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STUDIES ON PATHOGENESIS AND TREATMENT OF PREGNANCY TOXAEMIA IN SHEEP

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ABSTRACT
Pregnancy toxaemia was induced in 15 sheep by dietary restriction and insulin administration. A clinical syndrome simulating naturally occurring pregnancy toxaemia was produced. Listlessness, ataxia, grinding of teeth, apparent blindness, sternal and lateral recumbency and convulsions were the major clinical signs observed. Biochemically decrease in blood glucose and increase in blood ketone and free fatty acids was prominent. Various liver function tests viz. serum enzyme (AST, ALT, arginase) levels, serum bilirubin level, BSP clearance rate and propionate loading test indicated moderate to severe liver damage with decreased gluconeogenic capability of liver. Renal function tests namely BUN and serum creatinine levels showed moderate loss of kidney function. Glucose tolerance test revealed diabetes like changes in the glucose tolerance curve. This together with the changes in the pattern of blood glucose, ketone and free fatty acid levels was inferred to indicate impaired glucose utilization. Blood hormone profile exhibited a marked increase in cortisol and a marked decrease in insulin and glucagon levels. The histopathological examination revealed severe fatty changes with focal infiltration of lymphoid cells in liver, mild to severe nephritic changes in kidneys, fatty changes and hyperplasia in adrenals and congestion, leptomeningeal haemorrhages and neuronal degeneration in brain. Parenteral glucose therapy was unsuccessful with only 20 percent recovery rate while as oral glucose therapy following premedication with vasopressin was highly effective giving an 80 percent recovery rate against a 100 percent mortality rate in untreated animals.

KEYWORDS: AST, ALT, arginase, leptomeningeal haemorrhages.

INTRODUCTION
Ovine pregnancy toxaemia is a syndrome which occurs in the latter part of pregnancy in sheep, more commonly in ewes with multiple foeti and is characterized by neurological signs, motor weakness, apparent blindness and high mortality (Reid, 1968; Ferris *et al*., 1969). It is one of the most important causes of mortality in sheep. The disease was first reported by Seaman (1854) from East Anglian region of England. It is described under many names like stupid sickness, Afrikaans domsiekte, lambing sickness, twin lamb disease, sleepy sickness, pregnancy disease. The disease occurs spontaneously in field but can also be induced experimentally (Reid, 1968). Both spontaneous and experimentally induced pregnancy toxaemia present similar clinical manifestations (Hill *et al*., 1984). Up to 20 percent ewes may be manifestly afflicted with the disease and subclinically even more animals may be affected (Leopold, 1991). In individual flocks the disease can reach a level of incidence sufficient to be classified as an outbreak (Radiostitis *et al*., 1994). Pregnancy toxaemia is a highly fatal disease and mortality of up to 90 percent has been reported (Ford, 1983). The pathogenesis of the condition has been a subject of controversy and has not been fully elucidated (Bickhardt, 1988; Ford *et al*., 1990). Unlike bovine ketosis, in pregnancy toxaemia blood cortisol levels are elevated and there is severe acidosis. It is not clear as to whether the elevated cortisol is due to overproduction by the adrenals or less excretion by the liver. Relatively recently a possible role of glucostatic hormones in the genesis of bovine ketosis has been reported (Lean *et al*., 1991). Disturbances in glucostatic hormones may be playing an important role in pregnancy toxaemia also. Unlike acetonaemia of cattle which has some aspects in common with pregnancy toxaemia, the response to treatment is much less satisfactory. Induction of abortion or removal of foetus by caesarean section is recommended by some authors, but the results are variable (Radiostitis *et al*., 1994). Recently a high percentage of recovery in clinical cases of pregnancy toxaemia following oral glucose therapy

has been reported (El-Hamamsy *et al*., 1990). However, its basis has not been fully elucidated.

Ovine pregnancy toxaemia has many aspects in common with human pre-eclampsia and could, therefore, be used as a possible animal model for understanding its pathogenesis.

The present study was, therefore, undertaken with the following objectives

1. To study clinical courses of experimental pregnancy toxaemia in sheep.
2. To study pathogenesis of the disease in relation to haematological, biochemical and histopathological changes.
3. To attempt a suitable therapeutic regimen on the basis of above findings.
4. An attempt was made to evolve an oral therapeutic regimen in an effort to cut down the cost of treatment and make it more practicable under field conditions.

MATERIALS AND METHODS

Experimental Animals

Fifteen nondescript pregnant sheep were supplied by the local sheep farms of Derna city for carrying out the present study. The animals were in the age group of two to three years with body weight ranging between 27 and 35 Kg. On receipt the animals were dewormed with moxidectin @ 0.2 mg/Kg b.w. and were allowed two weeks period for acclimatization before starting the experiment. Throughout the study the animals were maintained under identical managemental conditions. Individual animals were handed daily for 10 – 15 minutes to acquaint them with sampling in an effort to reduce fluctuations in physiological parameters due to nervousness. The animals were subjected to experimentation when they were pregnant of 130 days and above. The animals were randomly divided into three groups of five animals each. Group I served as control group in which pregnancy toxaemia was induced and observations recorded till death or recovery. Group II and Group III served as treatment groups in which the disease was induced and different treatments administered.

