of pathological conditions of late pregnancy.

Celi *et al.* (2008) monitored the metabolic profile and oxidative status in 10 Red Syrian goats during the peripartum period and found that blood GSH-Px activity decreased during the post-partum period and its values were significantly lower on 14^{th} and 28^{th} d post-partum. No effect of time from delivery was noted on ROM and SOD activity. This study indicates that goats experienced moderate oxidative stress during the peripartum period.

Piccione *et al.* (2008) studied on the effect of the stage of lactation on serum homocysteine and oxidative stress in ewes and reported a significant increase of ROS on days 40 and 200 of lactation and during dry period compared to values obtained on 1^{st} d of lactation. This shows high oxidative processes which occur during lactation in ewes. Low values of ROS at the start of experimental period could be due to the negative energetic balance which promotes mobilization of lipids and gluconeogenesis thereby induced an increase of free radicals.

Turk *et al.* (2008) investigated serum paraoxonase-1 (PON1) activity and MDA concentration to assess the antioxidant status during pregnancy and post-partum period in cows. A significantly lower PON1 activity was found in late

pregnancy and early post-partum compared to the first and the second trimester of pregnancy and the mid-lactation. MDA level was significantly higher in the dry period compared to pregnant lactating and post-partum cows.

Nath and Baruah (2009) evaluated the metabolic correlation between preeclampsia and oxidative stress and found a significant increase in serum triglyceride, serum cholesterol along with LDL and very low density lipoprotein (VLDL) in pregnancy over nonpregnant controls and preeclampsia over pregnant control. In normal pregnancy and preeclampsia, serum vitamin E and serum lipid peroxidation as represented by whole blood MDA were negatively correlated indicating significant degree of inverse relationship between two.

Rajni *et al.* (2009) evaluated the oxidative stress in preeclampsia by estimating serum prooxidants and antioxidants and observed that preeclamptic patients had significant increase in serum MDA and decrease in serum vitamin E, SOD and erythrocyte GSH levels when compared to healthy controls.

Suriyasathaporn *et al.* (2009) studied the correlation between milk yield and MDA levels, an oxidative stress marker and reported that the loss of milk yield after intramammary infection may be highly mediated by increased oxidative stress status.

Celi *et al.* (2010) studied the effects of high and low energy diet on oxidative stress in 14 Red Syrian goats during the peripartum period and found that energy content of the diet did not seem to have an effect on the oxidative status biomarkers measured. They also reported that there was no correlation between the biomarkers measured and the goats were exposed to an increased risk of oxidative stress during the peripartum period, as suggested by the observed decreases in GSH-Px and SOD activities and by the increase in ROM concentration.

2.4 METABOLIC PROFILE DURING PREGNANCY AND LACTATION

Tainturier *et al.* (1984) studied the effects of pregnancy and of the onset of lactation on blood composition in 21 Friesian cows and found that blood glucose, cholesterol and serum iron decreased at the end of pregnancy while creatinine increased throughout the last six months and serum urea increased in the first month after calving.

Amer *et al.* (1999) studied the biochemical changes in serum and milk constituents during post-partum period in 10 Saudi Ardy goats and found that serum levels of each of total protein, calcium and amino acid nitrogen were stable throughout the whole post-partum period or showed little variation. Serum glucose levels were low at the beginning of lactation but increased on 14^{th} d. Serum total cholesterol and phosphorus had their peak values on 21^{st} d and their lowest value on 7^{th} d.

Azab and Abdel-Maksoud (1999) studied changes in some hematological and biochemical parameters during pre-partum and post-partum periods in female Baladi goats. They found that PCV significantly decreased during the last four weeks of pregnancy as well as during the post-partum period. The decrease in haemoglobin (Hb) concentration was not significant during the last four weeks of pregnancy and became significant during the post-partum period. Plasma Na decreased at 4, 3 and 2 weeks before parturition and this decrease became significant at 1 week before parturition and on the day of parturition. Plasma Na increased at 3 and 4 weeks post-partum. Meanwhile, plasma K decreased during late pregnancy and this decrease was significant on the day of parturition and 1 week post-partum.

El-Sherif and Assad (2001) examined the changes in blood constituents during pregnancy and lactation periods on Barki ewes. From 10^{th} week to parturition, PCV per cent and blood hemoglobin increased and blood glucose

increased from the 4th week of pregnancy to reach its maximum at parturition. Plasma protein with its two components, albumin and globulin, increased significantly at the 6th week, but dropped throughout the 16-18th week of pregnancy. Urea and creatinine began to increase significantly after 10-12 weeks of pregnancy. During the first month of lactation, PCV and blood Hb decreased sharply in lactating ewes and the levels of glucose, urea and creatinine returned to levels comparable to those in dry ewes. The same occurred with total plasma proteins, mainly due to a sharp decrease in globulin, while albumin remained higher than in dry ewes.

Firat and Ozpinar (2002) studied the metabolic profile in 20 multiple lambing Sakiz ewes during pre-pregnancy, pregnancy and early lactation. They reported that plasma cortisol level slightly increased at the 100th d of pregnancy, decreased during late pregnancy and continued to decrease during lactation while plasma glucose and 3-hydroxybutyrate levels were not significantly different during pre-pregnancy, pregnancy and the early lactation periods.

Krajnicakova *et al.* (2003) studied selected biochemical parameters in the puerperal period of goats. A significant decrease in the levels of Na, calcium and total lipids was recorded during this period. An increase in total protein was observed on 40th d post-partum.

Ozpinar and Firat (2003) studied the metabolic profile in 20 multiple lambing Sakiz ewes during pre-pregnancy, pregnancy and early lactation. They reported that blood plasma progesterone levels were significantly increased during late pregnancy and lactation. Plasma estradiol-17β and cholesterol levels were not significantly different in pre-pregnancy, pregnancy or early lactation periods. During pre-pregnancy, a positive correlation was found between plasma cholesterol and progesterone levels.

Ribeiro *et al.* (2004) studied the metabolic profile of 64 Border Leicester x Texel ewes during pregnancy and lactation. The values of total protein, globulin, albumin and urea had a reduction in the advancement of pregnancy and beginning of lactation. No changes were found on cholesterol and urea plasma levels.

Sandabe *et al.* (2004) studied the effect of pregnancy on some biochemical parameters in Sahel goats in semi-arid zones and found that in pregnant does, serum glucose concentration was significantly lower and the serum cholesterol concentration was higher, than non-pregnant does.

Turk *et al.* (2004) studied the serum paraoxonase activity and lipid parameters in the early post-partum period of dairy cows and found a decreased serum paraoxonase activity. The serum triglyceride and cholesterol concentrations in post-partum cows were markedly reduced compared to the cows in late non-pregnant lactation and have been considered that, it is due to an imbalance of the liver's ability to fatty acid uptake and its capacity to secrete lipoproteins synthesized from triglycerides mobilized from adipose tissue.

Balikci *et al.* (2007) investigated the serum glucose, cholesterol, total protein, albumin, globulin and urea concentrations in 30 healthy Akkaraman ewes at 60^{th}, 100^{th} and 150^{th} d of pregnancy and 45^{th} d post-partum. Lower serum glucose levels were recorded on 100^{th} and 150^{th} d of gestation, compared to 45^{th} d post-partum and 60^{th} d of pregnancy. Lower serum total protein and globulin levels were also recorded on 150^{th} d of pregnancy than in the other stages of pregnancy. A gradual increase in serum cholesterol occurred during pregnancy, compared to 45^{th} d post-partum. Serum urea levels increased on 60^{th} and 100^{th} d of pregnancy, compared to 150^{th} d of pregnancy and 45^{th} d post-partum.

Iriadam (2007) investigated the changes which occur in certain hematological and biochemical plasma parameters during the peri-partum period of female Killis goats and reported a significant decrease in plasma total protein

during late pregnancy and lactation and an increase in the total cholesterol content from 12th to 18th week of pregnancy, at parturition and 3 weeks post-partum.

Karapehlivan *et al.* (2007) studied the blood biochemical parameters during the lactation and dry period in 10 Tuj ewes. The serum urea concentrations on 30th d of lactation were higher than those on the 1st d of lactation and 3 weeks after drying off. The serum urea concentration on the 1st d of lactation was higher than those 3 weeks after drying off while the blood total protein levels showed a reverse trend. A decrease in serum albumin was recorded 3 weeks after drying off and on 30th d of lactation compared to those on the onset of lactation. Serum glucose concentrations on 1st and 30th d of lactation were higher than those 3 weeks after drying off.

Ramin *et al.* (2007) studied the distribution of subclinical pregnancy toxemia by the evaluation of serum glucose, BHBA, urea, and cortisol concentrations in pregnant ewes and reported that mean urea and BHBA concentrations in pregnant ewes were greater and glucose was lower than in lambed and aborted ewes. Cortisol concentrations in pregnant ewes were higher than lambed but lower than aborted ewes.

Celi *et al.* (2008) monitored the metabolic profile and oxidative status in 10 Red Syrian goats during the peripartum period and found that albumin levels were significantly lower on 3rd d before delivery and 1st and 28th d from delivery as compared with 21st d before delivery. Plasma urea levels significantly decreased starting from 3rd d before delivery. No effect of time from delivery was noted on non-esterified fatty acids, triglycerides, cholesterol and total protein concentrations.

Moghaddam and Hassanpour (2008) compared the serum concentrations of glucose, beta hydroxybutyric acid (BHBA), blood urea nitrogen (BUN) and calcium in the pregnant and lambed ewes. The mean level of BHBA and calcium

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concentration in serum in pre-partum period was higher than post-partum period significantly, while the mean level of glucose in pre-partum period was significantly lower than post-partum period. The level of BUN in pre-partum period was greater than post-partum period but that was not significant.

Abdelatif *et al.* (2009) evaluated the long term responses in blood parameters during normal reproduction cycle, pregnancy and lactation of desert ewes receiving supplement feeding. They concluded that pregnancy and lactation affect blood constituents of grazing sheep and the responses were modified by concentrate supplementation. The Hb concentration, serum urea and plasma glucose increased significantly with the advance of pregnancy while serum levels of total protein and albumin decreased during early and mid pregnancy. The PCV decreased with the advance of pregnancy, increased at parturition and decreased thereafter with the progress of lactation. Total protein, albumin, urea and plasma glucose increased at parturition and urea and plasma glucose increased gradually with the advance of lactation.

Gurgoze *et al.* (2009) investigated the possible effects of the reproductive status on the serum chemistry and minerals in Awassi ewes. They reported that serum urea, total bilirubin, direct bilirubin, total protein, albumin, alanine amino transferase and creatinine kinase concentrations increased significantly in pregnancy and serum glucose, creatinine, calcium, alkaline phosphatase and phosphorous concentrations were higher after parturition.

Piccione *et al.* (2009) assessed changes in selected biochemical parameters during different stages of pregnancy, post-partum, lactation and dry period in ewes. They observed that blood serum total protein as well as serum albumin increased significantly during pregnancy compared to dioestrus phase and blood urea showed a significant increase during late gestation and a significant decrease during dry period. Total blood lipids showed a significant increase during

pregnancy, post-partum and early lactation compared to dioestrus, while total cholesterol and triglycerides showed the opposite trend.

Materials and methods

3. MATERIALS AND METHODS

The experiment was conducted in the Department of Veterinary Biochemistry, College of Veterinary and Animal Sciences, Mannuthy to evaluate and compare the antioxidant status and metabolic profile of pregnant, lactating and non pregnant dry goats and to evolve any possible relationship between antioxidant markers and other relevant blood parameters.

3.1 EXPERIMENTAL ANIMALS

The study was carried out in 33 adult healthy Malabari and crossbred does, aged between 3 to 6 years maintained at the University Goat and Sheep Farm, College of Veterinary and Animal Sciences, Mannuthy. All the animals were apparently healthy, free of external parasites, dewormed regularly and vaccinated routinely against infectious diseases such as, foot and mouth disease (FMD) haemmorhagic septicemia (HS) and enterotoxemia. Animals were fed with concentrates in pellet form according to their physiological state and free range grazing for 5 hours a day.

Investigations were done at three stages,

i. During the period of pregnancy (between March and July).
ii. Lactation period (between June and November).
iii. A period in which animal is neither lactating nor pregnant (dry period).

The day of mating was considered as day one of pregnancy and the day of kidding was taken as day one of lactation. Two groups of dry animals were studied as control each consisting of 10 animals. First group was studied in March to June period and other in between July and November, to nullify the seasonal effect. All the 13 pregnant goats selected for study had normal kidding and no clinical abnormalities were seen during the post-partum period.

3.1.1 Collection of Blood Samples

Blood samples were collected from the animals with and without anticoagulants during the periods shown below.

1. Early gestation (between 40-60 d of pregnancy) period.
2. Mid gestation (between 90-110 d of pregnancy) period.
3. Advanced gestation (between 130-150 d of pregnancy) period.
4. Lactation period (7th, 45th and 70th d post-partum).
5. Dry period I (during the months between March and June).
6. Dry period II (during the months between July and November).

Blood was drawn from animals between 8.30 and 9.30 a.m. prior to morning feeding, by jugular venipuncture using sterile needle. Approximately, 8 ml of blood was collected into dry glass tube without anticoagulant and 2 ml blood was transferred into a heparinised vial. The tubes for serum collection were allowed to clot at room temperature for 2 h and the clear sera were harvested by centrifugation at 3000 r.p.m. for 15 min.

3.2. ASSESSMENT OF OXIDATIVE STRESS

3.2.1. Estimation of Lipid Peroxides in Serum

Level of lipid peroxides in serum was determined by the method of Yagi, (1984) by estimating MDA level.

Principle

Thiobarbituric acid (TBA) reacts with lipid peroxides and MDA to form a red coloured pigment that can be determined by colorimetry. 1,1,3,3 Tetramethoxypropane (TMP) was used as a standard since it can be converted to malondialdehyde quantitatively by reacting with TBA.

Reagents

TBA, phosphotungstic acid and TMP were purchased from Himedia Laboratories Pvt. Ltd, Mumbai. All the other chemicals required were purchased from Merck India Limited, Mumbai.

N/12 H_2SO_4

10% Phosphotungstic acid solution

TBA reagent: 0.67% aqueous solution of TBA was prepared and mixed with an equal volume of glacial acetic acid.

n-Butanol

Procedure

1. 4ml of *N*/12 H_2SO_4 was added to 200µl of serum, taken in a centrifuge tube and the mixture was shaken gently. To this 0.5 ml of 10% phosphotungstic acid was added and mixed. After standing at room temperature for 5 min. the mixture was centrifuged at 3000 r.p.m. for 10 min.
2. The supernatant was discarded, and the sediment was mixed with 2 ml of *N*/12 H_2SO_4 and 0.3 ml of 10% phosphotungstic acid. The mixture was centrifuged at 3000 r.p.m. for 10 min. and discarded the supernatant.
3. The sediment was suspended in 4 ml of distilled water and added 1 ml of TBA reagent. The reaction mixture was kept at 95°C in a water bath for 60 min.
4. After cooling with tap water, 5 ml of n-butanol was added and the mixture was shaken vigorously. Then centrifuged at 3000 r.p.m. for 15 min. and the absorbance of the n- butanol layer was measured at 532 nm.
5. A standard solution was prepared by taking 0.6 n*M* of TMP and followed steps from 3 – 4.

Calculation

The value was expressed in terms of MDA.

measured.

Reagents

$$\text{Level of MDA } (n\mathit{M}\text{/ml of serum}) = \frac{a}{A} \times \frac{0.6}{0.2}$$

Absorbance of Standard - A

Absorbance of sample - a

Concentration of standard solution - 0.6 n*M*

Volume of sample - 0.2 ml

3.2.2 Estimation of Blood Glutathione Peroxidase

Glutathione peroxidase was determined photometrically in semiautomatic blood analyzer (Microlab 200) using Ransel kit, (RANDOX Laboratories Ltd, U.K).

Principle

Glutathione peroxidase catalyses the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH the oxidized glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm is measured.

