

Biochemical evaluation of amniotic fluid during different stages of gestation in the goat

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Summary

To study changes in concentrations of some biochemical factors of amniotic fluid, 115 normal goat uteri at different stages of pregnancy were collected from slaughterhouse. After expelling of each fetus accompanied by fetal membranes, 10 ml amniotic fluid was taken from amniotic sac for biochemical analysis. Then approximate ages of fetuses were calculated by using age estimation formula. At five stages of five months of pregnancy, the following results were obtained, respectively: sodium concentrations were 123.3, 125, 115.9, 122 and 120.8 mmol/L; potassium, 6.2, 5.2, 5.8, 4.9 and 5.6 mmol/L; chloride, 101.4, 115.1, 117.3, 106.6 and 94.9 mmol/L; glucose, 7.1, 11.5, 8.8, 4.9 and 3.6 mg/dl; urea, 43.7, 69.3, 84.8, 117 and 91.7 mg/dl; uric acid, 0.4, 0.1, 0.1, 0.3 and 0.7 mg/dl; creatinine, 1.5, 0.6, 0.6, 5 and 5.9 mg/dl and total protein with the highest value of 54.2 mg/dl at the first month of pregnancy and the lowest value of 26.6 mg/dl at the second month.

Key words: Biochemical evaluation, Amniotic fluid, Gestation, Goat

Introduction

Fetal membranes and fluids are developed for the purpose of physiologic exchanges between fetal and maternal tissues. Fetal membranes are extra-embryonic in nature (Wahid *et al.*, 1991). Dynamic system of fetoplacental unit causes constant exchange of water and fluid constituents between fetal fluid compartments and the maternal circulation, which is reflected in changes in the physical, chemical and biochemical constituents of fetal fluids (Aidasani *et al.*, 1993). Fetal fluids are important in the efficient handling of fetal waste products and in preventing mechanical shock to the developing fetus during entire gestation (Amle *et al.*, 1992).

In cattle and sheep, formation of amnion occurs on 13–16 days of pregnancy and then the amniotic fluid fills the amniotic sac (Robert, 1986). A broad knowledge of

amniotic fluid is of the utmost importance in understanding fetal metabolism and identifying pathologic conditions during pregnancy (Prestes *et al.*, 2001). Studies on amniotic fluid composition have made in human (Wintour, 1986), sheep (McDougall, 1949; Alexander *et al.*, 1958; Mellor and Slater, 1972, 1973), cow (Wintour *et al.*, 1986) and goat (Aidasani *et al.*, 1992, 1993) that have been limited by the study of only few factors over a restricted period of pregnancy.

The present study was carried out to determine the values of some biochemical components including sodium, potassium, chloride, glucose, urea, uric acid, creatinine and total protein during five months of pregnancy in normal goats.

Materials and Methods

This study was undertaken in Ahvaz. One-hundred and fifteen gravid uteri of

native goat breeds from first to fifth months of gestation were collected randomly at a local slaughterhouse. Uteri were opened along the dorsal curvatures. Maternal caruncles were separated gently from fetal cotyledons. The intact amnion, chorion and allantois along with the embryo or fetus were separated. Amniotic sacs were punctured and 10 ml of amniotic fluids were aspirated from each amniotic sac by using a 10-ml disposable syringe. The aspirated fluids were stored in labeled plastic tubes and frozen at -18°C until biochemical analyses. Then, fetuses were expelled from enclosing membranes and the fetal ages were determined by applying the age estimation formula:

$$Y = 2.74 X + 30.15,$$

presented by Gall *et al.* (1994), where Y denotes the developmental age in days and X is the crown-rump length in cm. The pregnancies were divided into five stages as: stage I (0–30 days), stage II (31–60 days), stage III (61–90 days), stage IV (91–120 days) and stage V (121 days to term).

The following amniotic fluid components were measured: sodium and potassium (mmol/L) by flame photometry, using a Corning 410C flame photometer (USA), and chloride (mmol/L) by colorimetric method; glucose, urea and uric

acid (mg/dl) by enzymatic/colorimetric method; creatinine (mg/dl) by kinetic method and total protein (mg/dl) by colorimetric method (Prestes *et al.*, 2001). We used a Milton Roy spectrophotometer (USA) to assess all of them. In this study, analysis of data was done by SPSS 11.5. Comparisons between stages were performed by one-way ANOVA and Duncan's multiple range tests. The differences were considered significant when $p < 0.05$.

Results

The values for considered biochemical components of amniotic fluid are given in Table 1. With the exception of sodium and potassium, the concentrations of other factors showed significant differences ($P < 0.05$) at various months of pregnancy.

