BÚVÍSINDI
ICEL. AGR. SCI. *5, 1991:* 25–31

Metabolic disorders in ewes during late pregnancy

HELGI SIGURÐSSON
Institute for Experimental Pathology, University of Iceland, Keldur, Box 8540, IS-128 Reykjavík, Iceland

SUMMARY

Clinical signs and some biochemical blood constituents in paretic ewes during late pregnancy under field condition was studied. Metabolic disorders in 2 out of 6 flocks occurred. Blood samples were obtained from 5 clinically healthy pregnant ewes in each flock at approximately the same stage of gestation as the affected animals (reference groups). Blood samples were also drawn from 13–18 randomly selected pregnant ewes in the same flocks. In addition blood samples were collected from about 80 healthy pregnant ewes in the last month of pregnancy where no metabolic disorders were detected, for establishing "reference values". In flock A, 4 ewes showed symptoms of metabolic disorders. The concentration of calcium in the blood samples from the affected ewes was significantly lower than in the corresponding reference group. There was no hypoglycaemia nor hyperketonaemia so the disorders can be characterised as hypocalaemic paresis. In 4 out of 5 ewes showing symptoms of metabolic disorders in flock B, hypocalcaemia, hypoglycaemia and hyperketonaemia were found, i.e. combination of hypocalcaemia and pregnancy disease. A high concentration of cortisol was found in blood samples from affected ewes in both flock A and B.

Key words: hypocalcaemia, late pregnancy, paretic ewes, pregnancy disease.

YFIRLIT

Brengl í efnaskiptum áa á seinni hluta meðgöngu

Könnuð voru sjúkdómseinkenni og efnaþættir í blóði lambfullra áa sem sýndu einkenni lömunar á seinni hluta meðgöngu. Óregla í efnaskiptum fannst á tveimur bæjum af sex. Blóðsýni voru tekin úr fimm heilbrigðum, lambfullum ám í hópunum á svipuðu stigi meðgöngu og ærnar sem veiktust (viðmiðunarhópar). Blóðsýni voru einnig tekin úr 13–18 lambfullum ám í hópunum völdum tilviljunarkennt. Þá voru tekin blóðsýni úr 80 heilbrigðum, lambfullum ám í síðasta mánuði meðgöngu á bæjum þar sem enginn efnaskiptabrengl átti sér stað til að fá rétt ,,viðmiðunargildi".

Á bænum A voru fjórar ær með lömunareinkenni. Kalsíum í blóði þessara áa var raunhæft minna en í viðmiðunarhópnum. Blóðsykur og ketónefni í blóði voru innan eðlilegra marka þannig að þessi tilfelli flokkast sem doði. Í fjórum af fimm tilvikum, þar sem ærnar sýndu einkenni lömunar á bænum B, fannst lítið af kalsíum og blóðsykri í blóðsýnum og einnig mikið af ketónefnum. Þessi tilfelli flokkast því sem sambland af doða og fósturveiki. Mikið kortisól fannst í blóðsýnum áa með lömunareinkenni á báðum bæjunum A og B.

INTRODUCTION

A great deal of work has been done in studying blood constituents in ewes in late pregnancy under experimental conditions in an attempt to find some parameters which may help to explain the pathogenesis of pregnancy disease (McClymont and Setchell, 1955; Reid, 1968; Wastney *et al*., 1982; Sigurdsson, 1988a). Several papers have described clini-

cal studies of spontaneous cases of paresis in ewes in late pregnancy (Siemesen, 1971; Bostedt, 1976; Bath, 1983; Kjølleberg and Mogstad, 1984, Ford *et al*., 1990). Moreover, several studies seem to indicate that there exist three different metabolic disorders, namely: pregnancy disease (ketosis), hypocalcaemia and a combination of pregnancy disease and hypocalcaemia (Siemesen, 1971; Bath, 1983; Kjølleberg and Mogstad, 1984).

The purpose of the present work was to study clinical signs and some biochemical blood constituents in paretic pregnant ewes in late pregnancy under field conditions.

MATERIAL AND METHODS

Animals

This study was carried out in Denmark in the spring of 1985. It was undertaken in six flocks of pregnant Mask-Texel crossbred ewes. When there were suspected cases of metabolic disorders a clinical examination was carried out and blood samples were drawn before treatment. Blood samples were obtained from 5 clinically healthy pregnant ewes in each flock at approximately the same stage of gestation as the affected animals (reference groups). Blood samples were also drawn from 13–18 randomly selected pregnant ewes in the same flocks.