Reagents

R1a.	Reagent	
	Glutathione	4 m*M*/l
	Glutathine Reductase	≥ 0.5 U/l
	NADPH	0.34 m*M*/l
R1b.	Buffer	
	Phosphate Buffer	0.05*M*/l; pH 7.2
	EDTA	4.3 m*M*/l
R2.	Cumene Hydroperoxide	0.18 m*M*/l
R3.	Diluting Agent	

Assay Procedure

Wavelength	340 nm
Light path	1 cm
Temperature	37°C
Measurement	Against air

Procedure

Preparation of Reagents

R1 : Reconstituted one vial of Reagent R1a with 6.5 ml of buffer R1b.

R2 : Diluted 10 µl R2 with 10 ml of saline and mixed thoroughly by shaking vigorously.

R3 : Reconstituted the contents of one vial of Diluting agent R3 with 200 ml of redistilled water.

Preparation of Sample

Diluted 0.05 ml of heparinised whole blood with 3 ml of diluting agent.

	Blank	Sample
Diluted Sample	-	0.02 ml
Distilled water	0.02 ml	-
Reagent R1	1.00 ml	1.00 ml
Cumene R2	0.04 ml	0.04 ml

Mixed, read initial absorbance of sample and reagent blank after one minute. Read again after 1 and 2 min. Subtracted reagent blank value from that of the sample.

Calculation

U/l of Haemolysate = 8412 × ΔA 340 nm / min.

3.2.3 Estimation of Reduced Glutathione

Reduced glutathione in blood was measured by its reaction with 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB) to give a yellow coloured complex with absorption maximum at 412 nm (Moron *et al.*, 1979).

Reagents

Phosphate buffer	pH 8.0	0.2 *M*
TCA		25 %
TCA		5 %
DTNB		0.6 m*M*
GSH standard		10 mg/dl

Procedure

Preparation of Protein Free Filtrate from Blood

125 µl of 25 % TCA was added to 200 µl of blood samples taken in ependoff tubes, mixed well and cooled on ice bath for 5 min. The mixture was further diluted with 575 µl of 5 % TCA and centrifuged for 5 min. at 5000 r.p.m. The supernatant was taken for further procedure.

Supernatant		300 µl
Phosphate buffer	0.2 *M*	700 µl
Freshly prepared DTNB		2 ml

Mixed well and read the yellow colour formed at 412 nm.

Preparation of Standard Curve of GSH

Dissolved 10 mg standard reduced glutathione in 100 ml 5 % TCA and took the following different GSH concentration for the calibration curve.

Tube No	GSH standard (µl)	Phosphate buffer 0.2 *M* (µl)	Corresponding GSH concentration (µg)
S_1	10	990	1
S_2	20	980	2
S_3	30	970	3
S_4	40	960	4
S_5	50	950	5
S_6	60	940	6
S_7	70	930	7

2 ml of freshly prepared DTNB was added in all the above tubes. Mixed well and read the absorbance at 412 nm.

A graph was plotted between optical density and concentration of the standards. Knowing the optical density of the unknown samples, the corresponding concentration of GSH was read directly from the calibration curve and expressed as mg/dl.

3.2.4 Estimation of Oxidative Stress Factor

OSF was calculated by the following formula. (Bisla *et al*, 2003)

$$OSF = \frac{\text{MDA (n}M\text{/ml of blood)} \times \text{GSH (mg/dl of blood)}}{\text{PCV (per cent)}}$$

3.2.5 Estimation of Serum Ascorbic Acid.

Ascorbic acid was estimated by spectrophotometric method (Sonnenwirth and Jarett, 1980) and expressed in mg/dl.

Principle

Ascorbic acid is oxidized to dehydroascorbic acid which in acid solution reacts with 2, 4- dinitrophenyl hydrazine, forming the corresponding hydrazone. When treated with sulfuric acid the hydrazone develops an orange red color, which is measured spectrophotometrically.

Reagents

TCA	10 g/dl
H_2SO_4	4.5 *M*
H_2SO_4	12 *M*
2, 4- Dinitrophenyl hydrazine	2 g/dl in 4.5 *M* H_2SO_4
Thiourea	5 g/dl
Copper sulfate	0.6 g/dl

Dinitrophenyl hydrazine-thiourea-copper sulfate (DTC):

100 ml Dinitrophenyl hydrazine + 5 ml thiourea + 5 ml copper sulfate

Procedure

1. 0.5 ml serum was added to 2.0 ml TCA and mixed on a vortex mixer for 5 seconds.
2. After standing at room temperature for 3 - 4 min. the mixture was centrifuged at 3000 r.p.m. for 10 min.
3. 1.2 ml supernatant was pipetted out and mixed with 0.4 ml DTC reagent.
4. The reaction mixture was incubated at 37^0C for 3 hr and transferred to ice-water bath for 10 min.
5. 2.0 ml cold 12*M* H_2SO_4 was then added slowly with gentle mixing.

6. Allowed to stand at room temperature for 20 min and absorbance was measured at 520 nm.

Preparation of Standard Curve of Ascorbic acid

1. Stock standard of ascorbic acid (50 mg/dl) was prepared by adding 50 mg ascorbic acid to 50 ml TCA in a 100ml volumetric flask, mixed and then brought to volume with TCA.
2. Intermediary standard (5 mg/dl) was prepared by adding 10 ml stock standard to 50ml TCA in a 100ml volumetric flask, mixed and then brought to volume with TCA.
3. Following working standards of different ascorbic acid concentration was taken for the calibration curve.

Tube No	Intermediary standard (ml)	TCA (ml)	Concentration (mg/dl)
S_1	0.25	24.75	0.05
S_2	0.5	24.5	0.1
S_3	1	24	0.2
S_4	2	23	0.4
S_5	3	22	0.6
S_6	4	21	0.8

1.2 ml aliquots of each working standards was taken and test procedure repeated from step 3.

A graph was plotted between optical density and concentration of the standards. Knowing the optical density of the unknown samples, the corresponding concentration of sample was read directly from the calibration curve. The result obtained was multiplied by 5 to correct the serum dilution by TCA and expressed in mg/dl.

3.3 EXAMINATION OF BLOOD FOR HAEMATOLOGICAL PARAMETERS

3.3.1 Haemoglobin Estimation

Haemoglobin was estimated photometrically by cyanhaemoglobin method using Hemoglobin kit, Beacon Diagnostics Pvt. Ltd.

Principle

In alkaline medium Hb and its derivatives are oxidised in presence of potassium ferricyanide and converted to methaemoglobin which reacts then with potassium cyanide to form purple red coloured cyanhaemoglobin complex, the intensity of which is measured in a spectrophotometer (Spectronic 1001) at 546 nm.

Reagents

Drabkin's solution

Sodium bicarbonate	1 g
Potassium cyanide	50 mg
Potassium ferricyanide	200 mg
Distilled water	1000 ml

Procedure

	Blank	Test
Working Drabkin's solution	5 ml	5 ml
Blood	-	20 µl

Mixed well and allowed to stand at room temperature for 5 min. Absorbance of test was measured against blank at 546 nm. Took the absorbance

of cyanhaemoglobin standard directly (without adding working reagent), against blank at 546 nm.

Calculation

$$\text{Blood Hb in g/dl} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 15.06 \text{ (standard concentration)}$$

3.3.2 Packed Cell Volume

Packed cell volume was estimated by Wintrobe's method (Benjamin, 1985) and expressed as percentage.

3.4 ESTIMATION OF SERUM BIOCHEMICAL PARAMETERS

3.4.1 Glucose

Glucose concentration was determined photometrically, immediately after blood collection by GOD/POD method using GLUCOSE LS kit (Euro Diagnostic Systems Pvt. Ltd).

Principle

Glucose after enzymatic oxidation by glucose oxidase releases gluconic acid and H_2O_2.The colorimetric indicator, quinoneimine is generated from 4-aminoantipyrene and phenol by H_2O_2 under the catalytic action of peroxidase (Trinder's reaction).

Reagents

Enzyme Chromogen
Glucose Standard 100 mg/dl

Assay Procedure

Wavelength	500 nm
Light path	1 cm
Temperature	37°C
Measurement	Against reagent blank

Procedure

	Blank	Sample	Standard
Sample/ Standard	-	10 µl	10µl
Distilled water	10 µl	-	-
Reagent solution	1000 µl	1000 µl	1000 µl

Mixed, incubated for 10 min. at 37°C. Read the absorbance against the reagent blank within 30 min.

Calculation

$$\text{Glucose [mg/dl]} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

3.4.2 Cholesterol

Cholesterol was determined photometrically by CHOD-PAP method in semiautomatic blood analyzer (Microlab 200) using Ecoline Cholesterol kit, (Merck Specialities Pvt Ltd).

Principle

Cholesterol and its esters are released from lipoproteins by detergents. Cholesterol esterase hydrolyses the esters. In the subsequent oxidation by

cholesterol oxidase, H_2O_2 is liberated. The colorimetric indicator, quinoneimine is generated from 4-aminoantipyrene and phenol by H_2O_2 under the catalytic action of peroxidase (Trinder's reaction).

Reagents

Reaction solution:	PIPE's buffer	pH 7.5	99 mM/l
	Salicylic alcohol		3.96 mM/l
	4-aminoantipyrine		0.5 mM/l
	Cholesterol esterase		≥ 100 IU/l
	Cholesterol oxidase		≥ 100 IU/l
	Peroxidase		≥ 1000 IU/l
Cholesterol standard			200 mg/dl

Assay Procedure

Wavelength	500 nm
Light path	1 cm
Temperature	37°C
Measurement	Against reagent blank

Procedure

	Blank	Sample	Standard
Sample/ Standard	-	10 µl	10 µl
Distilled water	10 µl	-	-
Reaction solution	1000 µl	1000 µl	1000 µl

Mixed, incubated for 5 min. at 37°C. Read the absorbance against the reagent blank within 60 min.

Calculation

$$\text{Cholesterol [mg/dl]} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{Concentration of standard}}{\text{Volume of sample}} \times 100$$

3.4.3 Total Protein

Total protein was determined photometrically by Biuret method in semiautomatic blood analyzer (Microlab 200) using Ecoline Total Protein kit, (Merck Specialities Pvt Ltd).

Principle

Proteins which contain peptide linkage form a violet coloured complex with copper in alkaline medium. The intensity of the colour is directly proportional to the number of peptide linkages present which is a measure of the concentration of protein.

Reagents

Reagent 1: Sodium hydroxide	100 m*M*/l
Potassium sodium tartarate	16 m*M*/l
Reagent 2 : Sodium hydroxide	100 m*M*/l
Potassium sodium tartarate	16 m*M*/l
Potassium iodide	15 m*M*/l
Copper sulphate	6 m*M*/l
Reagent 3: Standard protein solution	5 g/dl

Assay Procedure

Wavelength	540 nm
Light path	1 cm

Temperature	37°C
Measurement	Against reagent blank

Procedure

Preparation of Reaction Solution

Mixed reagent 1 and 2 in the ratio 4:1 and proceeded as follows

	Blank	Sample	Standard
Sample/ Standard	-	20 µl	20 µl
Distilled water	20 µl	-	-
Reaction solution	1000 µl	1000 µl	1000 µl

Mixed, incubated for 5 min. at 37°C and read the absorbance against the reagent blank within 60 min.

Calculation

$$\text{Total protein [g/dl]} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{Concentration of standard}}{\text{Volume of sample}} \times 100$$

3.4.4 Albumin

Albumin was determined photometrically based on bromocresol green method in semiautomatic blood analyzer (Microlab 200) using Ecoline Albumin kit, (Merck Specialities Pvt. Ltd.).

Principle

Serum albumin forms a yellow-green to green-blue complex at a slightly acidic pH, which is measured photometrically.

Reagents

Reaction solution: Citrate buffer	pH 4.2	30 m*M*/l
Bromocresol green		0.26 m*M*/l
Albumin standard		5 g/dl

Assay Procedure

Wavelength	546 nm
Light path	1 cm
Temperature	37°C
Measurement	Against reagent blank

Procedure

	Blank	Sample	Standard
Sample/ Standard	-	10µl	10 µl
Distilled water	10 µl	-	-
Reaction solution	1000 µl	1000 µl	1000 µl

Mixed, incubated for 10 min. at 37°C and read the absorbance against the reagent blank within 60 min.

Calculation

$$\text{Albumin [g/dl]} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{Concentration of standard}}{\text{Volume of sample}} \times 100$$

3.4.5 Creatinine

Creatinine was determined photometrically based on Jaffe kinetic method without deproteinisation in semiautomatic blood analyzer (Microlab 200) using Merckotest Creatinine kit, (Merck Specialities Pvt Ltd).

Principle

Creatinine in alkaline medium reacts with picric acid and forms an orange red colour of creatinine picrate. The intensity of the colour is a measure of the amount of creatinine present. At a low concentration of picric acid used in this method, precipitation of proteins does not take place.

Reagents

Reagent 1: Sodium hydroxide	313 m*M*/l
Phosphate	12.5 m*M*/l
Reagent 2: Picric acid	8.73 m*M*/l
Reagent 3: Creatinine standard	1.0 mg/dl

Assay Procedure

Wavelength	492 nm
Light path	1 cm
Temperature	37°C

Procedure

Preparation of Reaction Solution

Mixed reagent 1 and 2 in the ratio 1:1.

Left the monoreagent for at least 10 min. at room temperature before using.

	Sample	Standard
Sample/Standard	100 µl	100 µl
Reaction solution	1000 µl	1000 µl

Mixed and read absorbance A1 and after 60 s read absorbance A2 after further 120 s.

Calculation

$$\text{Creatinine[mg/dl]} = \frac{\Delta A \text{ of sample}}{\Delta A \text{ of standard}} \times \frac{\text{Concentration of standard}}{\text{Volume of sample}} \times 100$$

ΔA = Mean absorbance change of A_1 and A_2

3.4.6 Estimation of Blood Urea

Blood urea was determined spectrophotometrically based on Diacetyl monoxime method as described by Chawla (1995).

Principle

Urea reacts with diacetyl monoxime to form yellow colored complex in strong acidic medium. The reaction is intensified by the presence of ferric ions and thiosemicarbazide and the red color so produced is read at 520 nm.

Reagents

Reagent A: 5 g of ferric chloride was dissolved in 20 ml distilled water. Transferred this to a graduated cylinder and 100ml of orthophosphoric acid were added slowly with stirring. Made up the volume to 250ml with distilled water and kept in brown bottle at 4^0C.

Reagent B: 200 ml of conc. H_2SO_4 was added to 800 ml distilled water in a 2 litre flask slowly with stirring, keeping it in ice bath.

Reagent C: Diacetyl monoxime 20 g/l of distilled water. Filtered and kept in a brown bottle at 4^0C

Reagent D: Thiosemicarbazide 5 g/l of distilled water.

Acid Reagent: 0.5 ml of reagent A was added to 1 litre of reagent B and kept in a brown bottle at 4^0C.

Color Reagent: 67 ml of each reagent C and reagent D was mixed and made it upto 1000 ml with distilled water and kept in brown bottle at 4^0C.

Stock standard urea: 100 mg / 100 ml of distilled water.

Working standard urea (1 mg %): Diluted 1 ml stock to 100 ml with distilled water

Procedure

	Sample (ml)	Standard (ml)	Blank (ml)
Serum (diluted 1:100)	1	-	-
Standard	-	1	-
Distilled water	1	1	2
Color reagent	2	2	2
Acid reagent	2	2	2

Mixed thoroughly and placed the tubes in a boiling water bath for 20 min. Cooled and read the absorbance of pink colored solution at 520 nm.

Calculation

$$\text{Blood urea [mg/dl]} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{Concentration of standard}}{\text{Effective volume of serum taken}} \times 100$$

3.4.7 Estimation of Sodium and Potassium

The sodium and potassium level in serum was measured in a flame photometer (Systronics 128).

Principle

The solution containing the substance to be measured is sprayed as a fine mist into a flame. In the flame the solution evaporates and the substance is converted to atomic state. The thermal energy of the flame excites the electrons so that they are able to absorb the thermal energy and move into the higher energy orbit. When the electrons return to lower energy orbits, the energy absorbed is released as quanta of light. The light intensity is measured at a wavelength of 589 nm for sodium and 768 nm for potassium which is directly proportional to the concentration of the substance in the flame. Before the analysis of unknown fluids, the system is standardised with solutions of known concentration.