Induction of Pregnancy Toxaemia

Pregnancy toxaemia was induced by semi-starvation and insulin administration. The animals were subjected to dietary restriction for four days prior to insulin administration. During this period the concentrates and greens were withdrawn and the animals were maintained on wheat straw. On 5^{th} day plain insulin was administered subcutaneously @ 1 U/Kg b.w. The insulin administration was invariably repeated after 12 hours at half the dose.

Clinical Observations

Following insulin administration the animals were closely observed for recording clinical symptoms till death or recovery.

Sampling

Sampling was done at six hourly intervals after insulin administration up to 36 hours, after which 12 hourly sampling was done. For haematology, blood glucose and ketone estimation about 1 ml blood was collected into heparinised vials. For serum collection about 20 ml blood was drawn into 50 ml glass test tubes. Serum was separated within half an hour after blood collection, divided into 1 ml aliquots and frozen until used.

Intravenous Glucose Tolerance Test

Glucose tolerance testing was conducted before and at 12 hourly intervals after induction of pregnancy toxaemia. The test was done by injecting a 50 percent solution of glucose 0.5 g/Kg b.w. intravenously. Blood samples were then collected at 0, 5, 15, 25, 35, 45 and 60 minute intervals. Blood glucose was estimated and the values were plotted on semi logarithmic coordinates from which half-time ($T_{1/2}$) and fractional turnover rate (k) were calculated as described by Kaneko (1989).

Propionate Loading Test

Propionate loading test was conducted before and at 12 hourly intervals after induction of disease. The test was done by injecting a sterile solution of sodium propionate (5 M) @ 2.5 mmol/Kg b.w. Blood samples were collected at 0, 7 and 10 minute intervals. Seven minute increase in blood glucose was calculated and used as a measure of hepatic function.

BSP Clearance Test

The test was conducted before and at 12 hourly intervals after induction of pregnancy toxaemia. A five percent solution of BSP was injected intravenously in jugular vein. Two blood samples were then drawn exactly at three minutes and seven minutes after injection in heparinised vials from the jugular vein of other side. Half-life ($T_{1/2}$) of BSP was then determined as per the method described by Kaneko (1989).

Haematology

Haemoglobin was estimated by cyanomethaemoglobin method (Raymond and Wilkinson, 1963). Packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC) and differential leukocyte count (DLC) were conducted employing standard techniques as described by Jain (1986).

Blood Biochemistry

Blood glucose was estimated by glucose oxidase/peroxidase method using reagent kits. Ketones were estimated as per the method described by Henry *et al*. (1974). Ketone estimation was done within an hour after sample collection. Free fatty acids were estimated by the method of Lowry and Tinsley (1976).

Blood urea nitrogen was estimated by diacetyl monoxime method using reagent kits.

Creatinine was determined by alkaline picrate method using reagent kits.

Bilirubin was estimated by diazo method using reagent kits.

Serum Enzymes
Arginase was estimated as per the method described by Mia and Koger (1978).

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined by dinitrophenyl hydrazine method using reagent kits.

Hormones
Insulin was estimated by radioimmunoassay method using reagent kits.

Glucagon and cortisol were estimated by radioimmunoassay method using reagent kits.

Electrolytes
Sodium, potassium, calcium and magnesium were determined by atomic absorption spectrophotometer. For this the samples were digested in triple acid solution consisting of nitric acid (conc.), perchloric acid (70%) and sulphuric acid (conc.) in the ration of 10:3:1.

Phosphorus was estimated as per the method of Gomori (1942) using reagent kits.

Pathomorphological Studies
Post-mortem of the animals died of the disease was conducted and liver, kidneys, adrenals and brain were collected in neutral buffered formalin for histopathological examination. Histopathology was done by following standard procedures using haematoxylin and eosin staining.

Therapeutic Studies
Following disease induction the treatment was administered when the animals were in lateral recumbency with convulsions.

GROUP II: (Parenteral Glucose Therapy)
The animals of this group were given 200 ml of 25 percent dextrose with 0.9 percent sodium chloride intravenously. The treatment was administered twice daily for two to three days.

GROUP III: (Oral Glucose Therapy)
Group III animals were drenched the following oral solution about one minute after intravenous administration of vasopressin @ 0.1 U/Kg b.w.
Glucose 40 g
Sodium chloride 9 g
Water to 100 ml

The treatment was repeated twice daily for two to three days depending upon the response of the animal.

Statistical Analysis
The results were subjected to student's t-test. Significance was tested at 1 percent and 5 percent level (Snedecor and Cochran, 1976).

RESULTS AND DISCUSSION

Clinical Observations
Listlessness, unresponsiveness, ataxia, apparent blindness, neuromuscular disturbances, lateral and sternal recumbency and convulsions were the major clinical symptoms observed in present study.