Metabolic disorder occured in two out of six flocks. In flock A, 500 ewes were fed indoors and received approximately 0.8–1.0 kg/day concentrates and straw *ad libitum* and in flock B, 260 pregnant ewes were on pasture and received in addition varying quantity of hay.

In addition blood samples were collected from about 80 healthy pregnant ewes in the last month of pregnancy in four flocks where no metabolic disorders were detected, for establishing “reference values”. In three of these flocks the ewes were on pasture and received in addition varying quantity of hay or hay and concentrates, but the individual amount consumed is not known and one flock received in addition to pasture about 1.0 kg/day hay, 0.3 kg/day barley and 0.2 kg/day soya the last 50 days of pregnancy.

Blood samples

Blood samples were collected in three different tubes from each ewe: tubes containing heparin (Vacutainer), siliconized tubes (Vacutainer, containing clot activator) and tubes containing fluoride and heparin (Vacutainer). Within 2 hours a 0.5 ml sample of the heparinized whole blood was pipetted into 2.0 ml of 0.6 mmol/l perchloric acid and stored at –20°C until analyzed. The blood samples were centrifuged within 2 hours and plasma, serum and heparin-fluorid plasma were transferred to plastic tubes and stored at –20°C until analyzed. Heparinized plasma was analyzed for calcium (Ca), magnesium (Mg), inorganic phosphate, urea, asparate aminotransferase (ASAT) and creatine kinase (CK). The precipitated whole blood was analyzed for acetoacetate (AA) and beta-hydroxybutyrate (BHB) and the heparin-fluoride plasma was analyzed for glucose. Non-esterified fatty acids (NEFA), insulin and cortisol were analyzed in serum.

Analyses

Calcium and magnesium were measured on an atomic absorption spectrophotometer, Perkin-Elmer 5000. Inorganic phosphate was analyzed on an Ultralab System 2074, calculating absorptiometer. Glucose, ASAT and CK were measured on a Reaction Rate Analyzer (LKB 8600). The reagents were from Merck. Urea was measured by a rapid method from Merck (Mercognost). NEFA was measured by spectrophotometer (Spectronic 21) by the method of Duncomb (1964). Insulin was analyzed by radioimmunoassay using double antibody system. Cortisol was measured using charcoal separation radioimmunoassay and I-125 labelled cortisol as tracer. The concentration of AA and BHB in samples were determined according to a method of Työppönen and Kauppinen

(1980) modified for a Reaction Rate Analyzer (LKB).

The accuracy of the methods was checked regularly against commercially available control samples K 83 (Nyegaard & Co.) and calculated using the differences between duplicate determinations. The control samples are a cooperative system among laboratories in Denmark and checked monthly. The maximal allowed mean coefficient of variation for calcium was 3.0%, for magnesium 2.0%, for inorganic phosphate, ASAT and glucose 5%, for urea 6% and for CK 15%.

The other constituents were not checked by control samples, but the precision of the method employed was calculated using the differences between duplicate determinations (interassay variation). The mean coefficient of variation for 30 determinations was as follows: BHB 8.9%, AA 11.2%, NEFA 17.6%, insulin 13.3% and cortisol 6.8%.

RESULTS

Metabolic disorders occurred in two out of six flocks. In flock A, where the ewes were fed indoors and received concentrates and straw *ad libitum*, 4 ewes showed symptoms of metabolic disorders and 2 were found dead. About 75% of the ewes had given birth when the disorders occurred.

In flock B, 5 ewes showed symptoms of metabolic disorders. The 260 pregnant ewes in flock B were on pasture and received in addition varying quantities of hay. Just before they should have given birth, they were transferred to the farm and sheared around the anal and vulva region. The disorders occurred the day after the ewes were transferred.

Clinical symptoms

In flock A, 4 ewes showed symptoms of metabolic disorders. The ewes where approximately 4–6 years old. They had either stilted gait, were atactic or recumbent in sternal recumbancy. In three cases the pupils were dilated and the pupillary light reflex slow. The eye preservation reflex was not affected. Body temperature was about 39°C and the pulse rate was more than 100 per minute. The ears were not cold and there were no signs of twitching and clonic seizures. The ewes showed no interest in food. Two of the ewes gave birth to singles, while two gave birth to twins.