Preparation of Standard Stock Solutions of Sodium and Potassium

Sodium

Dissolved 1.169 g of pure dry sodium chloride in 100 ml of deionised glass distilled water. This gave 200 mEq/l of solution (1 mEq/l = 23 ppm).

Potassium

Dissolved 74.6 mg of pure dry potassium chloride in 100 ml of deionised glass distilled water. This gave 10 mEq/l of solution (1 mEq/l = 39ppm).

Preparation of Working Standards

Sodium Standard I

Took 0.4 ml of the standard stock solution of sodium and diluted to 100 ml with deionised glass distilled water (0.8 mEq/l)

Sodium Standard II

Took 1.5 ml of the standard stock solution of sodium and diluted to 100 ml with deionised glass distilled water (3 mEq/l).

Potassium Standard I

Took 0.2 ml of the standard stock solution of potassium and diluted to 100 ml with deionised glass distilled water (0.02 mEq/l).

Potassium Standard II

Took 1.5 ml of the standard stock solution of potassium and diluted to 100 ml with deionised glass distilled water (0.15 mEq/l).

Preparation of Samples

0.2 ml of sera samples was diluted with 19.8 ml of deionised glass distilled water.

Procedure

Calibrated the instrument with lower and higher concentrations of sodium and potassium working standards. Diluted serum sample was then sprayed into the flame and the response obtained was compared with those obtained from the standards. The value was expressed as mEq/l.

3.5 STATISTICAL ANALYSIS

The results obtained from the experiment were analysed and tested for significance using paired t-test as described by Snedecor and Cochran (1994) using computerised software program SPSS (SPSS/PC + statistic 10.0 SPSS Inc. Chicago, IL., 2000).

Results

4. RESULTS

In the present study, antioxidant status and metabolic profile of goats during pregnancy and lactation were evaluated on 13 Malabari and crossbred does. The values were compared with two control groups of dry and nonpregnant goats correspond to the period of pregnancy and lactation, each with 10 animals. No significant difference was observed in the antioxidant markers and metabolic parameters between the two control groups. The results were analysed using paired t test and presented here.

4.1 ANTIOXIDANT STATUS AND METABOLIC PROFILE OF GOATS DURING PREGNANCY

Observations were made at three different periods; early (between 40-60 d), mid (between 90-110 d) and advanced (between 130-150 d) period of gestation.

4.1.1 Antioxidant Status Indicators

The indicators of antioxidant status such as MDA, GSH-Px, GSH, ascorbic acid and OSF were measured and their mean values were shown in Table. 1 and graphically presented in Fig. 1 to 5.

4.1.1.1 Malondialdehyde

The mean values for MDA during early, mid and advanced gestation observed in 13 healthy does were 34.79 ± 0.72, 43.48 ± 0.93 and 36.99 ± 0.99 n*M*/ml respectively. The value for the corresponding control group was 35.24 ± 0.86 n*M*/ml. There was a significant increase ($P<0.01$) in MDA level during mid-gestation period when compared to control. Between different stages of gestation significant increase was noted in mid ($P<0.01$) and advanced ($P<0.05$) gestation as compared to early stage. But during advanced gestation a significant decrease ($P<0.01$) in MDA level was observed from the mid-gestation period (Fig. 1).

Table 1. Antioxidant status indicators in blood of goats during pregnancy and lactation.

Parameters	Pregnancy (days) (n=13)			Lactation (days) (n=13)			Control 1 (n=10)	Control 2 (n=10)
	40-60	90-110	130-150	7th	45th	70th		
MDA (n*M*/ml)	34.79±0.72	43.48±0.93**	36.99±0.99	39.66±0.97	37.77±0.82	39.49±0.42*	35.24±0.86	37.80±0.73
GSH-Px (U/l)	179.57±3.74**	224.11±7.55	192.89±5.06**	178.73±6.66**	201.93±4.55	202.75±3.33	210.44±1.81	211.57±6.11
GSH (mg/dl)	3.27±0.30	5.54±0.48**	3.39±0.34	3.34±0.16	2.69±0.05	2.54±0.04	3.08±0.14	2.99±0.21
Ascorbic acid(mg/dl)	3.43±0.19*	3.04±0.15	3.10±0.23	2.44±0.19	2.11±0.11	2.39±0.25	2.69±0.22	2.47±0.24
OSF	3.63±0.41	7.08±0.59**	3.53±0.29	3.84±0.32	3.42±0.25	3.70±0.25	3.30±0.18	3.26±0.24

* - P<0.05; difference significant at 5% level ** - P<0.01; difference significant at 1% level
n – number of samples
Control 1- dry non-pregnant animals for the period of pregnancy
Control 2- dry non-pregnant animals for the period of lactation

4.1.1.2 Glutathione Peroxidase

The activity of GSH-Px increased significantly ($P<0.01$) in mid-gestation from initial stage and then decreased ($P<0.01$) in the advanced stage of gestation period (Fig. 2). The mean values for GSH-Px during pregnancy were 179.57 ± 3.74, 224.11 ± 7.55 and 192.89 ± 5.06 U/l respectively in early, mid and advanced gestation and that of control group was 210.44 ± 1.81 U/l. Significant reduction ($P<0.01$) in the values of GSH-Px was observed during early and advanced gestation when compared with the control group

4.1.1.3 Reduced Glutathione

The mean values were 3.27 ± 0.30, 5.54 ± 0.48 and 3.39 ± 0.34 mg/dl respectively during early, mid and advanced gestation and for control group it was 3.08 ± 0.14 mg/dl. The level of GSH was significantly increased ($P<0.01$) during mid-gestation when compared with control group (Fig. 3). Like GSH-Px activity, GSH level was increased significantly ($P<0.01$) in the mid-gestation from early gestation and then decreased ($P<0.01$) in the advanced stage of gestation.

4.1.1.4 Ascorbic Acid

The mean ascorbic acid concentrations in serum during early, mid and advanced gestation and control group were 3.43 ± 0.19, 3.04 ± 0.15, 3.10 ± 0.23 and 2.69 ± 0.22 mg/dl respectively. A significant increase in serum ascorbic acid level ($P<0.05$) was noted only during early gestation, when compared with control group (Fig. 4).

4.1.1.5 Oxidative Stress Factor

The mean value obtained for OSF of control group was 3.30 ±0.18. During early, mid and advanced gestation period the OSF obtained were 3.63 ± 0.41, 7.08 ± 0.59 and 3.53 ± 0.29 respectively. A significant increase ($P<0.01$) was observed during mid-gestation as compared to control group as well as with other stages of gestation and lactation periods (Fig. 5).

4.1.2 Metabolic Profile

Biochemical parameters like serum glucose, cholesterol, total protein, albumin, creatinine, urea, Na and K and haematological parameters like Hb and PCV were assessed at different stages of pregnancy and the mean values were presented in Table 2 and 3 and graphically shown in Fig. 6 to 15.

4.1.2.1 Glucose

A significant decrease ($P<0.01$) in blood glucose concentration was found during mid and advanced gestation when compared with control as well as with early period (Fig. 6). The mean values for glucose during early, mid and advanced gestation were 45.59 ± 1.74, 38.14 ± 1.17, 41.35 ± 1.33 mg/dl respectively and that of control group was 48.68 ± 1.33 mg/dl. A significant increase ($P<0.01$) was also observed during advanced gestation period when compared to mid-gestation.

4.1.2.2 Cholesterol

The serum cholesterol values presented in the Table 2 showed that there was a significant reduction ($P<0.05$) during early gestation and a significant increase ($P<0.01$) during advanced gestation when taken the control 1 as reference group. Early gestation period showed a significantly ($P<0.01$) low value when compared with other stages (Fig. 7). The mean cholesterol values were 56.62 ± 1.84, 64.54 ± 2.16, 81.62 ± 2.80 and 63.50 ± 2.54 mg/dl respectively for early, mid, advanced gestation and control group.

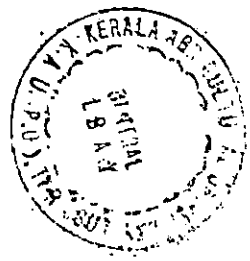
4.1.2.3 Total Protein

The mean values obtained for serum total protein during early, mid, advanced gestation period and the control group were 7.85 ± 0.22, 8.23 ± 0.17, 8.23 ± 0.23 and 7.80 ± 0.25 g/dl respectively. No significant changes were observed in total protein during pregnancy period when compared to the control as well as between different stages of pregnancy (Fig. 8).

Table 2. Blood biochemical parameters in goats during pregnancy and lactation

Parameters	Pregnancy (days) (n=13)			Lactation (days) (n=13)			Control 1 (n=10)	Control 2 (n=10)
	40-60	90-110	130-150	7th	45th	70th		
Glucose (mg/dl)	45.59±1.74	38.14±1.17**	41.35±1.33**	45.07±1.45	42.11±1.11**	43.86±1.47	48.68±1.33	46.92±1.00
Cholesterol (mg/dl)	56.62±1.84*	64.54±2.16	81.62±2.80**	70.62±3.27*	63.62±2.03	69.69±2.29*	63.50±2.54	61.70±1.91
Total protein (g/dl)	7.85±0.22	8.23±0.17	8.23±0.23	8.92±0.43	8.23±0.20	8.38±0.21	7.80±0.25	7.90±0.23
Albumin (g/dl)	3.18±0.05	3.35±0.09	3.09±0.05	3.48±0.08	3.05±0.12*	3.27±0.05	3.29±0.09	3.33±0.05
Creatinine (mg/dl)	1.34±0.03	1.40±0.03	1.91±0.09**	1.51±0.05	1.52±0.06	1.63±0.04*	1.43±0.03	1.49±0.03
Urea (mg/dl)	43.43±1.05	45.14±2.22	55.83±2.35**	50.59±1.67	53.39±2.37*	49.47±1.63	45.62±1.84	47.33±1.45
Sodium (mEq/l)	166.05±1.33	169.27±1.64	164.35±1.46*	168.26±2.10	169.80±1.56	163.11±1.56*	169.79±1.60	167.65±1.42
Potassium (mEq/l)	4.89±0.13**	4.69±0.14	4.36±0.12	4.47±0.11	4.54±0.19	5.03±0.12*	4.21±0.21	4.58±0.12

* - P<0.05; difference significant at 5% level ** - P<0.01; difference significant at 1% level
n – number of samples
Control 1- dry non-pregnant animals for the period of pregnancy
Control 2- dry non-pregnant animals for the period of lactation



4.1.2.4 Albumin

Though a significant decrease ($P<0.01$) was observed for serum albumin level in advanced gestation from the mid-gestation period, no other significant changes were observed during pregnancy period when compared with the control group (Fig. 9). The mean values for albumin during early, mid and advanced gestation were 3.18 ± 0.05, 3.35 ± 0.09, 3.09 ± 0.05 g/dl respectively and that of control group was 3.29 ± 0.09 g/dl.

4.1.2.5 Creatinine

Control group of animals had a mean creatinine value of 1.43 ± 0.03 mg/dl and the mean values of experimental group during early, mid and advanced gestation period were 1.34 ± 0.03, 1.40 ± 0.03 and 1.91 ± 0.09 mg/dl respectively. There was a significant increase ($P<0.01$) observed in creatinine level during advanced gestation when compared with the control group as well as with other stages of pregnancy (Fig. 10).

4.1.2.6 Urea

The mean values for urea during early, mid and advanced gestation were 43.43 ± 1.05, 45.14 ± 2.22 and 55.83 ± 2.35 mg/dl respectively and that of control group was 45.62 ± 1.84 mg/dl. A significant increase in blood urea level was observed (Fig. 11) during advanced gestation as compared to the control group, early gestation ($P<0.01$) and mid-gestation period ($P<0.05$).

4.1.2.7 Sodium

The mean serum Na values obtained during early, mid and advanced gestation period were 166.05 ± 1.33, 169.27 ± 1.64 and 164.35 ± 1.46 mEq/l respectively and that of control group was 169.79 ± 1.60 mEq/l. There was a significant reduction ($P<0.05$) in Na during advanced gestation when compared to the control group as well as with mid-gestation period (Fig. 12).

4.1.2.8 Potassium

The results showed a significant increase ($P<0.01$) in serum K level during early gestation as compared to the control and advanced gestation period (Fig. 13). The mean values for K during early, mid, advanced gestation period and control were 4.89 ± 0.13, 4.69 ± 0.14, 4.36 ± 0.12 and 4.21 ± 0.21 mEq/l respectively.

4.1.2.9 Haemoglobin

Haematological examinations revealed a significant increase ($P<0.05$) in Hb during early gestation as compared to the control group (Fig. 14). The mean Hb values noticed during early, mid and advanced gestation were 11.99 ± 0.42, 11.02 ± 0.29 and 10.37 ± 0.33 g/dl respectively and for the corresponding control group, it was 10.44 ± 0.33 g/dl. Among different stages of gestation, a significant decrease was observed in mid-gestation ($P<0.05$) when compared with early gestation as well as in advanced gestation when compared with both early ($P<0.01$) and mid-gestation ($P<0.05$).

4.1.2.10 Packed Cell Volume

During the study period, no significant difference was observed in PCV between the different stages of gestation and the mean values observed were 32.46 ± 1.36, 33.85 ± 0.90 and 35.00 ± 0.98 per cent respectively during early, mid and advanced gestation. Control group had a mean value of 33.20 ± 1.41 per cent. Also no significant changes were noticed during the whole gestation period as compared to the control group (Fig. 15).

4.2 ANTIOXIDANT STATUS AND METABOLIC PROFILE OF GOATS DURING LACTATION

Observations were made at three different periods of lactation; 7^{th}, 45^{th} and 70^{th} d post- partum.

