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Asian Journal of Animal and Veterinary Advances

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Journals Inc.

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Asian Journal of Animal and Veterinary Advances 6 (2): 175-181, 2011
ISSN 1683-9919 / DOI: 10.3923/ajava.2011.175.181

Biochemical Indices in Sheep During Different Stages of Pregnancy

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ABSTRACT

The biochemical evaluation of sheep and its foetal fluids was carried out at different stages of pregnancy. The whole study consisted of fifty two numbers of gravid genitalia collected from the local slaughter houses present in and around Srinagar city. In the present study the gravid uteri of sheep ranging from 14 to 140 days of gestation was divided into 5 groups e.g., Group A (GpA), Range: 14-57 days and Mean: 41.40±2.17; Group B (GpB), Range: 58-74 and Mean: 67.87±1.38; Group C (GpC), Range: 75-93 and Mean: 89.20±2.33, Group D (GpD), Range: 94-120 and Mean: 110.60±3.14 and Group E (GpE), Range: 121-140 and Mean: 130.0±2.79. The pH in amniotic and allantoic fluid showed shift from slight alkaline behaviour to slight acidic. While in maternal serum it remained almost alkaline throughout in different stages of pregnancy. Glucose level decreased in foetal fluids while increased in serum. A gradual increase in total protein was observed in foetal fluids and serum with a marked increase at late pregnancy. Total protein showed gradual increase with marked increase in serum during late pregnancy. Creatinine, urea and urea nitrogen showed marked increase in foetal fluids as well as in maternal serum. No change was detected in cholesterol level in amniotic as well as in allantoic fluid while a significant decrease in the level was observed in maternal serum during different stages of pregnancy. Level of transaminases (Alanine aminotransferase and Aspartate aminotransferase) remained almost constant in foetal fluids with a gradual decrease in maternal serum at various stages of pregnancy. Alkaline phosphatase showed increasing trend with gestation in foetal fluids and maternal serum as well.

Key words: Biochemical indices, foetal fluids, pregnancy, sheep, serum

INTRODUCTION

Ewes should be in good health during and after pregnancy so as to produce viable lambs. The identification of changes in the metabolism of such sheep in various production phases, the determination of abnormal metabolic states and the prediction of some metabolic disorders such as pregnancy toxaemia and fatty liver could provide some advantages to producers. Metabolic profiles have been used to predict prepartum and postpartum metabolic problems and for the diagnosis of metabolic diseases and the assessment of the nutritional status of animals. The study of biochemical profiles both in maternal serum and foetal fluids is a tool for pregnancy diagnosis and the status of growing foetus. Blood biochemical evaluation plays an important role in diagnosis of diseases. Serum biochemical parameters varied widely and preferably glucose and urea individually or in combination are the useful parameters in diagnosis of physiological and pathological conditions in

ewes. Glucose as a source of energy is necessary for production and reproduction performance (Radostits *et al.*, 2000). It is the major metabolite used by the sheep foetus and the energy requirements of the ewe increase during late pregnancy due to rapid growth of the foetus (Firat and Ozpinar, 2002). The concentration of glucose in ewes has been reported between 35 and 45 mg dL^{-1} (Nelson and Guss, 1992) and could be affected by physiological (Firat and Ozpinar, 1996) and disease conditions (Symonds *et al.*, 1986; Ford *et al.*, 1990). The presence of significant correlations among serum parameters including glucose, betahydroxybutyrate, cholesterol, total protein concentrations and urea in non-pregnant ewes could be useful to compare with values in late pregnant ewes in order to study need of the dam and pregnancy toxemia (Ramin *et al.*, 2005; Firat and Ozpinar, 2002; Nazifi *et al.*, 2002; El-Sherif and Assad, 2001; Brozostowski *et al.*, 1996). Therefore, understanding the normal values would be the useful index in the determination of the physiological aspects in non-pregnant or pregnant ewes. Biochemical profiles also carry important role not only during prenatal development but also during neonatal or postnatal period. Because the initial period of four weeks after birth of the lamb is critical for its survival if dietary deficiencies remain there by Agrawal *et al.* (2007). Determination of normal values are important for the clinical interpretation of laboratory data. These indices may vary depending on factors such as sex, age, weather, stress, season and physical exercise (Kaneko *et al.*, 1999). Mufti *et al.* (2000) reported that as the gestation age advanced, marked changes occur in total protein, glucose, urea and creatinine in allantoic, amniotic and maternal serum in ewes. Activity of enzymes like Aspartate aminotransferase, Alanine aminotransferase and Alkaline phosphatase was studied by Chauhan and Tiwari (1996) in the amniotic fluids of goat. The study thus was performed to investigate blood metabolite concentrations as an indicator of the metabolic needs at different stages of pregnancy.