In flock B, 5 ewes showed symptoms of metabolic disorders. The ewes were 3–8 years old. The gait was stilted, the ewes showed depression of consciousness or they were unable to stand. In one case fine myoclonic twitching of the ears and periorbital muscles was noted. The ewe walked into obstacles or was circling. Every now and then clonic contractions of muscle groups of the neck, back and forelimbs were noted as they spread from the head backwards. Body temperature was in all cases around 39°C and the pulse rate more than 100 per minute. The pupillary light reflex was slow and the pupils were dilated in all but one case, where it was constricted. The eye preservation reflex was not affected. The ewes showed no interest in food. The 3 oldest ewes (7–8 years) were undernourished, but the others (3 and 4 years) were over-fat. In three cases acetone could be smelled from their nostrils. Two of the ewes gave birth to twins but the others gave birth to triplets.

Biochemical blood constituents

Table 1 shows the results of analysis of blood samples obtained before treatment from the ewes diagnosed as suffering from metabolic disorders. It shows means and standard deviations of the same blood constituents as in the reference groups (5 ewes at approximately the same stage of gestation as the affected ewes). The table shows means and standard deviations of 13 and 18 randomly selected pregnant ewes in flock A and B, respectively. Table 1 also shows the results of blood samples taken in the last month of pregnancy from some 80 healthy pregnant

Table 1. Concentration of blood constituents of affected ewes in two flocks, in reference groups, randomly selected other ewes and reference values.
1. tafla. Styrkur efnaþátta í blóði veikra áa á tveimur bæjum, í viðmiðunarhópum, í öðrum ám völdum tilviljunarkennt og viðmiðunargildi.

	Calcium mmol/l	Magnesium mmol/l	Inorganic phosphate mmol/l	Aceto-acetate mmol/l	b-hydroxy-butyrate mmol/l	NEFA mEq/l
Flock A						
Ewe no. 1	1.34	1.80	1.67	0.16	0.54	1.09
Ewe no. 2	0.95	0.98	1.27	0.21	0.37	0.96
Ewe no. 3	0.67	0.85	0.61	0.21	0.31	0.93
Ewe no. 4	0.79	0.94		0.10	0.71	1.44
x̄: ewe 1–4	0.94±0.29***	1.44±0.44	1.18±0.53	0.17±0.05	0.48±0.18	1.11±0.23*
x̄: ref. group	2.40±0.28	0.95±0.05	1.58±0.15	0.17±0.04	0.61±0.23	0.75±0.15
x̄: other ewes	2.40±0.25	0.95±0.06	2.00±0.45	0.18±0.03	0.37±0.06	0.42±0.19
Flock B						
Ewe no. 5	1.76	0.74	2.40	0.36	3.17	1.97
Ewe no. 6	1.97	0.79	1.33	0.36	2.69	0.95
Ewe no. 7	1.91	1.00	1.95	0.35	2.30	2.20
Ewe no. 8	2.22	0.50	1.73	0.12	0.67	0.63
Ewe no. 9	1.78	0.87	1.54	0.37	2.37	1.16
x̄: ewe 5,6,7,9	1.86±0.10**	0.85±0.11	1.81±0.47	0.36±0.01*	2.63±0.40**	1.57±0.61
x̄: ref. group	2.22±0.15	0.98±0.13	1.75±0.23	0.18±0.13	0.95±0.59	1.19±0.62
x̄: other ewes	2.10±0.26	0.89±0.25	1.61±0.58	0.13±0.04	0.52±0.12	1.25±0.96
Ref. value	2.30±0.21	0.95±0.15	1.61±0.42	0.15±0.07	0.80±0.41	0.99±0.44