Depression of consciousness and neuromuscular disturbances have consistently been reported by earlier workers both in clinical and experimental pregnancy toxaemia (McClymont and Setchell, 1955; McClymont and Setchell, 1956; Mitchell and Stratford, 1987). Sternal and lateral recumbency has been reported both in experimental (Hill *et al*., 1984; Vihan and Rai, 1984) and natural (Lindsay and Pethick, 1983; Kimberling, 1988) pregnancy toxaemia in sheep. Apparent blindness has also been reported both in natural and experimental pregnancy toxaemia (Lindsay and Pethick, 1983; Hill *et al*., 1984; Singh *et al*., 1992). Grinding of teeth, a consistent observation in the present study has been reported only as an occasional symptom (Radiostitis *et al*., 1994). Convulsions, coma and death have also been reported by Lindsay and Pethick (1983), Singh *et al*. (1992) and Radiostitis *et al*. (1994).

Since sheep brain cannot use ketone body for energy supply (Lindsay and Setchell, 1976; Pell and Bergman, 1983), clinical manifestations particularly neurological signs could be due to hypoglycaemia which has been a consistent observation in the present study. Bergman (1971) and Schultz (1974) attributed nervous symptoms to hypoglycaemia in bovine ketosis. Hyperketonaemia may also have played a role in the development of nervous signs as it has been suggested to have a central toxic and depressing effect in bovine ketosis (Bergman, 1971; Radiostitis *et al*., 1994). Neurological symptoms could also be a result of elevated levels of isopropyl alcohol (Phillips, 1978; Foster, 1988), which is a breakdown product of 3-hydroxybutyrate and acetone. Nervous symptom might also be a reflection of widespread neuronal degeneration and haemorrhages in brain (Singh *et al*., 1992).

Haematology
The mean values for haemoglobin, packed cell volume and total erythrocyte account in healthy animals prior to induction of disease were 11.38 ± 0.349 g/dl, 36.16 ± 0.75 percent and 10.96 ± 0.24 x 10^6/mm^3 respectively. These values are within the range reported for normal animals (Jain, 1986; Pernthaner *et al*., 1993). There were no significant changes in these values following induction of pregnancy toxaemia. Similar observations

were recorded by Vihan and Rai (1984); Wierda *et al.* (1985) and Singh *et al.* (1992) both in experimental and spontaneous pregnancy toxaemia in sheep. There was a small but significant increase in leukocyte count following induction of pregnancy toxaemia. This is in agreement with the observations of Vihan and Rai (1984) who also reported leukocytosis in induced pregnancy toxaemia in sheep. Benjamin (1985) also reported leukocytosis in eclampsia. The leukocytosis could be due to stress and adrenal hyperactivity (Reid, 1960; Frobes and Singleton, 1964). This is substantiated by the increased cortisol levels observed in the present study. The differential leukocyte count exhibited a slight increase in neutrophil and eosinophil count. This agrees with the observation made by Vihan and Rai (1984). Radiostitis *et al.* (1994) reported eosinophilia in bovine ketosis.

Blood Biochemistry

Blood Glucose

In healthy animals the mean blood glucose level was 48.3 ± 11.122 mg/dl which is within the normal range reported for sheep (Kaneko, 1989; Singh *et al*., 1992; Radiostitis *et al*., 1994). Following insulin administration a variable response in different animals was observed. In some animals there was a sharp decline in blood glucose at six hours post-induction while as others maintained relatively higher levels up to 12 hours post-induction. On the whole there was a gradual decline in blood glucose following insulin administration. This goes in agreement with Vihan and Rai (1985) who reported a gradual decline in blood glucose up to 6 – 12 hours following subcutaneous administration of insulin in sheep. Reid (1951), however, observed a sharp decline in blood glucose within two hours of insulin injection with levels reaching up to 5 – 10 mg/dl. In the present study a minimum level of 6.2 mg/dl was recorded in only one of the 15 animals studied. Blood glucose was strongly correlated with clinical symptoms. Mean blood glucose level at the first appearance of clinical symptoms was 19.56 ± 2.509 mg/dl. Lateral recumbency was associated with a mean blood glucose level of 13.16 ± 2.7 mg/dl whereas at the convulsive stage the mean level was 16.12 ± 5.01 mg/dl. Procos and Gilchrist (1966) also reported hypoglycaemia prior to the onset of clinical symptoms in experimental pregnancy toxaemia in sheep. Our observations are also in agreement with McClymont and Setchell (1955); Lindsay and Pethick (1983) and Jaffery and Higgins (1992).