MATERIALS AND METHODS

Fifty two numbers of female genitalia from healthy pregnant sheep were used for the present study. The organs were collected from local abattoirs present in and around Srinagar City during months of August to October in the year 2007. The samples were collected immediately after slaughter and carried to the laboratory in thermos container for investigation. The animals were grouped on the basis of whole - embryo length (WEL for embryos) and crown-anus length (CAL for foetus) (Table 1).

Blood samples (10-15 mL) were collected via jugular venupuncture from dams in sterile glass tube. Serum was separated using standard procedures and divided in aliquots. For collection of allantoic and amniotic fluids, the gravid uteri were cut open through the dorsal curvature without damaging the foetal sacs. Sterile syringes and needles were used for collection of these fluids and stored in sterile screw caped glass tubes for further studies.

The pH of serum and foetal fluids was recorded by using digital pH meter. Glucose was estimated instantaneously using orthotoluidine method of Yee *et al.* (1971). Rest of the samples was stored at -20°C until used for biochemical analysis. Biuret method as described by Henry *et al.* (1974) was used for estimation of total protein. For the estimation of urea and cholesterol standard spectrophotometric methods were used (Natelson *et al.*, 1951; Zlatkis *et al.*, 1953), respectively and creatinine was estimated as per Jaffe's method (Owen *et al.*, 1954) simultaneously urea nitrogen was calculated.

Enzymes Alanine aminotransferase and Aspartate aminotransferase were estimated by the methods as described by Reitman and Frankel (1957). Alkaline phosphatase activity was determined using method of King and King (1954).

Table 1: Grouping of pregnant sheep on the basis of whole-embryo length (for embryos) and crown-anus length (for foetus) during gestation period

Groups	No. of gravid genitalia studied	W.E/C.A. length in cm		Age group in days	
		Range	Average	Range	Average
A	30	0.1-10.0	4.50±0.59	14-57	41.40±2.17
B	08	10.1-20.0	15.37±0.69	58-74	67.87±1.38
C	05	20.1-30.0	25.60±1.15	75-93	89.20±2.33
D	05	30.1-40.0	35.74±1.46	94-120	110.60±3.14
E	04	40.1-50.0	45.17±1.33	121-140	130.00±2.79

RESULTS AND DISCUSSION

It was observed that pregnancy has profound effects on some important blood and foetal fluid metabolites while fewer remain unaltered. The mean pH values from GpA to GpE in the amniotic fluid was almost alkaline in all the groups but in allantoic fluid it became acidic in GpE, otherwise it was alkaline in remaining groups (Table 2). In amniotic fluid GpA and GpC, GpA and GpE, GpB and GpE differed significantly ($p = 0.05$). In case of allantoic fluid GpA and GpB, GpA and GpC, GpA and GpD, GpA and GpE, GpB and GpE, GpD and GpE showed significant variation ($p = 0.05$). The mean value of pH in maternal serum was also alkaline in all the groups of gestation period. The differences in between GpA and GpE were significant ($p = 0.05$) (Table 2). The alkaline nature of amniotic fluid and slightly acidic nature of allantoic fluid during different stages of gestation could be due to the biochemical status of the two fluids. Maternal serum had alkaline pH. No related reports were available for comparison in the present study.