	Glucose mmol/l	Insulin ng/ml	Urea mmol/l	ASAT µkat/l	CK µkat/l	Cortisol nmol/l
Flock A						
Ewe no. 1	2.80	<0.10	28.00	4.08	22.38	207.00
Ewe no. 2	2.95	0.20	7.00	1.70	3.23	
Ewe no. 3	7.22	0.44	8.00	2.39	19.25	
Ewe no. 4		<0.10	8.00	2.47	10.60	151.00
x̄: ewe 1–4	3.24±2.97	Low	12.75±10.18	2.66±1.00*	13.87±8.67*	
x̄: ref. group	3.75±0.44	0.75±0.62	6.80±0.84	1.37±0.11	4.26±0.85	<26
x̄: other ewes	2.93±0.31	0.60±0.32	9.17±1.53			
Flock B						
Ewe no. 5	2.00	<0.10	12.00	2.95	25.25	45.00
Ewe no. 6	0.95	0.18	6.00	1.83	2.36	50.00
Ewe no. 7	1.47	<0.10	9.00	3.11	3.67	59.00
Ewe no. 8	3.68	4.98	6.00	0.44	3.47	40.00
Ewe no. 9	1.58	<0.10	7.00	2.86	3.88	33.00
x̄: ewe 5,6,7,9	1.50±0.43*	Low	8.50±2.65*	2.69±0.58	8.79±11.00	46.75±10.84
x̄: ref. group	2.42±0.55	0.24±0.13	4.80±1.64	1.90±0.63	3.22±3.82	<26
x̄: other ewes	2.39±0.81	0.12±0.04	5.33±1.24			
Ref. value	3.08±0.71	0.42±0.22	8.86±2.96	1.53±0.65	3.85±2.19	

*P<0.05; **P<0.01; ***P<0.001.

ewes in four flocks, where no metabolic disorders were detected, for establishing "reference values".

The concentration of calcium in the blood samples obtained from ewes showing symptoms of metabolic disorders in flock A, was significantly lower than in the reference group (0.94±0.24 mmol/l, P<0.001). There was no hypoglycaemia nor hyperketonaemia. The insulin concentrations of ewes with hypo-

calcaemia were low compared with the other ewes studied in the flock.

The results of the analysis of blood samples from ewes in flock B showing symptoms of metabolic disorders, revealed that more blood constituents were significantly different from those of the reference group in the same flock than in flock A. Thus low concentrations of calcium, glucose and insulin were found in blood samples from 4 out of 5 ewes showing symptoms. In addition significantly higher concentrations of acetoacetate, b-hydroxybutyrate and NEFA were found in the blood samples from these ewes than in the reference group. A low concentration of magnesium was found in one ewe (ewe no. 8; 0.50 mmol/l), but the concentration of glucose was within the reference range. In four out of five cases in flock B hypocalcaemia, hypoglycaemia and hyperketonaemia were found. The blood insulin concentration in ewes with both pregnancy disease and hypocalcaemia was low, but the concentration was also low in the blood of the other ewes studied in the flock.

In blood samples from 5 out of 18 randomly selected healthy, pregnant ewes in flock B calcium concentration lower than 2.0 mmol/l was found (1.77±0.14 mmol/l), while the other ewes studied (13 ewes) had 2.30±0.22 mmol/l. The ewes showed no symptoms of metabolic disorders. The calcium concentration in the reference group and the group of randomly selected pregnant ewes in flock B was lower than in corresponding groups of flock A.

A very high concentration of cortisol was found in blood samples from 2 hypocalcaemic ewes in flock A, 151 and 207 nmol/l, respectively. The mean concentration of cortisol in blood samples from ewes showing symptoms in flock B was 46.75 nmol/l, a little higher than in the reference group in the same flock (<26 nmol/l). The highest concentration of cortisol (59 nmol/l) was found in the blood sample from ewe no. 7, showing the most typical clinical signs of pregnancy disease.

DISCUSSION

The symptoms of metabolic disorders in flock A and B were not very different, although more acute in flock A. In flock A there was no hypoglycaemia nor hyperketonaemia so the disorders can be characterized as hypocalcaemic paresis. In four out of five cases in flock B hypocalcaemia, hypoglycaemia and hyperketonaemia were found, i.e. a combination of hypocalcaemia and pregnancy disease.