Blood Ketones

Blood ketone levels were consistently elevated following induction of pregnancy toxaemia and the increase was in proportion to the decrease in blood glucose. There was a strong negative correlation between blood glucose and blood ketone levels. This is in agreement with the observations of Lindsay and Pethick (1983); Vihan and Rai (1984); Ford and Evans (1986) and Singh *et al.* (1992). Ketones are primarily the products of intermediary metabolism and the main source of blood ketones is fatty acids including those with short, medium and long chains (Kaneko, 1989). Free fatty acids as the main substrate for increased ketogenesis in the present study is substantiated by the fact that free fatty acid levels were also elevated and a positive correlation between blood ketone and free fatty acid levels was observed. The increase in ketone body is a critical step in the glucose sparing metabolism of the ruminant. The ketones act to supply acetyl CoA for oxidation by the peripheral tissues and through this means decrease the demand of the peripheral tissues for glucose as a precursor for adenosine triphosphate (ATP) generation under circumstances when glucose supply is limited (Newscholme and Leech, 1983). In the event of hypoglycaemia, the pregnant ewe has an obligation to utilize non-glucose sources of energy for its own metabolic needs as the foetal drain of glucose continues and 60 – 70 percent of maternal glucose is still consumed by the foetus (Setchell *et al*., 1972; Prior and Christenson, 1978).

Increase in ketone body level was in proportion to the decrease in blood glucose. However, towards the terminal stages of the disease, the ketone level continued to rise even when the blood glucose levels were elevated. This could possibly be due to inhibition of glucose utilization and continued use of free fatty acids as energy source. This is further supported by the fact that the increase in free fatty acid levels continued even after the blood glucose showed as increasing trend towards the terminal stages of the disease. The observations of Ranaweera *et al.* (1979) that increase in blood glucose following treatment in ketotic sheep was not accompanied with decrease in ketone body concentration, further substantiates the view.

Free Fatty Acids

There was a gradual increase in free fatty acid levels of blood from a mean base level of 3.07 ± 0.407 mg/dl in healthy animals to 8.18 ± 0.922 mg/dl in animals with pregnancy toxaemia. This is in agreement with Lindsay and Pethick (1983) and Ford and Evans (1986) who reported increase in free fatty acid levels both in natural and experimental pregnancy toxaemia in sheep. The changes in free fatty acids occur in response to availability of carbohydrate as also the rate of utilization, which depends not only on supply (blood glucose) but also on hormones (Reid, 1968). Increasing trend of free fatty acids towards the terminal stages of the disease, when the blood glucose level was slightly elevated, indicates that glucose utilization is not increased in response to elevated blood glucose levels. This provides further evidence that there occurs an inhibition of glucose utilization in pregnancy toxaemia.

Blood Urea Nitrogen

Mean blood urea nitrogen level in normal animals was 18.64 ± 2.638 mg/dl which is within the range reported for normal animals (Rankins and Smith, 1991; Rankins *et al.*, 1991; Cole, 1992). Following induction of disease

there was an increase in blood urea nitrogen. Increase in blood urea nitrogen has been reported both in experimental and field cases of pregnancy toxaemia (Parry and Taylor, 1956; Lindsay and Pethick, 1983; Kimberling, 1988; Singh *et al*., 1992; Sargison *et al*., 1994). Wierda *et al*. (1985), however, recorded normal blood urea levels in clinical cases of pregnancy toxaemia. In the present study it was observed that blood urea nitrogen levels increased with the progression of the disease. There was a small but significant increase in blood urea nitrogen at the first clinical stage of the disease. However, the levels were greatly elevated (26.32 ± 4.128 mg/dl) at convulsive stage. During early stages of the disease the increase in blood urea nitrogen could have been due to increased gluconeogenesis from the mobilization of amino acids. However, marked increase towards terminal stages could be attributed to renal damage, which is further evidenced by histopathological changes in kidneys. However, terminal uraemia reported earlier (Radiostitis *et al*., 1994) was not observed in the present study.

Increase in blood urea nitrogen levels was not that marked as could be expected in some cases showing severe nephrotic changes on histopathological examination. In such cases it is possible that urea formation, which is almost exclusively a function of liver (White *et al*., 1973), was also affected owing to severe degenerative changes in liver. Some degree of impairment of liver function was also indicated by other liver function tests in the present study. Moreover about 75 percent of nephrons must be non-functional before values outside the normal range are obtained (Bernstein, 1965).

Creatinine

In healthy animals, prior to induction of disease, the serum creatinine was in the range of 0.7 to 1.8 mg/dl with a mean of 1.36 ± 0.301. These values are within the range reported for normal animals (Batra *et al*., 1991; Singh *et al*., 1992; Pernthaner *et al*., 1993). Following induction of disease there was a gradual increase in the serum creatinine levels, marked increase being recorded towards the terminal stages of the disease. Increase in serum creatinine has been reported both in experimental and clinical pregnancy toxaemia (Parry and Taylor, 1956; Kimberling, 1988; Singh *et al*., 1992). The initial mild increase in serum creatinine might be due to increased protein catabolism, to which the animal shifts its metabolism to raise blood glucose in the event of hypoglycaemia. However, marked increase in serum creatinine recorded towards terminal stages of the disease could be attributed to impaired renal function which is further substantiated by histopathological changes in kidneys and increased blood urea nitrogen levels.

Liver Function Tests

Serum enzymology

Aspartate Aminotransferase (AST)

Mean aspartate aminotransferase levels in normal animals were 45.73 ± 9.602 U/L which is within the range reported for normal animals (Pernthaner *et al*., 1993; Ramos *et al*., 1994). There was a gradual increase in aspartate aminotransferase levels following induction of disease. Highly significant increase, with levels reaching to 66.06 ± 10.592 U/L, was observed in advanced stages of the disease. This is in agreement with Vihan and Rai (1985) who also observed increase in aspartate aminotransferase following experimental induction of pregnancy toxaemia in sheep.