Mean values of glucose both in amniotic and allantoic fluid showed similar shift i.e., slightly declining trend as the gestation advanced but the changes were very less amongst groups. In case of amniotic fluid GpA and GpD, GpA and GpE, GpB and GpE, GpC and GpD, GpC and GpE, GpD and GpE had significant differences ($p = 0.05$), however in case of allantoic fluid only in between GpA and GpE there occured significant decrease ($p = 0.05$) (Table 2). The glucose level in maternal serum increased as the gestation period progressed and that increased significantly almost in all the groups except, in GpA and GpB, GpA and GpC, GpD and GpE (Table 2). The significant increasing trend in glucose level in advanced pregnancy in maternal serum while decreasing trend in foetal fluids with the advanced pregnancy was confirmed earlier by Mufti (1995). The change is an indicative of metabolic changes taking place from one stage of pregnancy to another. The increase in glucose level in maternal blood serum was also in agreement with those of Baetz *et al.* (1976), Lathura *et al.* (1987) and McCrabb *et al.* (1991).

The concentration of total protein in serum was much higher in comparison to foetal fluids (Table 2). The mean protein concentrations both in foetal fluids and maternal serum increased as the gestation period progressed. The values in maternal serum and allantoic fluid differed significantly ($p = 0.05$) in all the groups except, GpB and GpD, GpC and GpD in serum and GpA and GpB, GpA and GpC, GpB and GpC in allantoic fluid. In amniotic fluid total protein values in between GpA and GpE increased significantly ($p = 0.05$). An increased level of total protein in foetal fluids and maternal serum during pregnancy has been reported by Kaneko and Corneleous (1970), McDonald (1980), Lathura *et al.* (1987) and Mufti *et al.* (1995). Increased concentration of total protein in allantoic fluid than amniotic fluid has also been reported by Lathura *et al.* (1987) and Mufti *et al.* (1995). McDonald (1980) reported that increased protein concentration in maternal circulation indicates a positive association of pregnancy establishment.

Table 2: Values of biochemical constituents of foetal fluid and maternal serum during different stages of gestation in sheep

		Groups (Length of embryo and CA length of foetus in cm/Age in days)				
Parameters	Types of fluid	A (0.1-10/14-57)	B (10.1-20/58-74)	C (20.1-30/75-93)	D (30.140/94-120)	E (40.1-50/121-140)
pH	Amniotic	7.58±0.18^{a}	7.37±0.29^{af}	6.88±0.23^{cfg}	7.03±0.34^{afg}	6.53±0.09^{eg}
(Mean±SE)	Allantoic	7.46±0.17^{a}	6.89±0.21^{bf}	6.72±0.43^{cfg}	6.9±0.15^{df}	6.34±0.12^{eg}
	Serum	7.53±0.09^{a}	7.60±0.12^{af}	7.62±0.16^{ag}	±0.20^{ag}	7.38±0.17^{efg}
Glucose	Amniotic	4.33±0.42^{a}	4.24±0.62^{afg}	3.88±0.14^{af}	3.41±0.21^{dg}	2.92±0.15^{e}
(Mean±SE)	fluid (mg %)					
	Allantoic	3.57±0.34^{a}	3.49±0.78^{af}	2.8±0.85^{af}	3.09±0.93^{af}	2.30±0.43^{ef}
	fluid (mg %)					
	Serum (mg %)	56.85±2.22^{ac}	54.31±1.33^{a}	59.8±0.88^{c}	63.42±1.75^{df}	65.22±1.90^{ef}
Total protein	Amniotic	0.40±0.06^{a}	0.42±0.12^{af}	0.43±0.17^{af}	0.55±0.12^{af}	0.84±0.26^{ef}
(Mean±SE)	fluid (g dL^{-1})					
	Allantoic	0.48±0.06^{a}	0.47±0.03^{a}	0.56±0.087^{a}	0.93±0.09^{d}	1.42±0.20^{e}
	fluid (g dL^{-1})					
	Serum	6.94±0.82^{a}	11.1.0±0.62^{bf}	13.0±0.75^{eg}	13.11±.97^{dfg}	16.92±0.69^{e}
	(g dL^{-1})					
Creatinine	Amniotic	1.52±0.26^{a}	5.34±0.35^{b}	7.34±1.19^{ef}	9.30±0.65^{df}	13.67±0.75^{e}
(Mean±SE)	fluid (mg %)					
	Allantoic	2.21±0.37^{a}	6.09±0.43^{b}	11.36±0.88^{c}	15.01±0.10^{d}	16.77±0.31^{e}
	fluid (mg %)					
	Serum (mg %)	2.23±0.36^{a}	6.25±0.26^{b}	11.64±1.26^{c}	16.54±0.58^{d}	23.88±1.14^{e}
Urea	Amniotic	14.01±0.72^{a}	20.16±0.66^{b}	23.32±1.20^{c}	34.3±1.41^{d}	38.1±0.47^{e}
(Mean±SE)	fluid (mg %)					
	Allantoic	16.60±1.02^{a}	28.93±1.26^{b}	37.63±3.48^{c}	52.08±2.96^{d}	72.36±1.08^{e}
	fluid (mg %)					
	Serum (mg %)	61.93±2.46^{a}	88.19±3.42^{b}	96.95±0.39^{cf}	107.70±4.96^{dfg}	114.71±1.68^{eg}
Urea uitrogen						
(Mean±SE)	Amniotic					
	fluid (mg %)	6.54±0.34^{a}	9.41±0.31^{b}	10.89±0.56^{c}	16.02±0.66^{d}	17.79±0.22^{e}
	Allantoic					
	fluid (mg %)	7.75±0.48^{a}	13.51±0.59^{b}	17.57±1.62^{c}	24.32±1.38^{d}	33.79±0.50^{e}
	Serum (mg %)	28.92±1.15^{a}	41.19±1.60^{b}	45.28±0.18^{c}	50.30±2.32^{df}	53.57±0.79^{ef}
Cholesterol	Amniotic	4.26±0.15^{a}	4.25±0.09^{a}	4.2±0.09^{a}	4.36±0.16^{a}	4.15±0.10^{a}
(Mean±SE)	fluid (mg %)					
	Allantoic	3.13±0.19^{a}	3.13±0.08^{a}	3.16±0.08^{a}	3.18±0.09^{a}	3.15±0.10^{a}
	fluid (mg %)					
	Serum (mg %)	107.62±3.44^{a}	82±5.20^{b}	65.8±2.40^{cf}	63.8±1.91^{df}	57.75±1.31^{e}

