

STUDIES ON BIOCHEMICAL CONSTITUENTS OF GOAT ALLANTOIC FLUID DURING DIFFERENT STAGES OF GESTATION

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ABSTRACT

The objective of the present study was to determine changes in biochemical constituents of goat allantoic fluid in different stages of gestation. Allantoic fluid samples were collected from 115 normal pregnant goat uteri at various stages of pregnancy. After expelling of each fetus accompanied by fetal membranes, 10ml allantoic fluid was taken from allantoic sac. Approximate ages of fetuses were calculated by using related formula. At five stages of the five months of pregnancy, the following results were obtained, respectively: creatinine concentrations were: 4.7, 9.3, 9.2, 12.6 and 12.1 mg/dl; uric acid: 0.5, 0.2, 0.2, 0.3 and 3.8 mg/dl; urea: 122.7, 153.8, 77.3, 52.1 and 37.6 mg/dl; total protein: 97.8, 129.5, 99.7, 128.9 and 85 mg/dl; glucose: 10.3, 20, 9, 8.1 and 4.2 mg/dl; sodium: 75.3, 77.8, 75.3, 73.5 and 78.9 mmol/L; potassium: 5, 4.6, 5.3, 5.9 and 5.8 mmol/L; chloride: 70, 68.6, 46.9, 30.2 and 30.2 mmol/L.

INTRODUCTION

Extraembryonic membranes (yolk sac, chorion, amnion and allantois) provide the basic media which are common to all amniotes and can usually be recognized at least at the earlier stages of placental growth (Wooding and Burton, 2008). Both amnion and allantois

are sacs containing fetal fluids and covered by the chorion (Wintour et al., 1986).

The developing fetus, surrounded by the amniotic fluid compartment and connected with the allantoic sac via the urachus and placental vasculature, receives nutrient supplies mainly via the umbilical vein (Battaglia and Meschia, 1988).

Fetal fluids are important for physiologic exchanges between fetal and maternal tissues, so they are necessary for the efficient handling of fetal waste products and preventing mechanical shock to the developing fetus during entire gestation (Amle et al., 1992; Khadjeh et al., 2007).

The concentration of constituents in amniotic and allantoic compartments is influenced by the exchange through the placenta, metabolic products of the fetus, fetal urine formation and fluid flow through the urachus or urethra and fetal secretions from lung and salivary glands. Urine from the bladder of the fetus could pass through the urachus into the allantoic sac or through the urethra into the amniotic sac (Baetz et al., 1976; Javed, 1990; Foulds et al., 1998). However, both amniotic and allantoic fluids differ substantially in composition than that of fetal urine (Javed, 1990). The allantoic sac was considered traditionally and mainly as a reservoir for fetal wastes, however recent investigations with pigs have shown that allantoic sac plays an important role in the accumulation of nutrients and metabolism of

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some factors like uteroferrin and iron, suggesting a hitherto unrecognized function of the allantoic sac in fetal nutrition (Kwon et al., 2004).

Studies on fetal fluids composition had made in bovine (Baetz et al., 1976; Wintour et al., 1986; Wahid et al., 1991; Riding et al., 2008), in human (Wintour, 1986), in sheep (Wintour et al., 1986; Reddy et al., 1995; Prestes et al., 2001; Xia et al., 2009), in buffalo (Amle et al., 1992), in sow (Razdan et al., 2004) and in caprine (Aidasani et al., 1992 and 1993; Khadjeh et al., 2007).

It is estimated that among more than 808.9 million goat around the world, the contribution of Iran is 3.36% or 27 million of this population. Iran recognized as one of the 10 important countries in goat keeping in the world. The main purposes of its rearing have been to produce fiber (mohair and cashmere) and meat. Goat mostly kept mixed with sheep in all regions of the country (Eilami, 2000; Maghsoudi et al., 2009).

There is little information available on the reproductive characteristics of goat particularly regarding allantoic fluid composition. The objective of this study was to determine the values of different allantoic components during different stages of gestation in Iranian local goat, in order to gain a better understanding of their pregnancy physiology and fetal metabolism.