Alanine aminotransferase (ALT)

In healthy animals the mean alnine aminotransferase level was 14.6 ± 5.165 U/L, which is within the range reported for normal sheep (Batra *et al*., 1991; Rankins *et al*., 1991; Pernthaner *et al*., 1993; Ramos *et al*., 1994). Following induction of disease there was a gradual increase in serum alanine aminotransferase level as the disease advanced and a highest level of 29.93 ± 5.812 U/L was recorded at the convulsive stage of the disease. Increase in alanine aminotransferase and aspartate aminotransferase levels in bovine ketosis has been reported (Radiostitis *et al*., 1994). Liver of sheep contains high levels of aminotransferases particularly aspartate aminotransferase and these enzymes leak into the serum when there is a damage to this organ (Kaneko, 1989). Increase in aspartate aminotransferase and alanine aminotransferase in hepatic damage in sheep has been reported by many workers (Khan *et al*., 1989; Piper, 1989; Batra *et al*., 1991; Bhaumik and Sharma, 1993).

Arginase

Mean arginase level in healthy animals, prior to induction of disease was 0.6 ± 0.369 U/L, which is within the range reported for normal animals (Mia and Koger, 1979). Following induction of disease, there was a small but significant increase in serum arginase levels. Liver is the richest source of arginase (Aminlari and Vaseghi, 1992), particularly the liver of ureotelic animals (Cornelius *et al*., 1963; Dittrich *et al*., 1974) and its damage results in leakage of this enzyme into the serum. Increase in serum arginase following induced hepatic necrosis has been reported (Grohn *et al*., 1985; Aminlari *et al*., 1994).

On the whole a small but significant increase in the serum levels of aspartate aminotransferase, alanine aminotransferase and arginase indicate a moderate degree of hepatic damage in induced pregnancy toxaemia which is further supported by histopathological changes observed in liver.

Serum Bilirubin

In healthy animals the serum bilirubin was in the range of 0.18 to 0.39 mg/dl with a mean value of 0.28 ± 0.07 mg/dl. This is within the range reported for normal sheep

(Rao *et al*., 1984; Kaneko, 1989; Rankins *et al*., 1991; Nadir *et al*., 1993). Following induction of disease there was a gradual increase in serum bilirubin as the disease progressed. Increase in bilirubin has been reported in sheep with subclinical and clinical pregnancy toxaemia (Henze *et al*., 1994). In the present study increase in serum bilirubin provides further evidence for hepatic damage in pregnancy toxaemia. Increased serum bilirubin as an indicator of hepatic damage has been reported by many workers (Hansen, 1964; Grohn *et al*., 1985; Bhaumik and Sharma, 1993).

BSP Clearance Rate

The mean BSP ($T_{1/2}$) values in normal animals, before induction of disease, were 2.42 ± 0.416 minutes. This is within the range reported for normal sheep (Tucker *et al*., 1971; Sen *et al*., 1976; Grohn *et al*., 1985; Kaneko *et al*., 1989). There was a small but significant decrease in BSP clearance rate following induction of pregnancy toxaemia, though individual variations were observed. BSP clearance rate was found to be correlated with the clinical stage of the disease and uniformly decreasing clearance values (increased $T_{1/2}$ values) were observed as the disease advanced, with mean $T_{1/2}$ values reaching a maximum of 3.88 ± 0.453 minutes. This agrees with Cornelius *et al*. (1958) and Kaneko (1989) who reported decreased BSP clearance rate in sheep with clinical pregnancy toxaemia. The slow BSP clearance could be due to impediment to hepatic blood flow from extensive lipidosis and/or the presence of biochemical lesions associated with cellular necrosis (Cornelius *et al*., 1958). Delayed BSP clearance in liver damage in sheep has been reported (Hansen, 1964; Roberts, 1968; Grohn *et al*., 1985; Bhaumik and Sharma, 1993).

BSP clearance test in general has been recommended as a better measure of liver function than all other liver function tests (West, 1989; Lal *et al*., 1990).

Glucose Tolerance Test

In healthy animals a typical glucose tolerance curve showing peak glucose level at 5 minutes and reaching near normal level at 60 minutes was observed. Mean $T_{1/2}$ and fractional clearance rate (k) of glucose obtained during glucose tolerance testing were 32.33 ± 1.34 minutes and 2.14 ± 0.087 percent/minute respectively. These values are somewhat closer to the normal $T_{1/2}$ and k values of 35 and 1.98 percent/minute reported for cattle (Kaneko and Rhode, 1964). Following disease induction there was a peculiar change in the glucose tolerance curve: the 5 minute peak was distinctly higher and the 60 minute glucose level was relatively higher than the pre-induction value. Higher 5 minute peak and higher 60 minute value are indicative of glucose tolerance (Kaneko, 1989).