Values with different superscript are significant and having any one of the common superscript are non significant, p = 0.05. Comparison was made by applying students t-test

As shown in Table 2, creatinine, urea and urea nitrogen showed in general a significant increasing trend in dam serum and foetal fluids during different stages of pregnancy except in between GpC and GpD, GpD and GpE (urea level in serum), in between GpD and GpE (urea nitrogen level in serum) and in between GpC and GpD (creatinine level in amniotic fluid). Increased level of urea nitrogen during pregnancy has been reported by Mellor and Slatter (1971), Chauhan and Tiwari (1996) and Mufti *et al.* (2000). The increased level of creatinine in pregnant animal serum as well as in foetal fluids has been explained by Stainer (1965). Lack of exchange of solutes from allantoic fluid may be the cause of increase.

The mean cholesterol values in amniotic and allantoic fluids observed from GpA to GpE showed no significant difference among the groups (Table 2).The maternal serum cholesterol values showed a decreasing trend from 107.62±3.44 in GpA to 57.75±1.31 in GpE (Table 2). The observed values from GpA to GpE in serum decreased significantly ($p = 0.05$) except in between GpC and GpD. In present case serum cholesterol level is much higher than foetal fluids. With the advancement of pregnancy, serum cholesterol level shows a decreasing trend (Mufti, 1995). In whole gestation period, the level of cholesterol remained constant in foetal fluids serving need for synthesis of progesterone (Mufti, 1995). In contrast Ozpinodotnar and Finodotrat (2003) however reported that plasma cholesterol levels were not significantly different between pre-pregnancy, pregnancy and early lactation periods in ewes.

The mean concentrations of AST showed similar changes both in amniotic and allantoic fluids. In amniotic and allantoic fluids the concentrations showed decreasing trend from GpA to GpC and again increased in GpD and GpE (Table 3). In maternal serum concentrations decreased as gestation period advanced. In amniotic fluid in between GpA and GpB, GpA and GpC and in allantoic fluid in between GpA and GpC the values decreased significantly ($p = 0.05$). In serum in all the groups the enzyme concentration decreased significantly except in between GpC and GpD, GpD and GpE (Table 3). There was nonsignificant variation in the mean values of ALT in amniotic fluid as pregnancy advanced except the values in between GpA and GpB, GpA and GpE which differed significantly ($p = 0.05$). The mean values of GPT/ALT in allantoic fluid decreased from GpA to GpD with a significant decrease ($p = 0.05$) in between GpA and GpB (Table 3). In maternal serum the enzyme concentration decreased from GpA to GpE. Here, almost in all the groups the decrease was significant ($p = 0.05$) except in GpB and GpC, GpC and GpD, GpD and GpE. The mean concentrations of Alkaline phosphatase both in foetal fluids and maternal serum increased as the gestation period advanced (Table 3). Both in foetal fluids and maternal serum almost in all