MATERIALS AND METHODS

The present study was undertaken in Ahvaz city, southern Iran. One-hundred and fifteen pregnant goat's uteri of native goat breeds at the first to fifth months of pregnancy were collected immediately after slaughtering in a local slaughterhouse. By careful dissection of uteri along the dorsal curvatures, chorion and allantois were exposed. Maternal caruncles were separated gently from fetal cotyledons. The intact allantois along with the embryo or fetus was separated. Allantoic sacs were punctured and 10ml of allantoic fluids were aspirated from each allantoic sac by using 10ml disposable syringe. The aspirated fluids were stored in labeled plastic tubes and frozen

at -18°C until biochemical analyses. Then, embryos or fetuses were expelled from enclosing membranes and the fetal ages were determined by applying the age estimation formula: $Y=2.74X+ 30.15$, 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- 1 (0-30days), stage- 2 (31-60 days), stage- 3 (61-90days), stage- 4 (91-120days) and stage- 5 (121 days to term).

The following allantoic fluid contents were measured: creatinine (mg/dl) by kinetic method; uric acid, urea and glucose (mg/dl) by enzymatic/colorimetric method; total protein (mg/dl) by colorimetric method; sodium and potassium (mmol/L) by flame photometer using Corninge 410C flame photometer (USA); and chloride (mmol/L) by colorimetric method (Prestes et al., 2001). A Milton Roy spectrophotometer (USA) was used to assess of factors. Analysis of data was done by SPSS 15 software. Comparisons between stages were performed by one-way ANOVA and Duncan's multiple range tests. The differences were considered significant if $P<0.05$.

RESULTS AND DISCUSSION

The mean±SD values for various biochemical constituents of allantoic fluid are shown in Table 1. With the exception of sodium, potassium and phosphorus, the concentrations of other components showed significant differences ($P<0.05$) among various months of pregnancy.

In domestic animals, the fetus is surrounded by the amnion and allantois, which establish a perfect water and electrolyte balance (Prestes et al., 2001). In species placenta only form at multiple predetermined sites in the uterine wall (caruncles), fluid is required to fill the allantoic compartment, which enables the expansion of the chorion and overlying the allantois to such caruncles of both uterine horns (Liu et al., 2008).

Recently, a number of small membrane proteins (aquaporins) have been found to be

expressed in the placenta, fetal membranes and tissue/organs of the developing fetus which may play roles in fluid transport (Liu et al., 2008).

In this study, it was found that creatinine and uric acid are constituents that could change by increasing their concentration with gestational age in the allantoic fluid. Similar results had been observed by Baetz et al. (1976) in bovine. Alexander et al. (1958) also reported increases in creatinine in allantoic fluid with fetal aging in sheep. Studies by Stanier (1965) on pig's and rabbit's fetuses indicate a rapid exchange of water and urea with the maternal circulation when urea or water injected into the fetus or the amniotic fluid. This exchange is slower when water is injected into the allantoic sac and almost nonexistent for urea occurred in the allantoic sac. This lack of exchange of solutes from the allantoic fluid may explain the increase found in creatinine and uric acid with gestational age (Baetz et al., 1976).

Mellor et al. (1975) reported that urea concentration in bladder of sheep fetus increases during pregnancy. According to Tangalakis et al. (1995), until day 75 of pregnancy in sheep, fetal urine drains exclusively into the allantoic sac, while in the final third of gestation, it drains equally into both amniotic and allantoic sacs. So, tendency

of decreasing urea concentrations in last stages of gestation that observed in present study can be attributed to entrance of some urine into amniotic sac in goat, like sheep.

Values obtained for protein show that its concentration in allantoic fluid appears to steadily rise as gestation advanced. Clearance of redundant protein from this compartment is possibly more restricted and dependent on the proteolytic breakdown of proteins to smaller polypeptides and amino acids prior to removal (Riding et al., 2008).

Glucose concentrations decreased from early to late stages of gestation. These results are similar to findings recorded in bovine (Baetz et al., 1976).

Glucose from the maternal circulation is the main energy source for the fetus during a normal pregnancy. Part of this glucose is reduced to sorbitol in the placenta. The sorbitol is then oxidized to fructose in the placenta or the fetal liver. Fructose is not used by the fetus unless the flow of glucose from the mother is interrupted (Baetz et al., 1976; Javed, 1990).