Table 3. Hematological parameters in goats during pregnancy and lactation

Parameters	Pregnancy (days) (n=13)			Lactation (days) (n=13)			Control 1 (n=10)	Control 2 (n=10)
	40-60	90-110	130-150	7th	45th	70th		
Haemoglobin (g/dl)	11.99±0.42*	11.02±0.29	10.37±0.33	11.48±0.40	9.61±0.48*	9.56±0.43*	10.44±0.33	10.78±0.25
PCV (per cent)	32.46±1.36	33.85±0.90	35.00±0.98	35.69±1.52	30.92±1.66	28.54±1.87*	33.20±1.41	35.40±1.96

* - P<0.05; difference significant at 5% level ** - P<0.01; difference significant at 1% level
n – number of samples
Control 1- dry non-pregnant animals for the period of pregnancy
Control 2- dry non-pregnant animals for the period of lactation

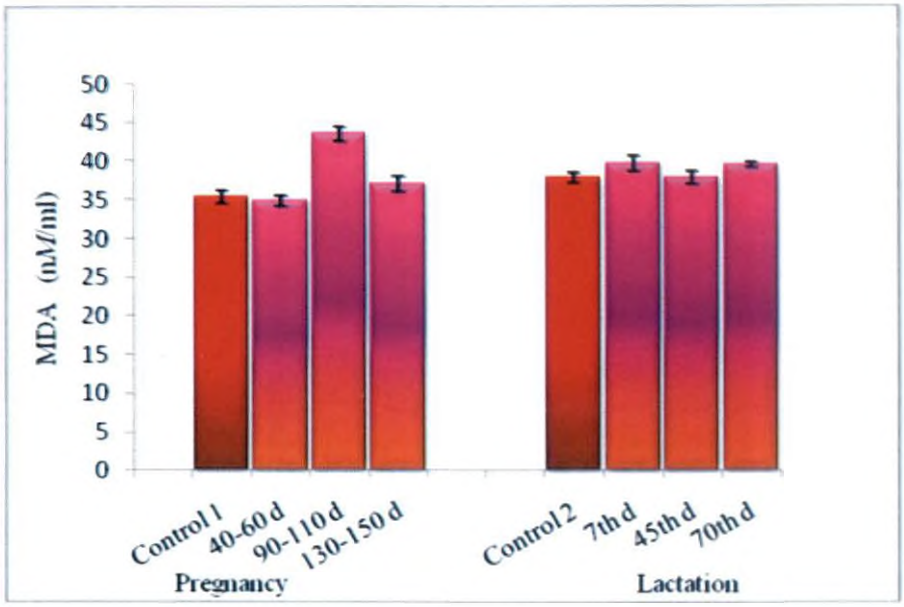


Fig. 1. Malondialdehyde (MDA) concentration in serum of goats during different periods of pregnancy and lactation

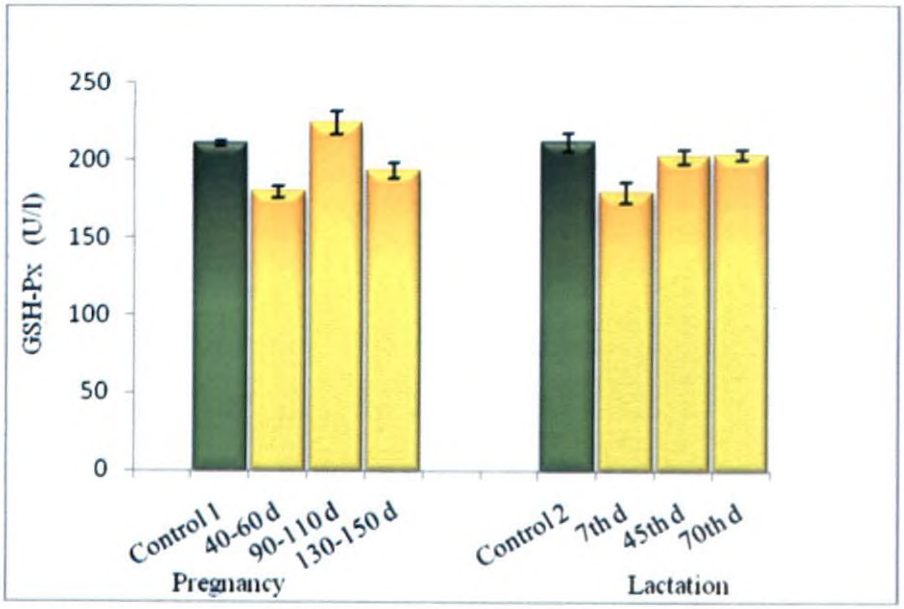


Fig. 2. Blood GSH-Px activity of goats during different periods of pregnancy and lactation

4.2.1.3 Reduced Glutathione

No significant differences were observed in GSH level during lactation period as compared to control group (Fig. 3). The mean values obtained were 3.34± 0.16, 2.69 ± 0.05 and 2.54 ± 0.04 mg per cent for 7^{th}, 45^{th} and 70^{th} d of lactation respectively and that of control group was 2.99 ±0.21 mg per cent. A significant decrease ($P<0.01$) was noted in 45^{th} and 70^{th} d when compared with 7^{th} d. GSH was significantly lower ($P<0.05$) in 70^{th} d when compared with 45^{th} d of lactation and also with early and advanced period of gestation. When compared with mid-gestation GSH was significantly reduced ($P<0.01$) during all the stages of lactation.

4.2.1.4 Ascorbic Acid

The results showed no significant differences in ascorbic acid level during lactation period as compared to its control group (Fig. 4). But there was significant decrease during lactation period when compared with different stages of gestation. Ascorbic acid was significantly reduced ($P<0.01$) in 45^{th} d of lactation when compared with all three stages of gestation. On 7^{th} and 70^{th} d significant decrease was noted, when compared to early ($P<0.01$) and mid ($P<0.05$) gestation period. The mean values during 7^{th}, 45^{th}, 70^{th} d of lactation and control group were 2.44 ± 0.19, 2.11 ± 0.11, 2.39 ± 0.25 and 2.47 ±0.24 mg/dl respectively.

4.2.1.5 Oxidative Stress Factor

The mean values for OSF during 7^{th}, 45^{th} and 70^{th} d of lactation were 3.84 ± 0.32, 3.42 ± 0.25, 3.70 ± 0.25 respectively and that of control group was 3.26 ±0.24. Though there were no significant changes in OSF during lactation period as compared to its control group, a significant reduction ($P<0.01$) was observed in all lactation stages when compared with mid-gestation period (Fig. 5).

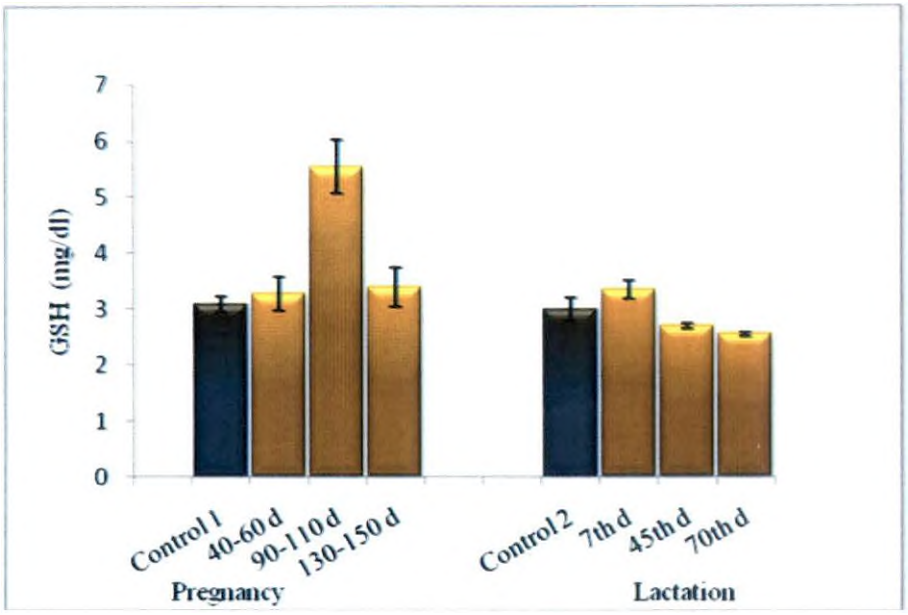


Fig. 3. Reduced glutathione (GSH) level in blood of goats during different periods of pregnancy and lactation

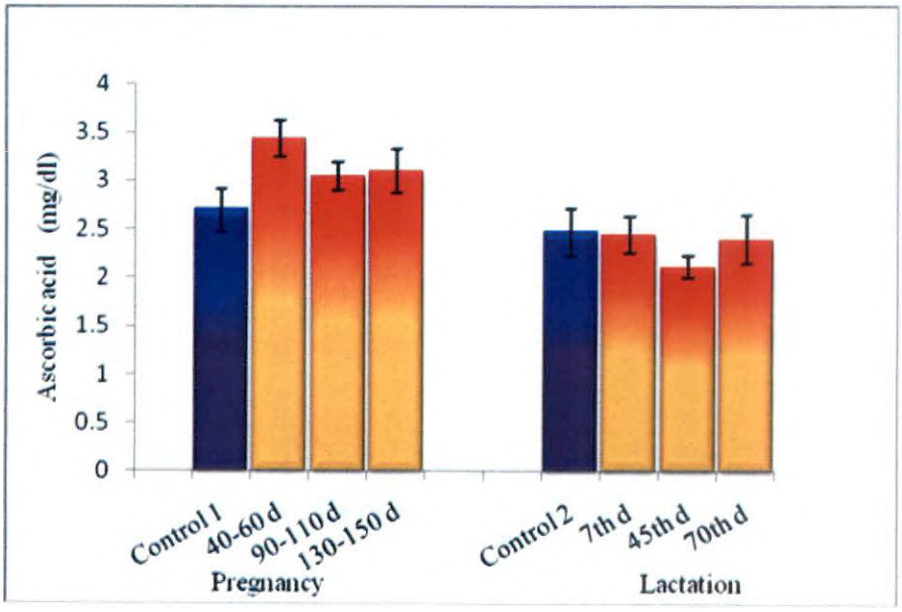


Fig. 4. Serum ascorbic acid concentration of goats during different periods of pregnancy and lactation

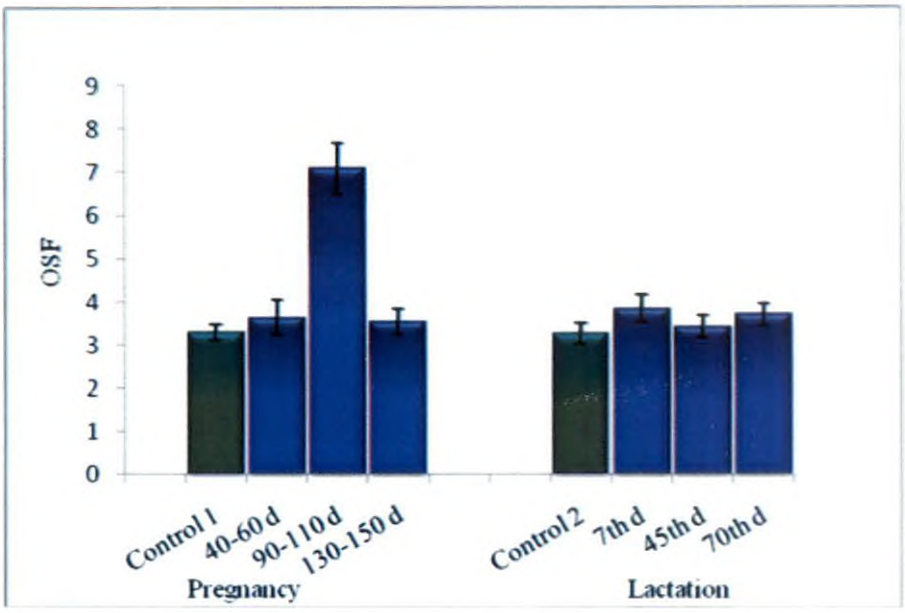


Fig. 5. Oxidative stress factor (OSF) in goats during different periods of pregnancy and lactation

4.2.2 Metabolic Profile

The mean values obtained for biochemical parameters like serum glucose, cholesterol, total protein, albumin, creatinine, urea, Na and K were listed in Table 2 and graphically presented in Fig. 6 to 13. The haematological parameters viz., Hb and PCV in Table 3 and graphical presentation in Fig. 14 and Fig. 15.

4.2.2.1 Glucose

The mean blood glucose concentration presented in Table 2 showed that there was a significant decrease ($P<0.01$) on 45^{th} d of lactation period as compared to control group (Fig. 6). A significant reduction ($P<0.05$) in glucose concentration was observed during this period when compared with 7^{th} d of lactation also. Glucose concentration was found significantly increased during lactation period (7^{th} and 70^{th} d ($P<0.01$) and 45^{th} d ($P<0.05$)) when compared with mid-gestation. The mean values were 45.07 ± 1.45, 42.11 ± 1.11, 43.86 ± 1.47 mg/dl respectively for 7^{th}, 45^{th} and 70^{th} d of lactation and that of control group was 46.92 ± 1.00 mg/dl.

4.2.2.2 Cholesterol

Serum cholesterol shows a significant increase ($P<0.05$) during 7^{th} and 70^{th} d of lactation as compared to its control group (Fig. 7). The mean values during 7^{th}, 45^{th} and 70^{th} d of lactation were 70.62 ± 3.27, 63.62 ± 2.03, 69.69 ± 2.29 mg/dl respectively and that of control group was 61.70 ± 1.91 mg/dl. After advanced gestation days cholesterol level declined significantly ($P<0.01$) during lactation period. But when compared to early gestation significant increase (7^{th} and 70^{th} day ($P<0.01$) and 45^{th} day ($P<0.05$)) was noticed during lactation period.

4.2.2.3 Total Protein

Though a significant increase ($P<0.05$) was observed in 7^{th} d as compared to early gestation period, no significant changes were observed during lactation period when compared with the control group (Fig. 8). The mean values for serum

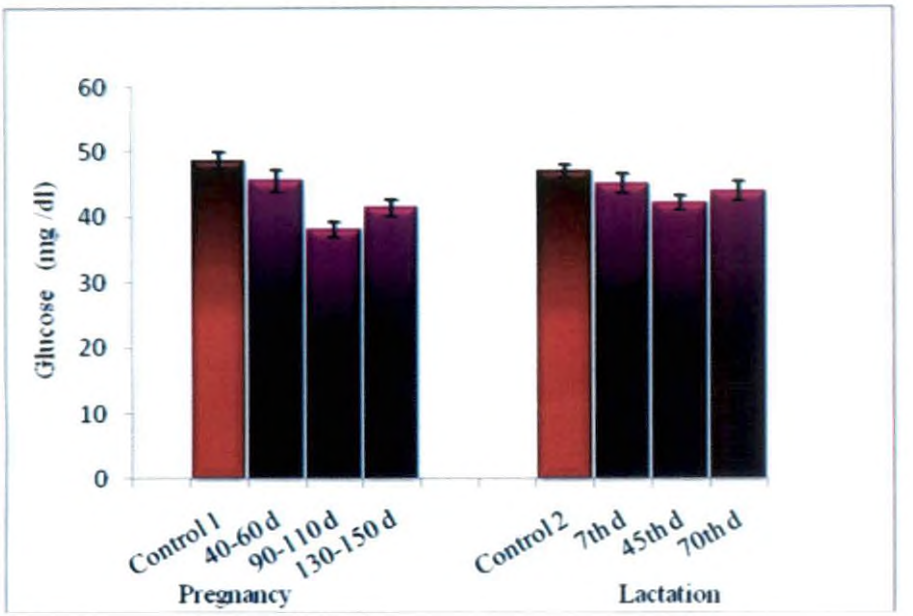


Fig. 6. Blood glucose level of goats during different periods of pregnancy and lactation

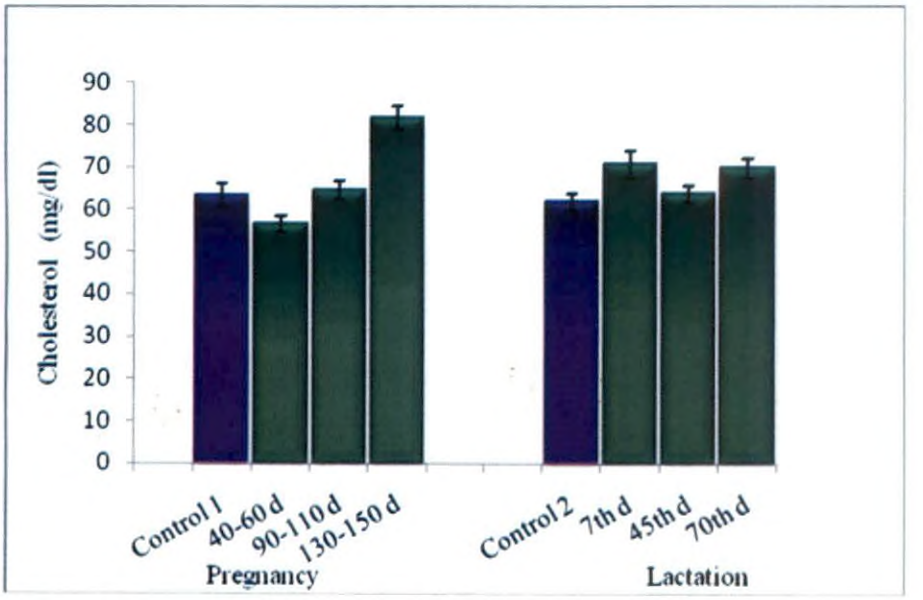


Fig. 7. Serum cholesterol concentration of goats during different periods of pregnancy and lactation

total protein during 7th, 45th and 70th d of lactation were 8.92 ± 0.43, 8.23 ± 0.20, 8.38 ± 0.21 g/dl respectively and that of control group was 7.90 ± 0.23 g/dl.

4.2.2.4 Albumin

The mean values for serum albumin during 7th, 45th and 70th d of lactation were 3.48 ± 0.08, 3.05 ± 0.12, 3.27 ± 0.05 g/dl respectively and that of control group was 3.33 ± 0.05 g/dl. There was a significant reduction ($P<0.05$) in albumin level during 45th d of lactation period as compared to the control group (Fig. 9). Significant changes like increase in albumin level during 7th d ($P<0.05$) compared to early gestation and 7th ($P<0.01$) and 70th d ($P<0.05$) compared to advanced gestation period were noticed. Also reduction in albumin level during 45th d compared to mid-gestation ($P<0.05$) and 7th d of lactation ($P<0.01$) were observed.