In just one case in flock B similar symptoms of pregnancy disease were noted as described by McClymont and Setchell (1955). The metabolic disorders in flock B can be characterized as being the subacute syndrome of pregnancy disease described by Reid (1960a) induced by fasting and psychological stress. The insulin concentration in these ewes was low, but the concentration was also low in the blood of the other ewes studied in the flock. The mean concentration of cortisol in blood samples from ewes showing clinical signs of pregnancy disease was little higher than in the reference group in the same flock. This, together with the development of the subacute syndrome is in agreement with observations made by Reid (1960b). Reid (1960b) postulated that a state of insulin deficiency in pregnancy disease is an attractive explanation of the pathogenesis and that the possibility remains that the inhibitory effects of cortisol on glucose utilization may be enhanced under conditions of severe insulin insufficiency. In that case the severity of ketosis could depend on the balance between cortisol and insulin rather than on the absolute amount of each hormone secreted. The degree of inhibition of glucose utilization and an appearance of clinical signs could then depend on this balance. In another study the author has made similar observations (Sigurdsson, 1988a). The ability of 7 pregnant ewes to maintain glucose homeostasis following a glucose tolerance test and during starvation was studied. Fol-

lowing an intravenous load 2 out of 7 ewes tested showed an impaired insulin secretion during the first 10–15 min. Two of the twin-bearing ewes developed symptoms of pregnancy disease following starvation, these being the same ewes that showed an impaired insulin secretion after the glucose load. The concentration of cortisol was also significantly higher in the blood of the ewes showing symptoms of pregnancy disease than in the blood of the other twin-bearing ewes that did not. It was concluded that the results are in agreement with those postulated by Reid (1960b).

Several authors have reported high plasma cortisol concentration in ewes suffering from pregnancy disease (Assali *et al.*, 1958; Lindner, 1959; Reid, 1960b; Ford *et al.*, 1990). On the other hand, Saba *et al*. (1966) could not observe such a high cortisol concentration in ewes with clinical signs of the subacute syndrome of pregnancy disease, but the insulin concentration was low. Saba and Cunningham (1971) reported elevated cortisol concentration in the blood of ewes with clinical hypocalcaemia. In the present work very high concentration of cortisol was found in blood samples of 2 hypocalcaemic ewes in flock A, much higher than in ewes in flock B suffering from metabolic disorders. The association of ovine hypocalcaemia with stress is well established. Moseley and Axford (1973) observed that changes in plasma calcium concentration were accompanied by reciprocal changes in the plasma concentration of NEFA. In the present work the NEFA concentration in ewes in flock A suffering from hypocalcaemia was significantly higher than in the reference group. In flock A the ewes were fed large amounts of concentrates and it has been shown that 0.4 kg/day of concentrates or more induces a drop in serum calcium (Jones and Luthman, 1978). This together with stress could be a possible explanation.

In flock B a calcium concentration lower than 2.0 mmol/l was noted in 5 out of 18 randomly selected ewes. The ewes showed no symptoms of metabolic disorders, but they had been exposed to stress. The calcium concentration in blood samples from ewes in flock B was rather low, which may be explained by different feeding practices in the flocks. In a study of the effect of flock, number of fetuses and age on some biochemical blood constituents in ewes during late pregnancy under field conditions, the effect of flock was significant for calcium and magnesium (Sigurdsson, 1988b). A low concentration of calcium in the blood of ewes suffering from pregnancy disease might play an important role in the pathogenesis of the disease and should always been accounted for when publishing studies on pregnancy disease. The possibility remains that pregnancy disease is always accompanied by a rather low concentration of calcium in the blood.

A normal secretion of insulin depends on an adequate calcium concentration in the blood (Ganong, 1977) and a relationship is seen between low calcium concentration and low insulin concentration in the blood of ewes with clinical symptoms in flock A. This limits the use of measurements of insulin concentration in the blood of ewes in late pregnancy to reveal a pressed glucose homeostasis.

In this study a great deal of work was done in establishing “reference values” from clinically healthy pregnant ewes in the same flock at approximately the same stage of gestation as the affected animals. This is an important basis for clinical chemical diagnosis often missed in studies under field conditions.

The results of this study are in agreement with results of other authors (Siemesen, 1971; Bath, 1983; Kjølleberg and Mogstad, 1984) showing a combination of hypocalcaemia and pregnancy disease under field conditions. This should be taken into account under practical circumstances both when treatment is carried out as well as when studying

experimental and spontaneous cases of metabolic disorders.

It is concluded that stress is apparently an important factor in the pathogenesis of both pregnancy disease and hypocalcaemia in ewes.

ACKNOWLEDGEMENTS

This work was supported by the Icelandic Science Foundation. I wish to thank members of the Clinical Central Laboratory, Royal Veterinary and Agricultural University, Copenhagen for their contribution.