Several studies have reported values for electrolytes in bovine and goat fetal fluids (Baetz et al., 1976; Khadjeh et al., 2007).

Table1: Means ± SD of the analyzed variables at different stages of pregnancy Stages of pregnancy*

	Stage1(n=20)	Stage2(n=20)	Stage3(n=28)	Stage4(n=28)	Stage5(n=19)
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Creatinine(mg/dl)	4.7±6.4 <i>de</i>	9.3±25.4	9.2±8.8	12.6±12.6 <i>a</i>	12.1±11.8 <i>a</i>
Uric acid(mg/dl)	0.5±1.8 <i>e</i>	0.2±0.4 <i>e</i>	0.2±0.2 <i>e</i>	0.3±0.4 <i>e</i>	3.8±10 <i>abcd</i>
Urea(mg/dl)	122.7±136 <i>cde</i>	153.8±163 <i>cde</i>	77.3±98 <i>ab</i>	52.1±47.8 <i>ab</i>	37.6±38.6 <i>ab</i>
Total protein(mg/dl)	97.8±149.4 <i>bc</i>	129.5±133.6 <i>cda</i>	99.7±147 <i>a</i>	128.9±128.2	85±135.2
Glucose(mg/dl)	10.3±14.2 <i>be</i>	20±21.2 <i>abcd</i>	9±9 <i>b</i>	8.1±10.6 <i>b</i>	4.2±4.6 <i>ab</i>
Sodium(mmol/l)	75.3±51.2	77.8±39.2	75.3±39.2	73.5±38.6	78.9±67.2
Potassium(mmol/l)	5±2.6	4.6±2.2	5.3±2.4	5.9±5	5.8±3.8
Chloride(mmol/l)	70±82.6 <i>cde</i>	68.6±38 <i>cde</i>	46.9±41.6 <i>ab</i>	30.2±46.6 <i>ab</i>	30.2±42.2 <i>ab</i>
Phosphorus(mg/dl)	5.1±4.4	7±6.8	7.5±13.4	6.7±12.8	4.1±14.6

a, b, c, d, e: The Values in each row with different letters differ significantly ($p < 0.05$). * The numbers of samples in each stage are shown in parenthesis.

These investigations suggest that amniotic fluid can be distinguished from allantoic fluid, because the latter has very low concentrations of sodium, chloride and phosphorus and high concentrations of potassium and calcium, when compared with the concentrations in amniotic fluid. These findings are similar with observations of the present study.