4.2.2.5 Creatinine

The results showed a significant increase ($P< 0.05$) in serum creatinine level during 70th d of lactation as compared to the control group (Fig. 10). The mean values during 7th, 45th and 70th d of lactation were 1.51 ± 0.05, 1.52 ± 0.06, 1.63 ± 0.04 mg/dl respectively and that of control group was 1.49 ± 0.03 mg/dl. While comparing the creatinine level during gestation and lactation period it was found that during advanced gestation period creatinine level increased significantly and thereafter it declines significantly (7th and 45th d ($P<0.01$) and 70th d ($P<0.05$)) during lactation period. But when compared to early gestation period creatinine level was found significantly increased during lactation period [7th and 45th d ($P<0.05$) and 70th d ($P<0.01$)].

4.2.2.6 Urea

Control group of animals during lactation period had a mean urea level of 47.33 ± 1.45 mg/dl and the mean values of experimental group during 7th, 45th and 70th d of lactation were 50.59 ± 1.67, 53.39 ± 2.37 and 49.47 ± 1.63 mg/dl

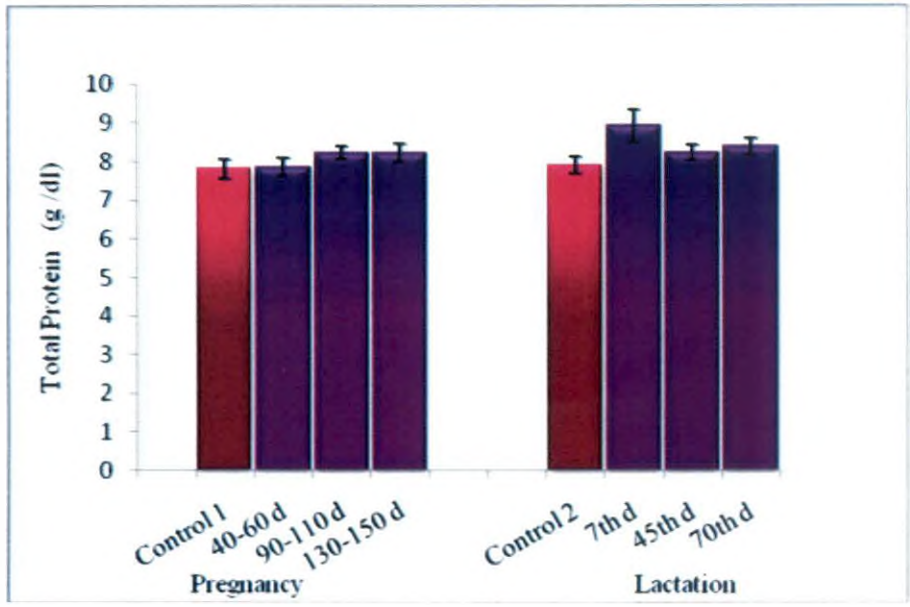


Fig. 8. Serum total protien level in goats during different periods of pregnancy and lactation

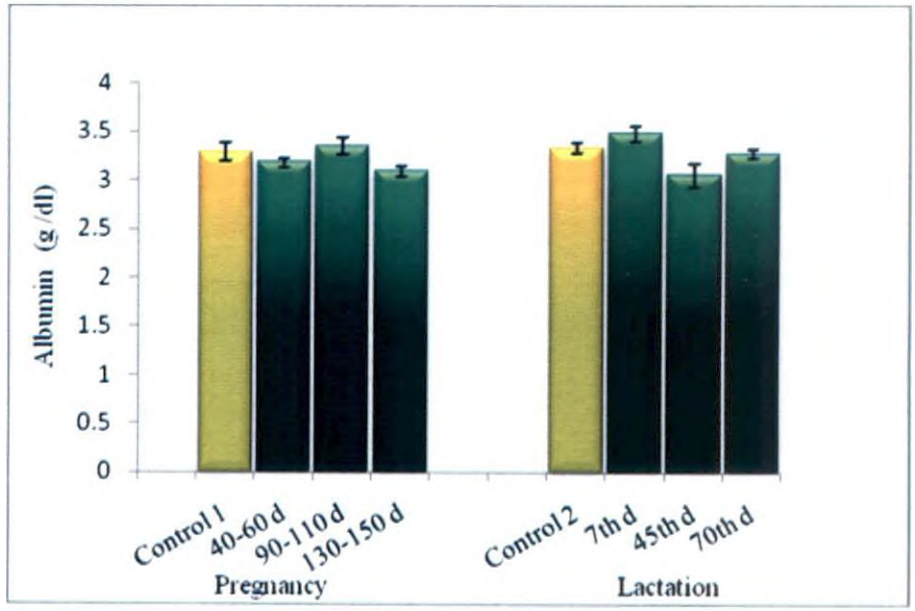


Fig. 9. Serum albumin concentration of goats during different periods of pregnancy and lactation

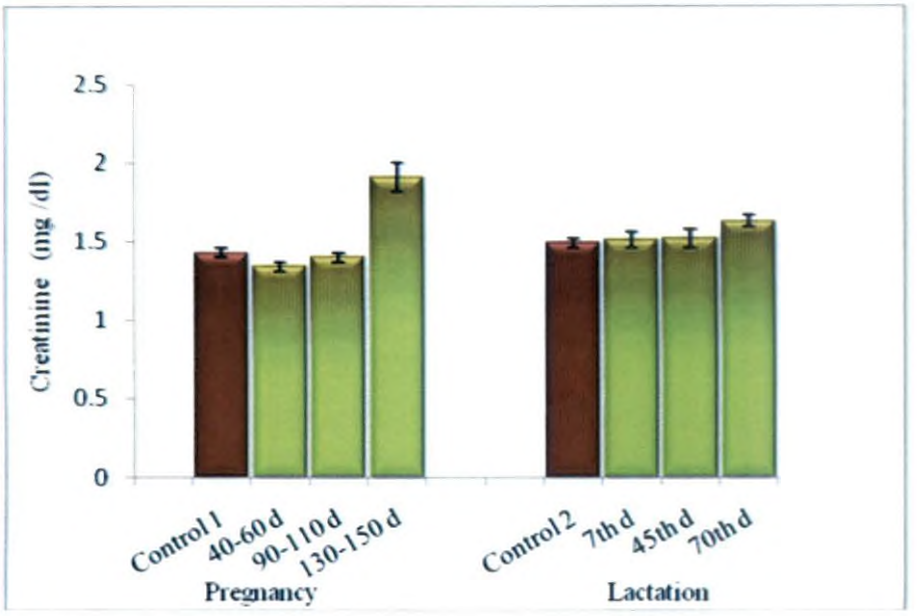


Fig. 10. Serum creatinine concentration of goats during different periods of pregnancy and lactation

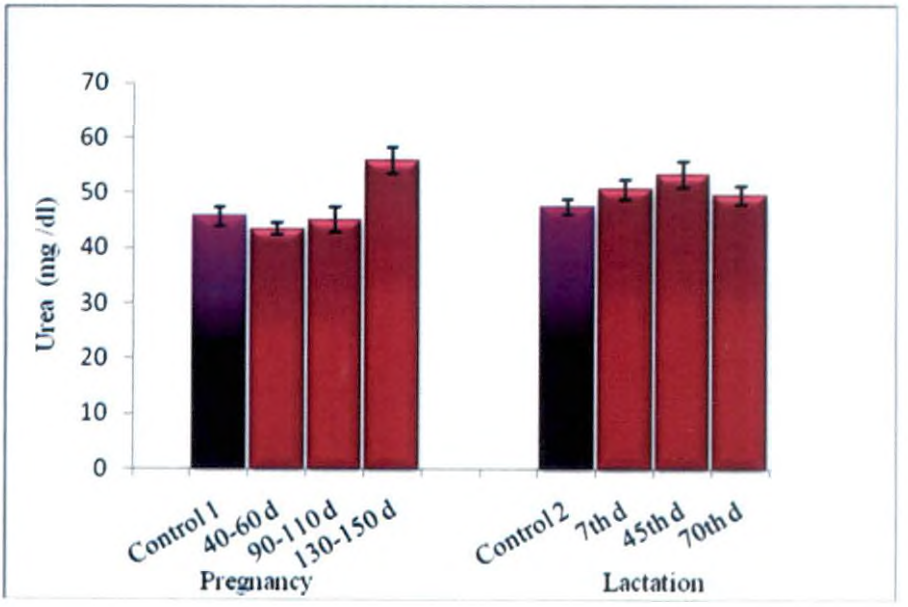


Fig. 11. Blood urea level of goats during different periods of pregnancy and lactation

respectively. There was a significant increase ($P< 0.05$) in urea during 45th d of lactation compared to the control group (Fig. 11). Similar to creatinine peak value of serum urea was found during advanced stage of pregnancy. Significant increase was noticed in 7th, 45th and 70th d ($P<0.01$) as compared to early gestation and in 7th and 45th d ($P<0.05$) as compared to mid-gestation.

4.2.2.7 Sodium

The mean values for Na during 7th, 45th and 70th d of lactation were 168.26 ± 2.10, 169.80 ± 1.56, 163.11 ± 1.56 mEq/l respectively and that of control group was 167.65 ± 1.42 mEq/l. There was a significant reduction in Na during 70th d of lactation as compared to the control group and 45th d of lactation ($P<0.05$) and mid-gestation period ($P<0.01$) (Fig. 12). Significant increase was noticed in 45th d ($P<0.05$) when compared with advanced gestation.

4.2.2.8 Potassium

During the study period, a significant increase in K was observed (Fig. 13) during 70th d of lactation as compared to the control group, 7th and 45th d ($P< 0.05$) and advanced gestation ($P<0.01$). A significant decrease ($P<0.05$) was noticed in 7th d when compared with early gestation. The mean values were 4.47 ± 0.11, 4.54 ± 0.19, 5.03 ± 0.12 and 4.58 ± 0.12 mEq/l during 7th, 45th and 70th d of lactation and control group respectively.

4.2.2.9 Haemoglobin

The Hb values presented in Table 3 showed that there was a significant reduction ($P< 0.05$) during 45th and 70th d of lactation as compared to the control group. The mean values were 11.48 ± 0.40, 9.61 ± 0.48 and 9.56 ± 0.43 g/dl respectively during 7th, 45th and 70th d of lactation and that of control group was 10.78 ± 0.25 g/dl. Among different stages of lactation, a significant decrease was observed in 45th and 70th d ($P<0.01$) as compared to 7th d of lactation (Fig. 14). Significant decrease in 45th and 70th d was noticed as compared to early gestation

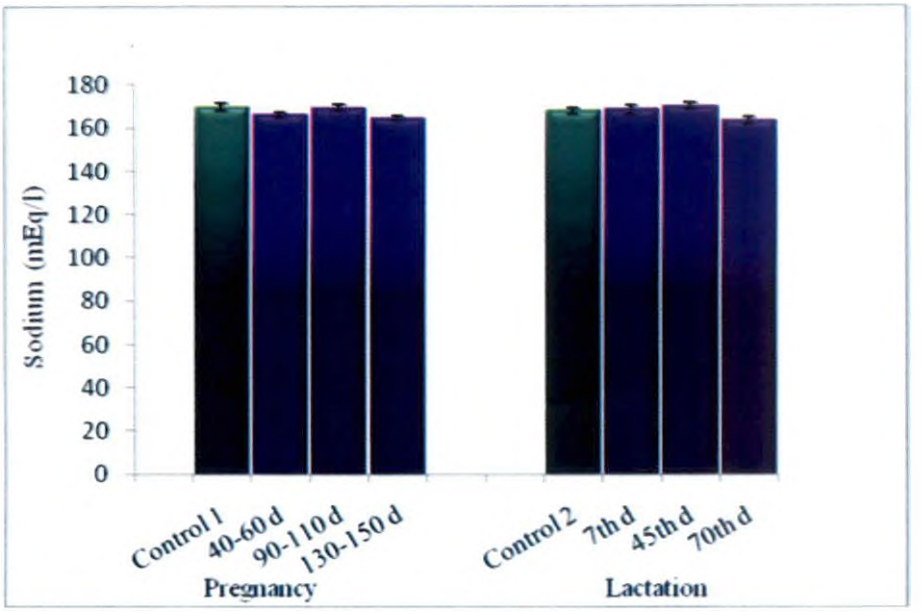


Fig. 12. Serum sodium level of goats during different periods of pregnancy and lactation

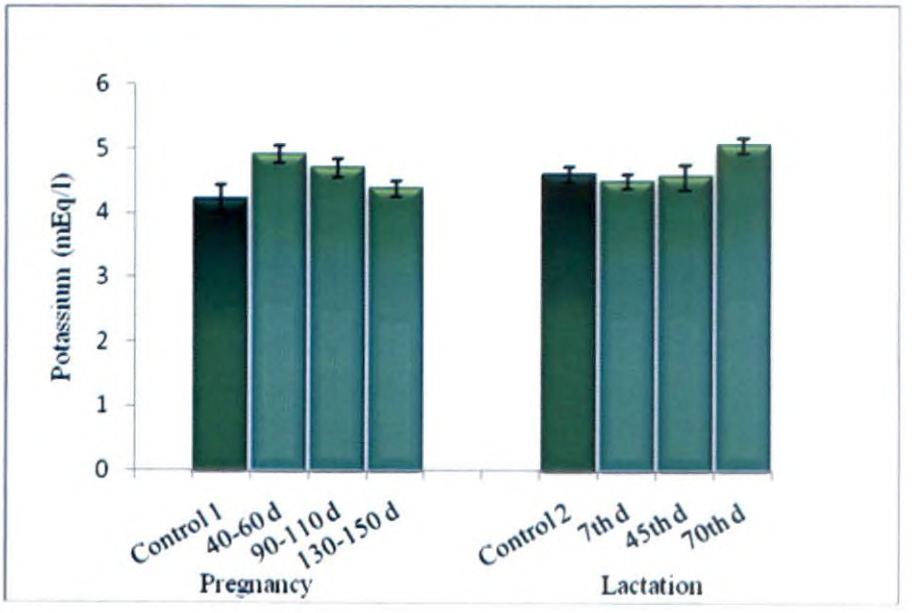


Fig. 13. Serum potassium level in goats during different periods of pregnancy and lactation

($P<0.01$) and mid-gestation (45^{th} d ($P<0.05$) and 70^{th} d ($P<0.01$)) . When compared with advanced gestation, significant increase ($P<0.01$) was observed in Hb during 7^{th} d of lactation.

4.2.2.10 Packed Cell Volume

Haematological analysis showed that a significant reduction ($P<0.05$) was observed in PCV during 70^{th} d of lactation period as compared to the control group (Fig. 15). The mean values for PCV during 7^{th}, 45^{th} and 70^{th} d of lactation were 35.69 ± 1.52, 30.92 ± 1.66, 28.54 ± 1.87 per cent respectively and that of control group was 35.40 ± 1.96 per cent. Like haemoglobin a significant decrease was observed in 45^{th} and 70^{th} d ($P<0.01$) as compared to 7^{th} d of lactation. When compared with early gestation, there was a significant increase in 7^{th} d ($P<0.05$) and decrease in 70^{th} d ($P<0.01$) of lactation.