There was an increase in $T_{1/2}$ and a decrease in k value following induction of pregnancy toxaemia. This is in agreement with the observations of Bickhardt *et al*. (1989) who observed reduced fractional clearance rate of glucose in ketotic ewes following intravenous glucose tolerance test. Kaneko and Rhode (1964) reported a remarkably increased $T_{1/2}$ value for glucose in spontaneously diabetic cows.

This test together with the changes in the pattern of blood glucose, ketone and free fatty acid levels provide ample evidence that an inhibition of glucose utilization occurs in advanced stages of the pregnancy toxaemia. Inhibition of glucose utilization in bovine pregnancy toxaemia has also been reported by Radiostitis *et al*. (1994).

Propionate Loading Test

A >2 mmol/L increase in blood glucose at seven minutes after propionate loading was maintained up to 12 hours following induction of disease. Thereafter there was a gradual decrease in 7-minute glucose levels till a significantly low value was obtained towards the terminal stages of the disease. Propionate loading test is a measure of liver function in general (Grohn *et al*., 1985) and gluconeogenic ability of liver in particular (Kaneko, 1989). Nearly all the propionic acid reaching liver is either oxidized or converted to glucose (Armstrong, 1965). In ruminants less than 10 percent of glucose is absorbed from the digestive tract and gluconeogenesis, therefore, provides more than 90 percent of the necessary glucose (Elliot, 1980).

The persent study, therefore, provides consistent evidence that the liver function is impaired and gluconeogenic ability of liver is reduced in pregnancy toxaemia. Our observations are in agreement with Kaneko (1989) who reported a decreased response to propionate loading in bovine ketosis and bovine hepatic necrosis.

Blood Hormone Profile

Cortisol

A very wide range of cortisol levels ranging from 0.18 to 1.68 µg/dl was recorded in normal animals, which is within the range reported for normal sheep (Linder, 1959; Reid, 1968; Hill *et al*., 1984). Following induction of pregnancy toxaemia there was a significant increase in serum cortisol levels, the levels being strongly correlated with the clinical stage of the disease. This is in agreement with the observations of Linder (1959); Saba *et al*. (1966); Hill *et al*. (1984); Kimberling *et al*. (1988) and Henze *et al*. (1994) who reported elevated levels of blood cortisol both in spontaneous and experimentally induced pregnancy toxaemia in sheep. The elevated cortisol level has been so consistent an observation that Ford *et al*. (1990) and Radiostitis *et al*. (1994) recommended its use as a valuable diagnostic aid for the diagnosis of pregnancy toxaemia.

The increase in cortisol level could be either due to reproduction by the adrenal glands or due to less excretion by the liver. Some degree of increase in cortisol occurs during advanced pregnancy (Kimberling,

1988; Lean *et al*., 1991). Cortisol is metabolized and excreted by liver. It seems both increased production and decreased excretion are responsible for elevated cortisol levels in pregnancy toxaemia in ewes. This view is supported by inconsistent histopathological changes observed in adrenal glands and biochemical and histopathological evidence of moderate to severe impairment of liver function in the present study.

Increased cortisol has been suggested to inhibit tissue glucose utilization (Reid, 1960). This further substantiates the view that glucose utilization inhibition occurs in advanced stages of pregnancy toxaemia, for which ample evidence is provided by the present study.

Insulin

In healthy animals insulin was found to be in the range of 0.45 – 4.13 ng/ml. These values are within the range of normal values reported for sheep (Christensen *et al*., 1991; Rankins *et al*., 1991). Following induction of disease the insulin levels fluctuated with large individual variations, but showed a declining trend with the advancement of the disease.

Both increased (Leng, 1965) and decreased (Cunningham, 1962) concentrations of insulin have been reported in toxaemic ewes. Leng (1965) and Saba *et al.* (1966), however, found no consistent relationship of insulin with pregnancy toxaemia of sheep. Kronfeld (1963) found increased insulin levels in ketotic cattle but Hove (1978) and Calhoun *et al.* (1962) found decreased levels of insulin in bovine ketosis.

The hypoinsulinaemia might be playing a role in inhibition of glucose utilization which was a consistent finding in the present study. Insulin plays an important role in glucose utilization by facilitating its entry into the cells and by stimulating the enzyme hexokinase (Murray *et al*., 1993). Hypoinsulinaemia might also be favouring ketosis by enhancing fatty acid mobilization and reducing ketone utilization by the peripheral tissues. Insulin reduces lipolysis (Brockman, 1978) and increases the utilization of ketone bodies by the peripheral tissues (Jerrett *et al*., 1974; Robinson and Williamson, 1980).

Glucagon

In healthy animals the blood glucose level, prior to the induction of disease, was in the range of 0.25 – 0.73 ng/ml. This agrees with Christensen *et al.* (1991) who reported a blood glucagon level of 0.27 ng/ml in normal sheep. Following induction of disease there was a gradual decline in blood glucagon level as the disease progressed. Data on role of glucagon in ketosis is sparse (Lean *et al*., 1991). Mills *et al*. (1986) observed decreased levels of glucagon in experimentally induced bovine ketosis. DeBoer *et al*. (1986) also suggested that deficiency of glucagon may be responsible for bovine ketosis.