REFERENCES

Assali, N.S., L. **Holm** & D.L. **Hutchinson**, 1958. Renal hemodynomics, electrolyte excretion and water metabolism in pregnant sheep before and after induction of toxaemia of pregnancy. *Circulation Research* **6**: 468–476.

Bath, G.K., 1983. Differentiation between hypocalcaemia and pregnancy ketosis of sheep. In: *Abstracts XXII, World Veterinary Congress*: 147.

Bostedt, H., 1976. Beitrag zum Problem des festliegens bei Schafen in der Zeit um die Geburt. *Berliner und Münchener Tierärztliche Wochenschrift* **89**: 156–161.

Duncomb, W.G., 1964. The colorimetric microdetermination of non-esterified fatty acids in plasma. *Clinical Chemical Acta* **9**: 122.

Ford, E.J.H., J. **Evans** & I. **Robinson**, 1990. Cortisol in pregnancy toxamia of sheep. *British Veterinary Journal* **146**: 539–542.

Ganong, W.F., 1977. *Review of Medical Physiology*. 6th edn. Lange Medical Publications, Los Altos, California: 577 pp.

Jones, B. & J. **Luthman**, 1978. Feeding induced hypocalcaemia. *Acta Veterinaria Scandinavica* **19**: 204–214.

Kjølleberg, K. & O. **Mogstad**, 1984. Mjölkefeber/ketose hos sau før lamming. *Norsk veterinærtidskrift* **96**: 2.

Lindner, H.R., 1959. Blood cortisol in sheep, normal concentration and changes in ketosis of pregnancy. *Nature, London* **184**: 1645–1646.

McClymont, G.L. & B.P. **Setchell**, 1955. Ovine pregnancy toxaemia. I. Tentative identification as a hypocalcaemic encephalopaty. *Australian Veterinary Journal* **31**: 53–68.

Moseley, G. & R.E.E. **Axford**, 1973. The effect of stress on the redistribution of calcium in sheep. *Journal of Agricultural Science, Cambridge* **81**: 403–409.

Reid, R.L., 1960a. Studies on the carbohydrate metabolism of sheep. X. Futher studies on hypoglycaemia and hyperketonaemia in ewes with pregnancy toxaemia. *Australian Journal of Agricultural Research* **11**: 346–363.

Reid, R.L., 1960b. Studies on the carbohydrate metabolism of sheep. XI. The role of the adrenals in ovine pregnancy toxaemia. *Australian Journal of Agricultural Research* **11**: 364–382.

Reid, R.L., 1968. The physiopathology of undernourishment in pregnant sheep with particular reference to pregnancy toxaemia. *Advances in Veterinary Science* **12**: 163–238.

Saba, N., K.N. **Burns**, N.F. **Cunningham**, C. **Nancy Hebert** & D.S.P. **Patterson**, 1966. Some biochemical and hormonal aspects of experimental ovine pregnancy toxaemia. *Journal of Agricultural Science, Cambridge* **67**: 129–138.

Saba, N. & N.F. **Cunningham**, 1971. Plasma corticosteroid levels in ovine pregnancy toxaemia and hypocalcaemia. *Research in Veterinary Science* **12**: 483–485.

Sigurdsson, Helgi, 1988a. Susceptibility to pregnancy disease and its relation to gestational diabetes. *Acta Veterinaria Scandinavica* **29**: 407–414.

Sigurdsson, Helgi, 1988b. The effect of flock, number of fetuses and age on some biochemical blood constituents in ewes in late pregnancy under field conditions. *Journal of Veterinary Medicine* **A35**: 417–423.

Siemesen, M.G., 1971. Undersøgelser vedrørende drægtighedssyge hos får. *Nordisk veterinærmedicin* **23**: 99–113.

Työppönen, J. & K. **Kauppinen**, 1980. The stability and automatic determination of ketone bodies in the blood samples taken in field conditions. *Acta Veterinaria Scandinavica* **21**: 55–61.

Wastney, M.E., A.C. **Arcus**, R. **Bickerstaffe** & J.E. **Wolff**, 1982. Glucose tolerance in ewes and susceptibility to pregnancy toxaemia. *Australian Journal of Biological Sciences* **35**: 381–392.

Manuscript received 23 September 1991,
accepted 10 December 1991.