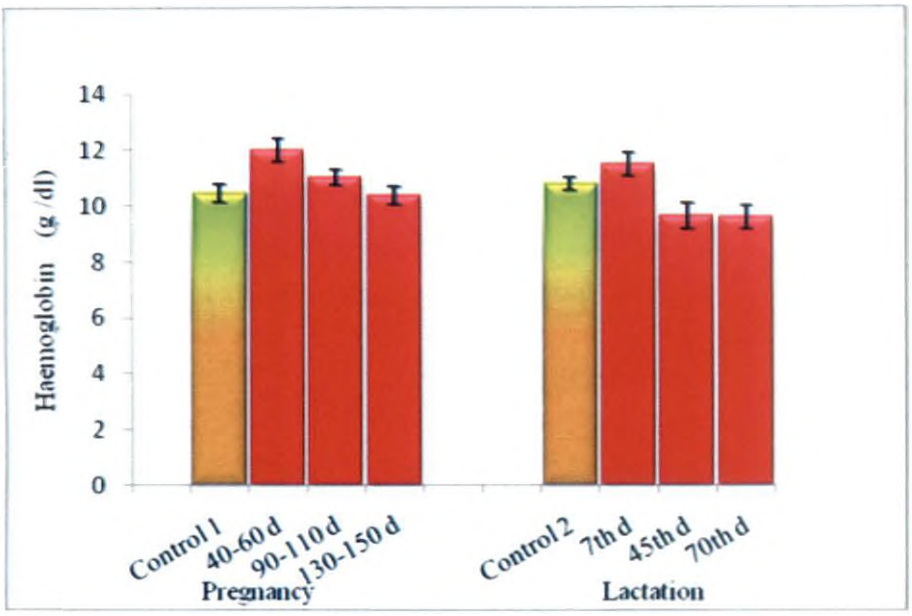


Fig. 14. Haemoglobin concentration in goats during different periods of pregnancy and lactation

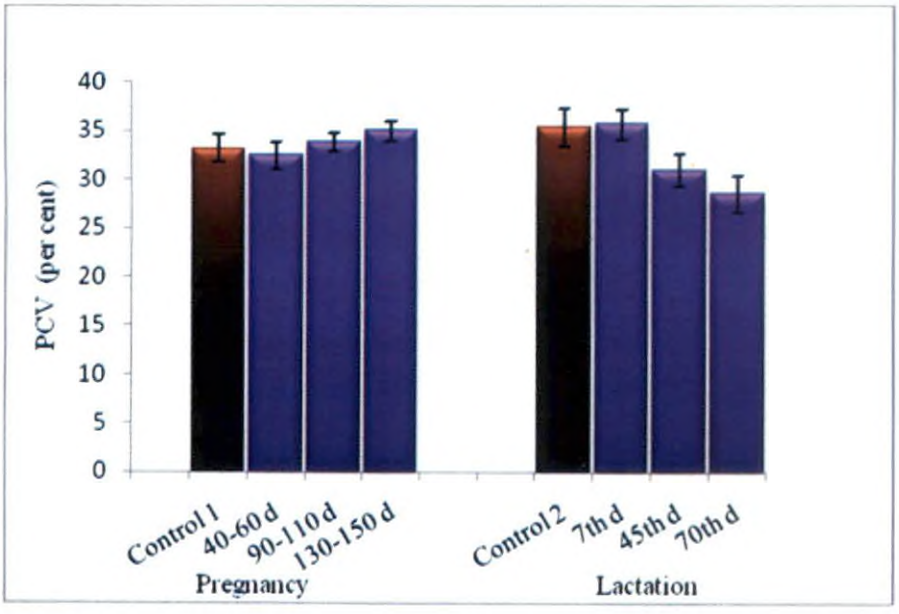


Fig. 15. Packed cell volume of goats during different periods of pregnancy and lactation

Discussion

5. DISCUSSION

Oxidative stress, a kind of chemical stress, is caused by an imbalance between the production of free radicals and the capability of an organism to absorb their excess (Piccione *et al.*, 2008). Increased oxidative stress has been reported in numerous studies during healthy pregnancy period of both animals and humans. Free radical induced damage of cells, lipids and proteins has been linked to the etiology of a number of disease conditions. It is suggested that the reason for increased oxidative stress in pregnant individuals may be probably due to the increased free radicals produced by increased metabolic activity and NEB. A reduction in the antioxidant reserve during pregnancy and metabolic adaptation during lactation could be another cause. These variations were related to the physiological adaptations of animals for their energy needs during pregnancy and lactation (Castillo *et al.*, 2006).

In the last few years, the evaluation of oxidative stress had increasingly contributed to our knowledge of the fundamental processes involved in metabolic disorders. In the present study, changes in antioxidant status indicators and metabolic parameters in the blood of goats during pregnancy and lactation were evaluated to assess oxidative stress in goats during these periods and to find out any relationship existing between antioxidant markers and other relevant blood parameters.

A total of 33 healthy Malabari and crossbred does were selected for this study, of which 13 were taken as experimental group, which were in the early stages of gestation. Two control groups, each with 10 animals were studied corresponds to the period of pregnancy (between March and June) and lactation (between July and November). No significant difference in the antioxidant status and metabolic parameters were found between these two control groups which suggested that seasonal variations did not affect the antioxidant and metabolic status of the animal.

5.1 ANTIOXIDANT STATUS AND METABOLIC PROFILE OF GOATS DURING PREGNANCY

During pregnancy, observations were made on three different periods; early (between 40-60 days), mid (between 90-110 days) and advanced (between 130-150 days) period of gestation.

5.1.1 Antioxidant Status Indicators

Production of free radicals is a physiological phenomenon which occurs continuously in the body during metabolism and the body has usually sufficient antioxidant reserves to cope with its production. However, when free radical generation exceeds the body's antioxidant production capacity, oxidative stress develops, especially during conditions of increased metabolic demand (Castillo *et al.*, 2005). In the present study MDA, GSH-Px, GSH, ascorbic acid and OSF were measured as antioxidant status indicators to assess the oxidative stress during pregnancy.

5.1.1.1 Malondialdehyde

Lipid peroxidation is a free radical mediated process. Proteins and DNA are often more significant targets of oxidative injury than are lipids, and lipid peroxidation often occurs late in the injury process (Halliwell and Chirico, 1993). In lipid peroxidation, a primary reactive free radical interacts with a PUFA to initiate a complex series of reactions that result in a variety of degradation products, most of which are cytotoxic aldehydes such as MDA and 4- hydroxynonenal (Slater, 1984). MDA remain after termination of lipid peroxidation and provides the basis for the thiobarbituric acid test for measuring lipid peroxidation end products in body fluids (Miller *et al.*, 1993). MDA level directly indicates level of lipid peroxidation and therefore oxidative stress.

In the present study, a significant increase ($P<0.01$) in MDA level was observed during mid-gestation period of goats compared to control. The result was in accordance with works of Turk *et al.* (2008) in dairy cows and Borisenkov *et al.* (2006) in ewes. Turk *et al.* (2008) reported that MDA concentration was significantly lower ($P< 0.05$) in the dry period compared to the second trimester of pregnancy in dairy cows. In ewes, lipid peroxidation was increased in the blood plasma in the middle pregnancy, reaching the maximum shortly before peak of progesterone production (Borisenkov *et al.,* 2006). However Castillo *et al.* (2005) reported that mean MDA levels did not differ significantly between cows in the late pregnancy and late lactation and did not show any clear trend within the pregnant cows at any stage.

In this study, between different stages of gestation significant increase was noted in mid ($P<0.01$) and advanced ($P<0.05$) gestation compared to early stage. But in advanced gestation, there was a significant decrease ($P<0.01$) in MDA level from the previous stage. The higher levels of lipoperoxidative products in the mid-gestation period suggested an exceeding degree of lipid peroxidation caused by ROS in that period. This indicated the imbalance between the oxygen radical production and their safe removal from critical sites, causing oxidative stress. In such conditions, the antioxidative capacity is inadequate to remove raised lipoperoxidative products. Lipid peroxidation is associated with acute phase response because oxidatively fragmented phospholipids are pro-inflammatory compounds. Over an extended period of time the disturbed antioxidative homeostasis might result in disease, particularly inflammation, and impair fertility. But in the present study, it was revealed that the animals possessed a good amount of antioxidant status and thereby increased MDA level was decreased in the advanced period of gestation when compared with mid-gestation.

5.1.1.2 Glutathione Peroxidase

Glutathione peroxidase is an antioxidant enzyme which catalyses the oxidation of glutathione at the expense of hydroperoxide like hydrogen peroxide. Other peroxides, including lipid hydroperoxides, can also act as substrates for these enzymes, which might therefore play a role in repairing damage resulting from lipid peroxidation. The enzyme requires selenium at the active site and deficiency might occur during severe selenium deficiency (Young and Woodside, 2001). The activity of this enzyme is dependent on the constant availability of GSH.

A significant reduction ($P< 0.01$) in the values of GSH-Px was observed during early and advanced gestation, when compared with the control group. Activity of GSH-Px increased significantly ($P<0.01$) in the mid-gestation from initial stage and then decreased ($P<0.01$) in the advanced stage of gestation. Reduction of GSH-Px activity in the advanced gestation was in accordance with the report of Gorecka *et al.* (2002), who found that in mares GSH-Px activity dropped 2 weeks before foaling to become extremely low a month after foaling. On the contrary, Bernabucci *et al.* (2005) reported increased ($P< 0.01$) GSH-Px activity in plasma from the week before calving until the 30 days after calving in dairy cows. In the present trial, the increase of plasma GSH-Px activity in the mid-gestation was an indirect indicator of the oxidative stress. Changes in the GSH-Px activity throughout the gestation period might reflect an altered oxidative status in that period.

5.1.1.3 Reduced Glutathione

Protein bound thiol groups are major chain breaking antioxidants in plasma. A major source of thiol group is GSH and it functions directly as an antioxidant, scavenging a variety of radical species, as well as acting as an essential factor for GSH-Px (Young and Woodside, 2001). In cells, GSH is

oxidized to oxidized glutathione (GSSG) in response to an increase in free radicals. Glutathione reductase restores GSH by reducing GSSG through the use of NADH and an increase in glutathione reductase activity suggests a response to oxidative stress (Clarkson and Thompson, 2000).

In the present study, GSH level was significantly increased ($P<0.01$) during mid-gestation when compared with control group. Similar to GSH-Px activity in the gestation period, GSH level was increased significantly ($P<0.01$) in the mid-gestation from early gestation and then decreased ($P<0.01$) in the advanced stage of gestation. Increased GSH in the mid-gestation might be due to an increased action of glutathione reductase, which regenerates GSH from GSSG to counteract the oxidative stress.

5.1.1.4 Ascorbic Acid

Ascorbic acid is a chain-breaking antioxidant which scavenges free radicals to inhibit the radical attack and break the chain oxidation. It acts as a potent reducing agent and reduces oxygen, nitrogen and sulphur centered radicals. Although ascorbic acid cannot scavenge lipophilic radicals within the lipid compartment by itself, it acts as a synergist with tocopherol for the reduction of lipid peroxyl radicals within the lipid compartment by reacting with tocopheroxyl radical and regenerating active tocopherol (Niki, 1991). During its antioxidant action, ascorbate undergoes a two electron reduction, initially to the semidehydroascorbyl radical and subsequently to dehydroascorbate.

Mean ascorbic acid concentrations in the present study did not differ significantly in between different stages of gestation and a significant increase ($P<0.05$) was noted only during early gestation, when compared with control group. So far no research was reported on changes in ascorbic acid concentrations during pregnancy and lactation in animals.

5.1.1.5 Oxidative Stress Factor

Oxidative stress factor is one of the parameters which indicate antioxidant status of an animal, calculated from the values of MDA, GSH and PCV. In the present study, significant increase ($P<0.01$) in OSF during mid-gestation was noticed compared to control group as well with other stages of gestation and lactation. This showed that during the entire period of gestation and lactation, animals had undergone a stage of maximum oxidative stress in mid-gestation period. In a study conducted by Bisla *et al.* (2003) on therapeutic effect of α-tocopheryl acetate and sodium selenite combination as an antioxidant in the treatment schedule of trans-abdominal diaghragmatic herniorrhaphy in buffaloes, reduction in OSF in the treatment group compared to diseased control group indicated reduction in oxidative stress during the therapy.

5.1.2 Metabolic Profile

Pregnancy and lactation are physiological statuses considered to modify metabolism in animals (Krajnicakova *et al.* and 2003, Iriadam, 2007). During pregnancy, maternal tissues are involved in providing energy for reproduction processes, which may affect blood serum chemistry values. In order to evaluate metabolic homeostasis during pregnancy, biochemical parameters like glucose, cholesterol, total protein, albumin, creatinine, urea, Na and K and haematological parameters such as Hb and PCV were assessed.

5.1.2.1 Glucose

Blood glucose is known as metabolic profile test, thus, it has distinguishable value in pregnancy toxemia, retarded growth, weight loss, production and reproduction defects. The level is varied in pregnancy, lactation and non-lactation. The variation is related to nutrition, production and

reproduction. The differences in glucose concentration among pregnant and lactating animals reveals the consumption of glucose by fetus and milk yield, so glucose administration before and after parturition resulted in the reduction of hypoglycemia and pregnancy toxemia (Ramin *et al.*, 2007).

Many authors reported lower blood glucose levels in pregnant animals. Tainturier *et al.* (1984) reported a decrease in blood glucose concentration at the end of pregnancy in dairy cows. In ewes, lower serum glucose levels were recorded on day 21, 120 and 145 of pregnancy compared to 7 and 14 days post-partum and glucose concentrations were lower than reference value until parturition (Gurgoze *et al.*, 2009). Ramin *et al.* (2007) reported glucose concentrations in pregnant ewes were lower ($P<0.01$) than lambed and aborted ewes. Lower ($P< 0.001$) serum glucose levels were recorded on day 100 and 150 of pregnancy, compared with day 45 post-partum and day 60 of pregnancy in ewes (Balicki *et al.*, 2007). Fırat and Ozpınar (1996) recorded plasma glucose levels to be lower in pregnant than in non-pregnant sheep. The results of the present study were parallel to these observations.

In the present study, glucose concentration showed a significant decrease ($P<0.01$) in mid and advanced gestation when compared with early period as well as control group. In contrast, El-Sherif and Assad (2001) reported blood glucose was increased from the 4^{th} week of pregnancy to reach its maximum at parturition in ewes. However, some authors reported no significant changes during pregnancy. Plasma glucose levels were not significantly different during pre-pregnancy and pregnancy (Firat and Ozpinar, 2002 and Castillo *et al.*, 2005). The decreased glucose concentration observed in mid and advanced gestation might be due to the increased requirements of glucose in late pregnancy in relation to non-pregnant stage.

5.1.2.2 Cholesterol

The serum total cholesterol values showed that there was a significant reduction ($P<0.05$) during early gestation and a significant increase ($P<0.01$) during advanced gestation when compared with control. Mid and advanced gestation period shows a significantly ($P<0.01$) high value when compared with early gestation as the values are increasing gradually from early to advanced gestation. In parallel to the current result, Iriadam (2007) reported total cholesterol content increased ($P<0.01$) from the 12^{th} to 18^{th} week of pregnancy and at parturition in goats. Similar results were also reported by other authors. When compared to day 45 post-partum the cholesterol increased, especially at 100 and 150 days of pregnancy (Balicki *et al.*, 2007). Krajnicakova *et al.* (1993) reported higher cholesterol concentrations during late pregnancy. In contrary to the present result Tainturier *et al.* (1984) reported total serum cholesterol decreased at the end of pregnancy in dairy cows. Cholesterol levels showed an apparent declining trend between six weeks before calving and one week after calving in cows (Castillo *et al.*, 2005). In dairy cows total cholesterol concentration was significantly lower ($P<0.05$) in the dry period compared to the first trimester of pregnancy (Turk *et al.*, 2008). However Ozpinar and Fırat (2003) reported mean plasma cholesterol levels were not significantly different for pregnant and non-pregnant ewes.

In the present study, increase in the cholesterol level during pregnancy was associated with increased oxidative stress. This increased cholesterol level during late pregnancy may be due to insulin, which plays a direct role in adipose tissue metabolism during pregnancy and its responsiveness is significantly reduced during late pregnancy. The diminished responsiveness of the target tissue to insulin during late pregnancy predisposed to increase of cholesterol, triglyceride and lipoproteins concentrations (Castillo *et al.*, 2005). Cholesterol metabolism requires cytochrome P-450, which is an important source of reactive oxygen metabolites that consume antioxidants (Miller *et al.*, 1993). This acquires great

importance if consider the negative correlation observed between antioxidant status and cholesterol.