Glucagon plays a gluconeogenic role (Brockman, 1984; DeBoer *et al*., 1986). Decreased glucagon levels observed in the present study might be hampering gluconeogenesis and therefore, precipitating hypoglycaemia. This is further substantiated by the observations of Wastney *et al*. (1983) that severely undernourished ewes, which were susceptible to pregnancy toxaemia, had a reduced gluconeogenic response as compared to the resistant ones. Kronfeld (1970) suggested inadequate rate of gluconeogenesis to be the main metabolic lesion responsible for pregnancy toxaemia.

Serum Electrolytes

Sodium

Mean values for serum sodium recorded in healthy animals were 153.6 ± 6.344 mEq/L, which is within the range reported for normal animals (Kaneko, 1989; Garcy *et al*., 1990; Rankins and Smith, 1991). The serum sodium levels declined gradually following disease induction till a level of 143.3 ± 4.73 mEq/L was recorded towards terminal stages of the disease. This decrease in sodium level could be due to renal damage (Rose and Carter, 1979; Rose, 1984; Rose *et al*., 1986) which is further substantiated by histopathological changes in kidneys observed in the present study.

Potassium

Serum potassium was in the range of 4.2 – 5.8 mEq/L in healthy animals prior to disease induction. The values are within the range reported for normal animals (Garcy *et al*., 1990; Rankin s and Smith, 1991). Following induction of disease there was an initial decline in serum potassium level which gradually increased as the disease progressed till a moderately significant increase was recorded during terminal stages of the disease. Kimberling (1988) and Henze *et al*. (1994) reported decreased values for serum potassium in clinical and subclinical pregnancy toxaemia in sheep.

The initial hypokalemia might be a result of anorexia (Taskar, 1980), which generally accompanied the clinical syndrome, as also the influx of serum potassium into the cells due to insulin administration (Tannen, 1984). The hyperkalemia observed in advanced stages of the disease could be attributed to cation shift (shifting of H^+ into the cells in exchange for K^+) due to metabolic acidosis. Metabolic acidosis is always observed in advanced stages of pregnancy toxaemia (Radiostitis *et al*., 1994).

Calcium

In healthy animals serum calcium was in the range of 9 – 13 mg/dl with a mean of 11.36 ± 1.262 mg/dl. This is within the range reported for normal animals (Vihan and Rai, 1984; Kaneko, 1989; Garcy *et al*., 1990). There was a small but significant decrease in serum calcium following induction of disease. Decreased serum calcium has been reported both in experimental and clinical pregnancy toxaemia in sheep (Vihan and Rai, 1985; Radiostitis *et al*., 1994).

The decline in serum calcium could probably due to increased loss of base in urine to compensate for acidosis which is generally associated with advanced pregnancy toxaemia (Radiostitis *et al*., 1994).

Magnesium

Serum magnesium in healthy animals was in the range of 1.5 – 2.2 mg/dl. This is within the range reported for normal sheep (Vihan and Rai, 1984; Cole, 1992). Following induction of disease there was a mild increase in serum magnesium, particularly towards the terminal stages. These results are in agreement with the observations of Vihan and Rai (1985) who recorded an increase in serum magnesium following experimental induction of pregnancy toxaemia in sheep. However, Kimberling (1988) reported decreased values of serum magnesium in pregnancy toxaemia.

Phosphorus

Pre-induction serum phosphorus levels in healthy animals were in the range of 5 – 6.9 mg/dl, which is within the range reported for normal animals (Garcy *et al*., 1990; Rankins and Smith, 1991; Rankins *et al*., 1991). Following induction of pregnancy toxaemia, there were mild fluctuations in serum phosphorus but a small decline was observed towards the terminal stages of the disease. Vihan and Rai (1984) observed a non-significant increase in serum phosphorus in experimental pregnancy toxaemia in sheep.

Decline in serum phosphorus observed in the present study could probably due to renal loss of phosphorus as phosphate to counter metabolic acidosis that generally accompanies pregnancy toxaemia.

Pathomorphological Studies

The liver was pale to congest in different animals. There were moderate to severe fatty changes, infiltration of lymphoid cells in some animals and disruption of architecture of hepatic cords in others. This goes in agreement with Vihan and Rai (1985); Tontis and Zwahlen (1987) and Kimberling (1988).

The changes in liver could be due to overwork imposed upon it by excessive mobilization of free fatty acids, amino acids, ketones, cortisol, together with excessive fatty infiltration.

In kidneys nephrotic changes and fatty infiltration was observed. Such changes have also been reported by Hill *et al*. (1984); Vihan and Rai (1985) and Tontis and Zwahlen (1987). Glomerular pathological changes observed in present study disagrees with Hill *et al*. (1984) who did not find glomerular changes in pregnancy toxaemia in sheep. However, our studies are in agreement with Ferris *et al*. (1969) and Vihan and Rai (1985).

The renal changes could possibly be a result of hypoglycaemia.