REFERENCES

- Aidasani, R., A. S. Chauhan, S. Tiwari and S. P. Shukla, (1992). Some metabolic constituents of caprine foetal fluids and foetal serum. *Indian. J. Anim. Sci.*, 62: 335-336.
- Aidasani R., R. A. S. Chauhan, S. Tiwari and S. P. Shukla, (1993). Studies on electrolytes of caprine foetal fluids and foetal serum. *Indian. Vet. J.* 70: 239-241.
- Alexander, D. P. D., A. Nixon, W. F. Widdas and F. X. Wohlzogen, (1958). Gestational variations in the composition of the foetal fluids and foetal urine in the sheep., *J. Physiol.* 140: 1-13.
- Amle, M. B., S. R. Chinchkar, V. B. Hukeri and V. L. Deopurkar, (1992). Studies on fetal fluids of buffaloes. *Indian. J. Anim. Res.*, 13: 165-167.
- Baetz, A. L., W. T. Hubbert and C. K. Graham, (1976). Changes of biochemical constituents in bovine fetal fluids with gestational age. *American. J. Vet. Res.*, 37: 1047-1052.
- Battagalia, F. C and G. Meschia, (1988). Fetal nutrition. *Annual. Rev. Nutrition.*, 8: 43-61.
- Billon, P. (2003). Milking management. In: *Encyclopedia of dairy sciences*. Academic press, Cornwall, pp: 1243-1253.
- Eilami, B. (2000). Feedlot performance and carcass characteristics of Fars native goats. In: *Proceedings of 7th international conference on goats*, France, pp: 830-832.
- Khadjeh, Gh. H., R. Ranjbar, M. Salehi and S. M. Banan Khojasteh, (2007). Biochemical evaluation of amniotic fluid during different stages of gestation in the goat. *Iranian. J. Vet. Res.*, 8: 266-269.
- Kwon, H. J, (2004). Amino acids, polyamines and nitric oxide synthesis in the ovine conceptus. M.S.c thesis, Texas University, United States of America.
- Foulds, L. M., D. M. De Kretser, P. Farnworth, D. Buttress, D. Jenkin, N. P. Groome and J. R. McFarlane, (1998). Ovine allantoic fluid contains high concentrations of activin A: partial dissociation of immunoactivity and bioactivity. *Bio. Reprod.*, 59: 233-240.
- Javed, M. H, (1990). Bovine amniotic and allantoic fluids for the culture of murine embryos and determination of pentose phosphate and Embden–Meyerhof pathway activities in bovine embryos. Ph.D thesis, Washington State University, United States of America.
- Liu, H., Z. Zheng and E. M. Wintour, (2008). Aquaporins and fetal fluid balance. *Placenta.*, 29: 840-847.
- Maghsoudi, A and R. V. Torshizi, (2009). Estimates of (co) variance components for productive and composite reproductive traits in Iranian cashmere goats. *Livestock. Sci.*, 126: 162-167.
- Mellor, D. J., J. S. Slater and C. I. Matherson, (1975). Effects of changes in ambient temperature on maternal plasma and allantoic fluid from chronically catheterized ewes during the last two months of pregnancy. *Res. Vet. Sci.*, 18: 219-221.
- Prestes, N. C., M. C. L Chalhoub, M. D. Lopez and R. K. Takahira, (2001). Amniocentesis and biochemical evaluation of amniotic fluid in ewes at 70, 100 and 145 days of pregnancy. *Small. Rumin. Res.*, 39: 277-281.
- Razdan, P., P. Tummaruk, H. Kindhal H. Rodriguez-Martinez, F. Hulten and S. Einarsson, (2004). The impact of induced Stress during days 13 and 14 of pregnancy on the composition of allantoic fluid and conceptus development in sows. *Theriogenology.*, 61:757-767.

- Reddy, A. P., V. S. C. Reddy, A. S. Rao, G. P. Sharma, G. V. N. Reddy, T. J. Reddy and V. S. Reddy, (1995). Biochemical studies in the ewe (*OVIS ARIES*) amniotic fluid during different phases of gestation. *Int. J. Anim. Sci.*, 10: 321-323.
- Riding, G. A., S. A. Lehnert, A. G. French and J. R. Hill, (2008). Conceptus-related measurements during the first trimester of bovine pregnancy. *Vet. J.*, 175: 266-272.
- Tangalakakis, K., K. Mortiz, L. Shandley and E. M. Wintour, (1995). Effect of maternal glucocorticoid treatment on ovine fetal fluid at 0.6 gestation. *Reprod. Fertil. Develop.*, 7: 1595-1598.
- Stanier, M. W, (1965). Transfer of radioactive water, urea and glycine between maternal and foetal body fluids in rabbits and pigs. *J. Physiol.*, 178: 127-149.
- Wahid, M. M., N. A. Iiemeid, A. M. R. Shalash and E. M. Isamil, (1991). Reproduction in native Egyptian cows: fetal membranes and fluids. *Reprod. Domestic. Anim.*, 26: 270-273.
- Wintour, E. M, (1986). Amniotic fluid-our first environment. *NIPS.*, 1:95-97.
- Wintour, E. M., B. M. Laurence and B.E. Lingwood, (1986). Anatomy, physiology and pathology of the amniotic and allantoic compartments in the sheep and cow. *Australian. Vet. J.*, 63: 216-221.
- Wooding, P and G. Burton, (2008). *Comparative placentation*. Springer Verlag, Berlin Heidelberg, pp: 3-8.
- Xia, Y., T. O'shea and J. R. McFarlane, (2009). Changes in Concentrations of follistatin in maternal plasma and foetal fluids during pregnancy and parturition in sheep. *Anim. Reprod. Sci.*, 114:148-15.

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