5.1.2.3 Total Protein

In the present study, no significant changes were observed in serum total protein during pregnancy period when compared to the control as well as between different stages of pregnancy. In a study conducted by Castillo *et al.* (2005), they found no significant difference in total protein content between pregnant and late lactating (dry) cows at any stage. On the contrary, in the ewes total protein levels decreased, especially at 100 and 150 days of pregnancy (Balicki *et al.*, 2007). A significant decrease in plasma total protein was recorded from early pregnancy, to late pregnancy and parturition in does (Iriadam, 2007). Plasma protein with its two components, albumin and globulin, increased significantly at the 6th week, but dropped throughout the 16–18th week of pregnancy in ewes (El-Sherif and Assad, 2001). Gurgoze *et al.* (2009) reported serum total protein concentrations increased significantly during pregnancy in ewes.

5.1.2.4 Albumin

Albumin is the major extracellular source of thiols, which are scavengers of free radicals allowing albumin to function as an antioxidant. In the present study, though a significant decrease in albumin ($P<0.01$) was observed in advanced gestation from the mid-gestation period, no other significant changes were observed during pregnancy period when compared with the control group. Albumin did not differ significantly between dry and pregnant cows at any stage, and remained roughly constant in the pregnant cow group (Castillo *et al.*, 2005). In contrast serum albumin concentrations increased significantly during pregnancy in ewes (Gurgoze *et al.*, 2009). Their results revealed that it was significantly higher on day 145 of gestation,

compared to other stages of gestation and was within the reference range. A significant increase in albumin level also reported during the late gestation compared to dry period in ewes and it proves the higher energy requirement for the foetal growth (Piccione *et al.*, 2009).

5.1.2.5 Creatinine

The quantity of creatinine formed each day depends on the total body content of creatine, which in turn depends on dietary protein intake, rate of synthesis of creatine, and muscle mass (Kaneko *et al.*, 2008). A major source of mobilised amino acids is protein breakdown in skeletal muscles, although skin, uterine involution and myometral protein degradation may have some contribution. Traditionally, the dry period has been thought to be necessary for replenishment of body reserves, regeneration of mammary tissue, and for maximal benefits from lactogenic endocrine events.

In the present study, there was a significant increase ($P< 0.01$) observed in creatinine during advanced gestation when compared with the control group as well as early period of pregnancy. According to the study of Tainturier *et al.* (1984) creatinine increased throughout the last six months of pregnancy in Friesian cows. Creatinine began to increase significantly after 10–12 weeks of pregnancy in ewes (El-Sherif and Assad, 2001). Mean serum creatinine content was most of the time significantly higher in pregnant cows than in dry cows, but did not show any clear trend within the pregnant cow group (Castillo *et al.*, 2005). The observed increase in creatinine levels in the last month of pregnancy probably reflected the increase in fetal musculature and muscle damage in the dam.

However Gurgoze *et al.* (2009) reported that serum creatinine concentrations were higher after parturition and the level remained below reference ranges for sheep during pregnancy. According to Piccione *et al.*

(2009), creatinine content of serum was significantly higher in dry period than in late gestation in ewes.

5.1.2.6 Urea

The concentrations of urea are used to assess protein metabolism. This metabolite originates from the ammonium absorbed from the rumen or the catabolism of amino acids and its level depends upon the protein intake and energy demand. An increase in blood urea may reflect an accelerated rate of protein catabolism rather than decreased urinary excretion of urea (Kaneko *et al.*, 2008).The results showed a significant increase in urea during advanced gestation, compared to the control group ($P< 0.01$) and among other stages of gestation. The high requirement for energy by pregnant goats during their second half of pregnancy led to an increase in urea level which was evident during late pregnancy in this study.

Ramin *et al.* (2007) observed a significantly greater ($P< 0.01$) mean urea concentrations in pregnant ewes than lambed and aborted ewes. According to Gurgoze *et al.* (2009) serum urea ($P<0.001$), concentrations increased significantly in pregnant ewes and it could be related to either high protein metabolism during pregnancy or nutritional management. El-Sherif and Assad (2001) found that plasma urea concentration was increased in pregnant ewes from 10^{th} week of pregnancy, reaching a maximum level at parturition. Piccione *et al.* (2009) reported an elevated value of urea in ewes during late gestation compared to dioestrus. The result of the present study correlated with these findings.

However, Balicki *et al.* (2007) observed lowest urea level at 150 days of pregnancy than at 60 days of gestation in ewes. Castillo *et al.* (2005) recorded that mean serum urea content dropped significantly two weeks before calving in dairy cows.

5.1.2.7 Sodium

In the present study there was a significant reduction ($P< 0.05$) in Na during advanced gestation compared to the control group as well as with mid-gestation. Azab and Abdel-Maksoud (1999) obtained similar decreased Na content at 4, 3 and 2 weeks before parturition in goats. This decrease became significant ($P<0.05$) at 1 week before parturition and on the day of parturition. The declining tendency of Na level might be linked to changes of its contents in the uterine wall (Krajnicakova *et al.*, 2003).

5.1.2.8 Potassium

A significant increase ($P< 0.01$) was observed in K during early gestation compared to the control group as well as with advanced gestation. The K levels in the present study were similar to those observed in female Baladi goats by Azab and Abdel-Maksoud (1999). In their study, plasma K decreased during late pregnancy which was significant on the day of parturition and 1 week post-partum.

The observed decrease in the plasma Na and K near parturition might be a result of loss of these ions through the colostrum. Colostrum is secreted and stored in the mammary gland during the last 2 to 7 days of gestation and first 2 to 3 days post-partum. Also there was a loss of large amount of fluids during parturition, in addition to decrease of food intake during late pregnancy and partuition (Azab and Abdel-Maksoud, 1999).

5.1.2.9 Haemoglobin

Haematological examinations revealed a significant increase ($P< 0.05$) in Hb during early gestation compared to the control group which then gradually declined up to the advanced stage of gestation. Iriadam (2007) recorded no

significant changes in Hb concentration at 11 and 18 weeks of gestation and the 3rd week after parturition in does. According to Azab and Abdel-Maksoud, (1999) the decrease in Hb concentration was not significant during the last four weeks of pregnancy and became significant during the post-partum period in goats. On the contrary, El-Sherif and Assad (2001) reported an increased ($P<0.01$) blood hemoglobin from 10^{th} week to parturition, resulted from increased PCV per cent and mean corpuscular haemoglobin concentration.

Oxygen diffusion from the maternal blood to the fetal blood depends on the difference in oxygen tension in the maternal and fetal blood (Guyton and Hall, 2000), thus a marked decrease in Hb of the maternal blood may cause reduction of oxygen supply to the fetus.

5.1.2.10 Packed Cell Volume

During the study period, no significant difference was observed in PCV between the different stages of gestation. Also no significant changes were noticed during this period compared to the control group. While El-Sherif and Assad (2001) opined that PCV per cent and MCHC in pregnant ewes increased from 10^{th} week to parturition. On the contrary, Azab and Abdel-Maksoud, (1999) reported that PCV decreased significantly ($P<0.05$) during the last four weeks of pregnancy as well as during the post-partum period and suggested that it could be attributed to the hemodilution effect resulting from an increase in plasma volume. The observed hemodilution in late pregnancy of the domestic animals may have a physiological importance as it reduces the blood viscosity, thereby, greatly increasing the blood flow in the small blood vessels (Guyton and Hall, 2000). Thus hemodilution may improve the blood flow through placental blood vessels, especially at late pregnancy to increase the diffusion of nutrients and oxygen to the fetus.

5.2 ANTIOXIDANT STATUS AND METABOLIC PROFILE OF GOATS DURING LACTATION

After kidding the antioxidant status and metabolic parameters of the same animals were investigated on 7th, 45th and 70th d of lactation, and were compared with dry and non-pregnant animals, selected from the corresponding season.

5.2.1 Antioxidant Status Indicators

In order to assess the oxidative status of the goats during lactation period, MDA, GSH-Px, GSH, ascorbic acid and OSF were measured.

5.2.1.1 Malondialdehyde

The level of MDA in the 70th day of lactation showed a significant higher value, when compared to the control ($P<0.05$) as well as with values of early ($P<0.01$) and advanced ($P<0.05$) period of pregnancy. All the values during lactation period were higher when compared with early gestation and lower when compared with mid-gestation. According to Castillo *et al.* (2006), mean MDA levels in cows did not differ significantly between lactating and dry period. The present study revealed that during the period of gestation and lactation, animals had higher lipid peroxidation during the mid-gestation period.

5.2.1.2 Glutathione Peroxidase

The activity of GSH-Px significantly reduced ($P<0.01$) during 7th day of lactation when compared with the control group as well as with other two stages of lactation. In the 7th day of lactation GSH-Px activity was significantly lower than advanced gestation ($P<0.05$) also. In mares GSH-Px activity dropped 2 weeks before foaling to become extremely low a month after foaling (Gorecka *et*

al., 2002). Blood GSH-Px activity significantly decreased at weeks 2 and 4 after parturition in goats (Celi *et al.*, 2010). Also blood GSH-Px activity decreased in goats during the post-partum period and its values were significantly lower on days 14 and 28 post-partum (Celi *et al.*, 2008). On the contrary plasma GSH-Px showed higher ($P<0.01$) values after calving compared with data registered before calving in dairy cows (Bernabucci *et al.*, 2005). But in the current study, significant increase ($P<0.01$) was noted in 45^{th} and 70^{th} day when compared with 7^{th} day of lactation and early gestation.

The GSH-Px activity is considered as an indicator of oxidative stress and is also related to plasma lipid peroxide content (Halliwell and Chirico, 1993). These variations might have induced an imbalance between production of ROM and their safe disposal (reduction of antioxidants), and initiated chain reactions and lipid peroxidation. Blood GSH-Px was found to decrease during the immediate post-partum period in the present study, which suggested that the goats may have experienced some degree of oxidative stress and lipid peroxidation in other two stages. Antioxidant depletion is considered to be the consequence and not the cause of oxidative stress.

5.2.1.3 Reduced Glutathione

When compared with mid-gestation, GSH was significantly reduced ($P<0.01$) during all the stages of lactation yet these values were not significantly different as compared to the corresponding control group. A significant decrease ($P<0.01$) was noted in 45^{th} and 70^{th} day when compared with 7^{th} day. The GSH concentration was significantly lower ($P<0.05$) in 70^{th} day when compared with 45^{th} day as well as early and advanced gestation. Similar to MDA and GSH-Px , peak level of GSH was also observed during mid-gestation period.

5.2.1.4 Ascorbic Acid

The results showed no significant differences in ascorbic acid level during lactation period as compared to control group. But there were significant decrease in values of lactation period when compared with different stages of gestation. Ascorbic acid was significantly reduced ($P<0.01$) in 45th day of lactation when compared with all three stages of gestation and in 7^{th} and 70^{th} day also significant decrease noted, as compared to early ($P<0.01$) and mid ($P<0.05$) gestation. The overall results showed a tendency to increase the ascorbic acid level in the initial stages of pregnancy, thereafter it returned to normal level during the lactation period. So it is evident that ascorbic acid also acts as an antioxidant to reduce negative influence of free radicals during pregnancy.

5.2.1.5 Oxidative Stress Factor

Though there were no significant changes in OSF during lactation period as compared to control group, significant reduction ($P<0.01$) observed in all lactation stages when compared with mid-gestation.

Values of various antioxidant status indicators like MDA, GSH-Px, GSH and OSF showed their peak values during the same period, the mid-gestation period. This indicated that during the entire period of pregnancy and lactation animals experienced a maximum oxidative stress during mid-gestation period. The pre-partum and lactation period are considered as physiologically critical stages characterized by high energy demand. The physiological changes started from the early period of pregnancy and as a result the oxidative imbalance become more evident in the mid-gestation period and during the later stages oxidative stress decreases due to body's antioxidant defense mechanisms.

5.2.2 Metabolic Profile

5.2.2.1 Glucose

Glucose concentration significantly increased ($P<0.05$) in 7^{th} day of lactation as compared to advanced gestation. Significant increase was also seen in 7^{th} and 70^{th} day ($P<0.01$) and 45^{th} day ($P<0.05$) as compared to mid-gestation and the mean glucose concentration shows a significant decrease in 45^{th} day of lactation period as compared to control group ($P<0.01$) and 7^{th} day of lactation ($P<0.05$). In contrast to this result, Turk *et al.* (2008) reported significantly lower serum glucose concentration ($P< 0.001$) in early and late puerperium compared to the median values during pregnancy in dairy cows. In mid-lactation, glucose concentration was significantly higher as compared to early and late puerperium. According to Amer *et al.* (1999) glucose levels were constant in most periods in Saudy Ardy goats in lactation but showed a high level on d 14 and a low level on d 1. In lactating ewes, levels of glucose decreased from the maximum values at parturition and returned to levels comparable to those in dry ewes. (El-Sherif and Assad, 2001) In accordance with the result of the present study, Karapehlivan *et al.* (2007) reported serum glucose concentrations on days 1 and 30 of lactation were higher than those 3 weeks after drying off in ewes ($P< 0.01$). There were some reports which showed no change in glucose levels during lactation. Serum glucose did not differ significantly between cows in the late lactation (dry cows) and lactating, or among different lactation stages in lactating group. (Castillo *et al.*, 2006) Plasma glucose levels were not significantly different in ewes during early lactation periods (Firat and Ozpinar, 2002).

During the present study period minimum blood glucose level was seen in the mid-gestation stage which indicates animal had the lowest energy status within the same period.

5.2.2.2 Cholesterol

In goats, Krajnicakova *et al.* (2003) reported that the mean concentration of total cholesterol had an increasing tendency to the day 40 post partum after a slight significant decrease on day 14. In the present study serum cholesterol showed a significant increase ($P<0.05$) during 7th and 70th days of lactation as compared to the control group and when compared with advanced gestation, significant reduction ($P<0.01$)) was observed in all 3 stages of lactation period. Following an insignificant decrease on day 45 post partum, the mean concentration of total cholesterol had an increasing tendency to the end of the observed period (70 days post partum) during lactation. On the contrary, Turk *et al.* (2008) reported total cholesterol concentration was significantly higher in mid-lactation than in early and late puerperium in dairy cows. According to Amer *et al.* (1999) serum total cholesterol in goats had their peak values on 21st d and their lowest value on 7th d. The mean cholesterol concentrations were significantly lower in early post-partum period when compared to the mean values in the cows in late non-pregnant lactation. (Turk *et al.*, 2004) However Celi *et al.* (2008) reported no effect of time from kidding on cholesterol concentrations in goats.

During the entire period of gestation and lactation, lowest level of cholesterol was seen in early stages of gestation which then attained a peak level in the advanced gestation period.

5.2.2.3 Total Protein

El-Sherif and Assad (2001) reported that total plasma proteins returned to levels comparable to those in dry ewes in lactating period. This was in accordance with the findings of present study, where no significant changes were observed during lactation period when compared with the control group.

But a significant increase ($P<0.05$) was observed in the 7th day of lactation as compared to early gestation period. In goats, Celi *et al.* (2008) and Amer *et al.* (1999) reported no significant variations in serum levels of total protein from delivery throughout the post-partum period.

However many authors have reported fluctuations in the serum total protein during lactation period. Serum total protein showed a significant increase during all (early, mid and end) lactation periods when compared to pregnancy period and in dry period an increase in total protein content was found compared to gestation and early lactation in ewes (Piccione *et al.*, 2009). Similarly the blood total protein levels on the 1st day of lactation were found lower when compared to those on 3 weeks after drying off in ewes (Karapehlivan *et al.*, 2007). But in dairy cows the value of total protein 2 weeks after calving was significantly higher than the late lactating (dry) cows (Castillo *et al.*, 2006). In their study, mean serum total proteins showed significant fluctuation during the lactating period increasing from one week after calving to 2 week after calving. A significant increase in total proteins was recorded on 40th d post partum ($P< 0.05$) in goats and at the beginning of the post-partum period, their concentrations ranged within the reference values (Krajnicakova *et al.*, 2003). The higher values of total protein in lactating animals prove the high energy need due to milk synthesis which exists in animals especially during the early lactation (Piccione *et al.*, 2009). The overall study showed that no significant variation in total serum protein during entire gestation and lactation period.