Discussion

The developing fetus is surrounded by amniotic fluid. Fetal urination and drinking as well as the permeability of fetal membranes (amnion and chorion) are important determinants of amniotic fluid composition and volume. Hormones, such as

Table 1: Means ± SD of the analysed variables at different stages of gestation

	Stages of pregnancy					
	Stage I (n=20)	Stage II (n=20)	Stage III (n=28)	Stage IV (n=28)	Stage V (n=19)	
	a	b	c	d	e	
	Mean	SD	Mean	SD	Mean	SD
Sodium (mmol/L)	123.3±10.9		125.0±8.3		115.9±14.6	
Potassium (mmol/L)	6.2±1.6		5.2±1.3		5.8±0.9	
Chloride (mmol/L)	101.4±31.1 ^c		115.1±15.6 ^e		117.3±17.8 ^{ac}	
Glucose (mg/dl)	7.1±4.5 ^{bc}		11.5±5.7 ^{adc}		8.8±3.6 ^{dc}	
Urea (mg/dl)	43.7±30.3 ^{cde}		69.3±25.6 ^d		84.8±24.2 ^a	
Uric acid (mg/dl)	0.4±0.6 ^{bce}		0.1±0.1 ^{ac}		0.3±0.1 ^c	
Creatinine (mg/dl)	1.5±1.9 ^{de}		0.6±0.8 ^{de}		0.6±0.2 ^{de}	
Total protein (mg/dl)	54.2±79.9 ^{bc}		26.6±14.1 ^a		29.3±15.7 ^a	
					117±68.5 ^{ab}	
					122.0±12.1	
					120.8±17.8	
					4.9±0.9	
					5.6±0.09	
					106.6±23	
					94.9±18.5 ^{bc}	
					3.6±3.3 ^{abc}	
					91.7±101.3 ^a	
					0.7±0.3 ^{abcd}	
					5.9±4.6 ^{abc}	
					44.4±20	
					37.6±6.9	

^{a, b, c, de} The values in each row with different letters differ significantly ($P < 0.05$). * The numbers of samples in each stage are showed in parenthesis

prolactin and cortisol, may play a major role by affecting membrane permeability (Wintour, 1986). In this study, it was found that sodium and potassium levels were highest in stage II and I, respectively, then decreased toward term. Prestes *et al.* (2001) through amniocentesis on 70, 100 and 145 days of pregnancy in sheep, reported that sodium and potassium values decrease from 70 to 145 days of pregnancy; these findings are in agreement with our results with the exception of that they also found a significant difference in the above-mentioned three stages which is different from our observations. The same researchers believed that the mineralocorticoids activity of intrauterine fetal maturity acts on fetal kidneys, increasing potassium and decreasing sodium concentrations in fetal urine. According to Wintour *et al.* (1986), a classical sodium pump may be responsible and alterations in the relative permeability to sodium and potassium may affect the transport.

Chloride values decreased from stage III to stage V. There was significant differences between stage V with stages II and III ($P < 0.05$). Similar observations were made by Prestes *et al.* (2001) in sheep. According to Mellor and Slater (1973), fetal orosomucoid secretion could be a source of chlorine (found in the amniotic fluid).

Glucose concentrations decreased from early to late stages of gestation and significant differences were found between stages IV and V with stages I, II and III ($P < 0.05$). The results obtained in this study are similar to those recorded in sheep by Prestes *et al.* (2001), Bradley and Mistretta (1973) and Reddy *et al.* (1995), but are different from those reported by Aidasani *et al.* (1992) in the goat and Tangalakis *et al.* (1995) in sheep—although the last two authors used different methods to measure glucose at different stages of pregnancy. Prestes *et al.* (2001) stated that decrease of glucose in amniotic fluid during gestation may be due to fetal intake of glucose as a consequence of the fetal swallowing reflex. According to Bauman and Curri (1980), glucose from the maternal circulation is the main energy source for the fetus during pregnancy in farm animals and it comprised 50 to 70% of total substrates oxidized by the

fetus.

Statistical analysis of urea revealed increasing values as gestation advanced; its value, however decreased in stage V. Stages III, IV and V had significant differences with stages I and II. Similar results were recorded by Wintour *et al.* (1986) in sheep, but our observations are not in agreement with those reported by Prestes *et al.* (2001) in sheep, who recorded decreasing values of urea on 70, 90 and 145 days of pregnancy in sheep. According to Mellor *et al.* (1972, 1973 and 1975), urea concentration in fetal bladder increases during pregnancy. Wintour *et al.* (1986) stated that in midgestation, fetal urine, high in urea and low in chloride enters the amniotic fluid, but the high permeability of the membranes allows equilibration with the extracellular fluid of mother and/or fetus. Late in gestation, fetal urine with high urea and even lower chloride concentrations enters amniotic fluid. At this time, neither the urea can leave so readily, nor chloride can enter. Thereby, equilibration with extracellular fluid can not occur. This dynamic situation is continued. The fetus removes some amniotic fluid by drinking, and fetal urine continues to enter, which results in an increase in amniotic fluid urea and decrease in chloride.

Values obtained for uric acid and creatinine showed that their concentrations increased from stages II and III to IV and V, so that significant differences observed between early and late stages. Similar results were reported by Prestes *et al.* (2001) in sheep. Lovell *et al.* (1995) and Aidasani *et al.* (1992) recorded low creatinine levels in the amniotic fluid of goat at the beginning of pregnancy. Aidasani *et al.* (1992) observed that the concentrations of creatinine and uric acid in the allantoic fluid were significantly higher than in the amniotic fluid and fetal serum in the goat. Tomoda *et al.* (1987) reported that fetal urine production and composition are influenced by the volume of body fluids and the availability of water and electrolytes in the maternal compartment. The progressive maturity and efficient activity of fetal kidney also justifies these findings.

The results of this study showed that the highest values of total protein were in stage I and then decreased so that significant

differences were observed between stage I and stages II and III. Reddy *et al.* (1995), reported that the low concentrations of total protein in amniotic fluid could be attributed to the absence of fibrinogen and other proteins due to fetal liver immaturity.

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