Table 3: Enzymes of foetal fluid and maternal serum during different stages of gestation in sheep

		Groups (Length of embryo and CA length of foetus in cm/age in days)				
Parameters	Types of fluid	A (0.1-10/14-57)	B (10.1-20/58-74)	C (20.1-30/75-93)	D (30.140/94-120)	E (40.1-50/121-140)
GOT/AST (Mean±SE)	Amniotic ($U\ L^{-1}$)	15.26±0.45^{a}	13.18±1.07^{bf}	13.40±0.4^{ef}	14.80±0.86^{af}	15.00±1.08^{af}
	Allantoic ($U\ L^{-1}$)	13.48±0.42^{a}	12.05±1.13^{af}	12.43±0.50^{ef}	13.4±0.93^{af}	13.75±0.95^{af}
	Serum ($U\ L^{-1}$)	104.10±4.03^{a}	94.38±3.05^{b}	86.6±3.79^{ef}	83.6±3.83^{dfg}	78.5±1.32^{eg}
GPT/ALT (Mean±SE)	Amniotic fluid ($U\ L^{-1}$)	7.56±0.24^{a}	6.36±0.39^{bf}	6.98±0.76^{af}	7.04±0.50^{af}	6.83±0.33^{ef}
	Allantoic fluid ($U\ L^{-1}$)	6.34±0.24^{a}	5.49±0.37^{bf}	5.62±0.82^{af}	5.82±0.51^{af}	5.90±0.19^{af}
	Serum ($U\ L^{-1}$)	34.63±1.19^{a}	29.75±0.97^{bf}	28.20±0.80^{efg}	26.00±1.30^{degh}	24.00±2.04^{eh}
Alkaline phosphatase (Mean±SE)	Amuiotic flnid (KA Uuits)	30.73±1.51^{a}	35.75±1.63^{b}	40.2±1.50^{ef}	41.2±1.59^{dfg}	43.75±1.31^{eg}
	Allantoic fluid (KA Uuits)	27.33±1.42^{a}	31.88±1.44^{bf}	33.60±1.60^{efg}	32.40±2.40^{df}	36.50±1. 26^{eg}
	Serum (KA Uuits)	60.70±3.43^{a}	71.88±2.96^{bf}	76.80±4.99^{efg}	78.80±2.75^{dg}	92.50±4.99^{e}

Values with different superscript are significant and having any one of the common superscript are non significant, $p = 0.05$. Comparison was made by applying students t-test

the groups the values increased significantly (p = 0.05) except, GpC and GpD, GpD and GpE in amniotic fluid GpB and GpC, GpB and GpD, GpC and GpD, GpC and GpE in allantoic fluid GpB and GpC, GpC and GpD in serum. A decreased level of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) enzymes in serum is in agreement with the study carried out by Manish-Mahawar *et al.* (2004) and Pouroucholtamane *et al.* (2005). Changes in blood concentrations of glucose, urea, proteins or enzymes may all reflect alterations in liver function during pregnancy. The subnormal or elevated levels of enzymes in foetal fluid and serum is one of the important tools to assess liver functioning and healthy state of pregnancy.

Based upon the evaluation of biochemical parameters, it may be possible to detect the early aberrations in metabolism and thereby appropriate corrections could be made to overcome the metabolic disturbances during pregnancy.

ACKNOWLEDGMENTS

Thanks to Professor G.M. Wani and Professor M.Z. Khan for their guidance, kind supports, help and suggestions during this study. S.K. University of Agricultural Sciences and Technology of Kashmir has been kind enough for the financial support for this research sanctioned under University Grant No. ROC AU/FVS/ARD/C-7/120-21 dated 15-07-07. This study was extracted from Ph.D. Thesis of A. Khatun.

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