5.2.2.4 Albumin

There was a significant reduction ($P<0.05$) in albumin during 45th day of lactation period compared to the control group in the present trial. Significant changes like increase in albumin level during 7th ($P<0.01$) and 70th day ($P<0.05$) compared to advanced gestation and reduction in 45th day compared to 7th day

($P<0.01$) were also noted. Many researchers have reported increased albumin level immediately after parturition. A decrease ($P<0.001$) in serum albumin levels were recorded in ewes, 3 weeks after drying off and on day 30 of lactation compared to those on the onset of lactation (Karapehlivan *et al.*,2007). Albumin in lactating period remained higher than in dry ewes with a slow decline (El-Sherif and Assad, 2001). According to Turk *et al.* (2008) albumin concentration in the late puerperium was significantly lower than in the early puerperium in dairy cows. On the contrary, albumin levels were significantly lower on 1st and 28th d from delivery compared with 21st d in goats (Celi *et al.*, 2008). However, according to Castillo *et al.* (2006) albumin did not differ signicficantly between dry and lactating cows or among different lactation stages.

5.2.2.5 Creatinine

Mean creatinine concentrations in 7th and 45th day of lactation did not show any significant change when compared with control group while significant increase ($P<0.05$) was present in 70th day of lactation. But when compared with advanced gestation all the values in lactation period were significantly reduced. This result was in close agreement with the results reported by El-Sherif and Assad (2001). They found that after an increase in the gestation period the creatinine concentration in lactating ewes returned to levels comparable to those in dry ewes. But Castillo *et al.* (2006) reported that creatinine levels were significantly higher in early lactation (1 and 2 weeks after calving) than in dry cows and at the lactation peak creatinine level was similar to that in dry cows. Serum creatinine ($P<0.001$) concentrations were significantly higher on day 14 post-partum than on days 21 and 120 pregnancy and creatinine levels remained below reference ranges for sheep during lactation period (Gurgoze *et al.*, 2009). The peak level of creatinine attained in the late pregnancy period returned to normal level after kidding.

5.2.2.6 Urea

In the present study a significant increase ($P<0.05$) in serum urea concentration during 45th day of lactation was noticed compared to the control group and no significant changes were observed among different lactation stages. Significant increase ($P<0.01$) in urea level was noticed in all three stages of lactation compared to early gestation. The serum urea concentration observed in all lactation stages was lower than that of advanced gestation but the difference was insignificant. Castillo *et al.* (2006) reported that urea did not differ significantly between dry and lactating cows, or among different lactation stages. According to El-Sherif and Assad (2001), urea concentration in lactating ewes returned to levels comparable to those in dry ewes. Tainturier *et al.* (1984) recorded an increased serum urea in the first month after calving in dairy cows. Karapehlivan *et al.* (2007) observed that serum urea concentrations on 30th d of lactation were higher than those on the 1st d of lactation and 3 weeks after drying off in ewes. Similarly, the serum urea concentration on the 1st d of lactation was higher than those 3 weeks after drying off. Urea showed a significant decrease at the end of lactation and in dry period compared to dioestrus, pregnancy, post-partum, early and mid lactation, and during mid lactation *vs* late gestation in ewes (Piccione *et al.*, 2009). Plasma urea levels started to decrease significantly from 3 days before delivery in goats (Celi *et al.*, 2008). Serum urea concentrations decreased as goats progressed from late pregnancy to early lactation. However the findings of Balicki *et al.* (2007) was not in agreement with the result of present study. They observed a significantly lower plasma urea level ($P<0.01$) at 45 days post-partum than at 60 days of gestation in ewes.

When compared to early stages of pregnancy a general tendency of increased urea concentration was observed in the advanced stages of gestation and lactation period. These variations might be due to the low energy status during this period.

5.2.2.7 Sodium

There was a significant reduction in Na during 70th day of lactation compared to the control group as well as 45th day ($P<0.05$) and mid-gestation ($P<0.01$). From advanced gestation an increase was noticed in 7th and 45th day however these values did not show any significant change from control group. Azab and Abdel-Maksoud (1999) reported an increased plasma Na at 3 and 4 weeks post-partum in goats. Krajnicakova *et al.* (2003) evaluated the Na concentrations in goats in comparison with 1st d post partum and reported a statistically significant decrease ($P<0.05$) with the lowest values on 28th d after parturition and then no significant change was observed until 40 days. Slightly decreasing value of Na observed in the study could be due to passage of Na to milk (Kaneko *et al.*, 2008).

5.2.2.8 Potassium

During the study period, a significant increase in K was observed during 70th day of lactation as compared to the control group, 7th and 45th day of lactation ($P< 0.05$) and advanced gestation ($P<0.01$). There was an insignificant decrease in advanced gestation period as compared to the previous stages and then gradually increased in lactation stages that become significant in 70th day. Krajnicakova *et al.* (2003) reported that concentrations of K did not show statistically significant differences in the puerperal period of goats and their lowest values found on days 3rd and 32nd d after parturition did not exceed the limit of the reference values. According to Azab and Abdel-Maksoud (1999), plasma K decreased ($P>0.05$) on the day of parturition and 1 week post-partum. The observed decrease in the plasma K near parturition may be a result of loss of these ions through the colostrum. Colostrum is secreted and stored in the mammary gland during the last 2 to 7 days of gestation and first 2 to 3 days post-partum.

5.2.2.9 Haemoglobin

The Hb values showed that there was a significant reduction ($P< 0.05$) during 45^{th} and 70^{th} day of lactation compared to the control group. When compared with the advanced gestation a significant increase ($P<0.01$) was observed in Hb during 7^{th} day and then a significant decrease was observed in 45^{th} and 70^{th} day ($P<0.01$). Azab and Abdel-Maksoud (1999) reported that the decrease in Hb concentration became significant ($P<0.001$) during the post-partum period in goats. Iriadam (2007) recorded no significant change in Hb concentration at 11 and 18 weeks of gestation and the 3^{rd} week after parturition in does. El-Sherif and Assad (2001) reported a drop in blood Hb at the 4^{th} week of lactation along with a sharp decline in PCV.

5.2.2.10 Packed Cell Volume

A significant decrease ($P<0.05$) in PCV on 70^{th} day of lactation period as compared to the control group was noticed. Like Hb a significant decrease in PCV was observed in 45^{th} and 70^{th} day ($P<0.01$) as compared to 7^{th} day of lactation. El-Sherif and Assad (2001) observed a sharp decrease in PCV during the first month of lactation, which was significantly lower than in dry ewes at the 3^{rd} week of lactation. Azab and Abdel-Maksoud (1999) reported that PCV significantly ($P<0.05$) decreased during the last four weeks of pregnancy as well as during the post-partum period.

The present study showed that the animals were in a low energy state mainly in mid-gestation period which is evident from the variations in the level of glucose, cholesterol and urea. During the same critical period of pregnancy indicators of oxidative stress like MDA and OSF were maximum. In order to overcome the oxidative stress, antioxidants like GSH-Px and GSH were found to be increased at this stage, providing the protection of maternal organs and fetus against the negative influence of free radicals. When the animals reached peak

lactation, the metabolic status was stabilized and that was reflected in the antioxidant status. From all these findings we can assume that low energy metabolic status of animal is correlated with the oxidative stress. In addition, measurement of MDA and OSF can provide complement information regarding metabolic status of the animal.

Summary

6. SUMMARY

The present study was undertaken with the objectives of evaluating and comparing the antioxidant status and metabolic profile of pregnant, lactating and non pregnant dry goats and also to evolve any possible relationship between antioxidant markers and other relevant blood parameters.

The study was carried out during the period from March to November, 2009 in 33 adult healthy female Malabari and crossbred goats aged between 3 and 6 years, maintained at University Goat and Sheep Farm, College of Veterinary and Animal Sciences, Mannuthy. Investigations were done at three stages; during the period of pregnancy (between March and July), lactation period (between June and November) and a period in which animal is neither lactating nor pregnant (dry period). Blood samples were collected from 13 goats of study group during early (between 40-60 d), mid (between 90-110 d) and advanced (between 130-150 d) period of gestation and on 7^{th}, 45^{th} and 70^{th} d post-partum to evaluate the antioxidant status and metabolic profile. Two groups of dry, non-pregnant animals were selected as control, each consisting of 10 animals. First group was studied in March to June period and other in between July and November, corresponding to the period of gestation and lactation respectively to nullify the seasonal effect.

Ascorbic acid, MDA, GSH-Px, GSH, and OSF were measured as antioxidant status indicators. Biochemical parameters like blood glucose, cholesterol, total protein, albumin, creatinine, urea, Na and K and haematological parameters such as Hb and PCV were analysed to assess the metabolic profile.

The research findings showed a significant increase in MDA level during mid-gestation period ($P<0.01$) and 70^{th} d of lactation ($P<0.05$) as compared to control. The higher level MDA concentration in the mid-gestation period in this study suggests an exceeding degree of lipid peroxidation caused by ROS in that

period. This indicates the imbalance between the oxygen radical production and their safe removal from critical sites provoking oxidative stress.

The activity of GSH-Px increased significantly ($P<0.01$) in the mid-gestation from initial stage and then decreased ($P<0.01$) in the advanced stage of gestation. Significant reduction ($P< 0.01$) in the values of GSH-Px activity was observed during early and advanced gestation and 7th d of lactation when compared with the control group. Changes in the GSH-Px activity throughout gestation period might reflect an altered oxidative status in that period. GSH level was significantly increased ($P<0.01$) during mid-gestation when compared with control group and during lactation, values were not significantly different from control group. Similar to GSH-Px activity in the gestation period, GSH level was increased significantly ($P<0.01$) in the mid-gestation from early gestation and then decreased ($P<0.01$) in the advanced stage of gestation. Increased GSH in the mid-gestation might be due to increased action of glutathione reductase, which regenerates GSH from GSSG to counteract the oxidative stress.

A significant increase ($P<0.05$) in ascorbic acid level was noted only during early gestation, when compared with control group, but they did not differ significantly in between different stages of gestation Also no significant differences in ascorbic acid level during lactation period as compared to control group. Significant increase ($P<0.01$) in OSF during mid-gestation was noticed compared to control group as well with other stages of gestation and lactation. This showed that during entire period of gestation and lactation, animals had undergone a stage of maximum oxidative stress in mid-gestation period.

Glucose concentration showed a significant decrease ($P<0.01$) in mid and advanced gestation and 45th d of lactation period when compared with control group. Significant increase noticed in 7th d of lactation from the advanced gestation period. The decreased glucose concentration observed in mid and

advanced gestation might be due to the increased requirements of glucose in pregnancy in relation to non-pregnant stage.

The serum cholesterol values showed that there was a significant reduction ($P<0.05$) during early gestation and a significant increase during advanced gestation ($P<0.01$) and 7th and 70th d of lactation ($P<0.05$) when compared with control. Increase in the cholesterol level during pregnancy was associated with increased oxidative stress and it might be due to the requirement of cytochrome P-450 in cholesterol metabolism, which is an important source of reactive oxygen metabolites that consume antioxidants. This increased cholesterol level during late pregnancy may be due to a direct role of insulin in adipose tissue metabolism during pregnancy and its responsiveness is significantly reduced during late pregnancy.

No significant changes were observed in serum total protein during pregnancy and lactation period. But in the albumin level a significant reduction ($P<0.05$) during 45th d of lactation period was noticed compared to control group. Also a significant decrease in albumin ($P<0.01$) was observed in advanced gestation from the mid-gestation period.

A significant increase was observed in creatinine during advanced gestation ($P< 0.01$) and 70th d of lactation ($P< 0.05$) when compared with the control group. The observed increases in creatinine levels in the last month of pregnancy probably reflect the increase in fetal musculature and muscle damage in the dam. There was a significant increase in urea during advanced gestation ($P< 0.01$) and 45th d of lactation ($P< 0.05$) compared to the control group. The high requirement for energy by pregnant goats during their second half of pregnancy led to an increase in urea level which is evident during late pregnancy in this study.

There was a significant reduction ($P< 0.05$) in Na during advanced gestation and 70^{th} d of lactation compared to the control group. The declining tendency of Na level might be linked to increased passage of Na to milk and with changes in its contents in the uterine wall. Serum K was significantly increased during early gestation compared to the control group and then it is gradually decreased in pregnancy period. From advanced gestation K level gradually increased in the lactation period with a significant increase during 70^{th} d of lactation. The observed decrease in the plasma Na and K near parturition might be a result of loss of these ions through the colostrum. Also there was loss of large amount of fluids during parturition, in addition to decrease of food intake during late pregnancy and partuition.

Haematological examinations revealed a significant increase ($P< 0.05$) in Hb during early gestation which reduced ($P< 0.05$) during 45^{th} and 70^{th} d of lactation compared to the control group. No significant difference was observed in PCV during pregnancy period but a significant decrease during 70^{th} d of lactation period as compared to the control group. As in the case of Hb, a significant decrease was observed in PCV on 45^{th} and 70^{th} d ($P<0.01$) as compared to 7^{th} d of lactation.

Hence the present study revealed that the animals were in a low energy state mainly in mid-gestation period and during the same critical period oxidative status was also altered. When the animals reached peak lactation, the metabolic status was stabilized and that was reflected in the antioxidant status. From all these findings we can assume that low energy metabolic status of animal is correlated with the oxidative stress. In addition, measurement of antioxidant parameters can provide complement information regarding metabolic status of the animal.

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ANTIOXIDANT STATUS AND METABOLIC PROFILE OF GOATS DURING PREGNANCY AND LACTATION

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ABSTRACT

The present study was undertaken with the objective to assess the changes in antioxidant status indicators and metabolic profile of goats during pregnancy and lactation and to evolve any possible relationship between antioxidant markers and other relevant blood parameters. The study was carried out in 33 adult healthy female Malabari and crossbred goats aged between 3 to 6 years maintained at University Goat and Sheep Farm, College of Veterinary and Animal Sciences, Mannuthy during the period of March to November 2009. Blood samples were collected during early (between 40-60 d), mid (between 90-110 d) and advanced (between 130-150 d) period of gestation and on 7^{th}, 45^{th} and 70^{th} d post-partum. Antioxidant and metabolic parameters were analyzed and were compared with dry and nonpregnant goats.

The research findings showed a significant increase in MDA level during mid-gestation period and 70^{th} d of lactation as compared to control. The activity GSH-Px and GSH level increased significantly in the mid-gestation from initial stage and then decreased in the advanced stage of gestation. Significant reduction in the values of GSH-Px activity was observed during early and advanced gestation and 7^{th} d of lactation and GSH level was significantly increased only during mid-gestation when compared with control group. During the entire study period a significant increase in ascorbic acid concentration was noted only during early gestation, when compared with control group. Significant increase in OSF was noticed during mid-gestation compared to control group as well with other stages of gestation and lactation.

Glucose concentration was significantly decreased in mid and advanced gestation and 45^{th} d of lactation period when compared with control group. A significant reduction was observed in serum cholesterol during early gestation and a significant increase during advanced gestation, 7^{th} and 70^{th} d of lactation when compared with control. No significant changes were observed in serum

total protein during pregnancy and lactation period. But in the albumin level a slightly significant reduction during 45th d of lactation period was noticed. When compared to control group, significant increase was observed for creatinine during advanced gestation and 70th d of lactation and for blood urea, during advanced gestation and 45th d of lactation. There was a significant reduction in Na during advanced gestation and 70thd of lactation but a significant elevation in serum K during early gestation and 70th d of lactation compared to the control group. Haematological examinations revealed a significant increase in Hb during early gestation and reduction during 45th and 70th d of lactation and PCV was decreased during 70th d of lactation compared to the control group.

In the present study, animals were in a low energy state mainly in mid-gestation period which is evident from the variations in the level of glucose, cholesterol and urea. During the same critical period of pregnancy, indicators of oxidative stress like MDA and OSF were maximum. In order to overcome the oxidative stress, antioxidants like GSH-Px and GSH were found to be increased at this stage, providing the protection of maternal organs and fetus against the negative influence of free radicals. When the animals reached peak lactation, the metabolic status was stabilized and that was reflected in the antioxidant status. The present study concludes with the finding that low energy metabolic status of animal is correlated with the oxidative stress.