The adrenals showed congestion, enlargement, fatty changes mild to moderate hyperplasia and disruptions in zona fasciculata. This is in agreement with Hill *et al*. (1984); Vihan and Rai (1985); Tontis and Zwahlen (1987) and Kimberling (1988). However, change in adrenals was not a consistent finding in the present study. In some animals only mild changes were observed. Gross enlargement of adrenals and hyperplasia of zona fasciculata provide an explanation for increased cortisol levels which was a consistent finding in the present study. It implies that excessive secretion alone was not responsible for elevated blood cortisol levels. Probably hepatic removal of cortisol was also affected.

Severe congestion of brain was a consistent observation in the present study. Histopathologically there was severe vascular congestion, leptomeningeal haemorrhages, moderate to severe neuronal degeneration and spongiosis.

Congestion, haemorrhages and neuronal degeneration have also been reported by Vihan and Rai (1985) in experimental pregnancy toxaemia in sheep. Our results are also in agreement with Jeffrey and Higgins (1992) who observed cerebrocortical neuronal necrosis and vacuolation of cerebral and cerebellar subcortical white matter. Leptomeningeal haemorrhages and widespread neuronal degeneration have also been reported by Singh *et al*. (1992) in experimental pregnancy toxaemia in sheep.

The changes in brain could be a result of persistent hypoglycaemia. In addition inhibition of glucose utilization, a consistent observation in the present study, might also have exerted an influence over brain metabolism in causing the lesions observed. Reid (1960) and Santara and Singh (1980) attributed brain lesions to cortisol-induced inhibition of glucose uptake by the central nervous system.

Therapeutic Studies

Though a somewhat satisfactory response to treatment in early stages of the disease has been reported (Reid, 1968; Lindsay and Pethick, 1983), the treatment in advanced stages of the disease has largely been disappointing (Lindsay and Pethick, 1983; Wierda *et al*., 1985; Radiostitis *et al*., 1994). In the present study treatment was administered only in advanced stages of the disease when the animals were in lateral recumbency. This is the stage at which most of the field cases are presented for treatment.

Parenteral glucose therapy was found largely ineffective giving a mere 20 percent recovery rate. This goes in agreement with Dayus and Weighton (1931); Cameron and Goss (1940); Reid (1968).

Failure of parenteral glucose therapy might probably be due to its inability to maintain blood glucose levels for sufficient time to allow for the metabolic disturbance to

stabilize. Moreover, severe hyperlycaemia, induced during first half an hour after parenteral glucose therapy, might be exerting excessive metabolic burden on the already damaged liver and kidney. Hormonal imbalances particularly suppression of glucagon might also probably be responsible for poor response to treatment.

Eighty percent recovery rate was recorded with oral glucose therapy following premedication with vasopressin. This agrees with El-Hamamsy *et al*. (1990) who reported a 100 percent recovery rate with oral glucose therapy following premedication with vasopressin. Buswell *et al*. (1986) also reported 68 percent recovery rate from pregnancy toxaemia with concentrated oral rehydration solution containing glucose and minerals. However, it is not indicated whether it was preceded by any premedication. Ordinarily without any premedication the glucose would be fermented by the rumen microbes and would not be available to the animal as such. Scholz (1990) successfully treated 23 cases of bovine ketosis, which are refractory to parenteral glucose therapy, with oral glucose following premedication with vasopressin.

Vasopressin triggers reticular groove contraction thus allowing the passage of drugs directly into the abomasum (Mikhail, 1982). Glucose administered orally, following premedication with vasopressin, goes directly into the abomasum and is absorbed as in simple stomach animals. Some degree of success with oral administration of propylene glycol (Reid, 1968; Kimberling, 1988) seems to have the same basis as it resists ruminal fermentation and is converted to glucose following absorption.

Glucose administered orally maintained elevated blood glucose levels for a prolonged period of over eight hours, thereby allowing sufficient time for body's compensatory mechanisms to counter the metabolic disturbance. Moreover, gut hormones, released as a result of oral glucose administration, might also be assisting in regularization of the glucostatic hormones and hence the glucostasis. Oral glucose stimulates release of both insulin and glucagon through the action of gastrointestinal hormones like gastrin, secretin, cholecystokinin (CCK), vasoactive intestinal polypeptide (VIP), gastric inhibitory polypeptide (GIP) and enteroglucagon (Clarenburg, 1992; Murray *et al*., 1993). Oral glucose has been found to cause more insulin secretion than the parenteral glucose presumably through the action of gastrointestinal hormones (Clarenburg, 1992).

CONCLUSIONS

1. A clinical syndrome simulating naturally occurring pregnancy toxaemia can be produced experimentally with dietary restriction and insulin administration.
2. Liver and kidney function is impaired in induced pregnancy toxaemia.
3. Glucose intolerance occurs in advanced stages of pregnancy toxaemia, which accounts for the failure of parenteral glucose therapy.
4. Hormonal imbalance plays an important role in the pathogenesis of pregnancy toxaemia.
5. Oral glucose therapy following reticular groove closure effectively treats pregnancy toxaemia and is recommended for field use.

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