VETERINARSKI ARHIV 75 (5), 431-437, 2005

Comparative biochemical assessment of the amniotic fluid and maternal plasma of pregnant rabbits

Peter Chuka Ozegbe

Department of Veterinary Anatomy, University of Ibadan, Ibadan, Nigeria

OZEGBE, P. C.: Comparative biochemical assessment of the amniotic fluid and maternal plasma of pregnant rabbits. Vet. arhiv 75, 431-437, 2005.

ABSTRACT

The following were determined for pregnant New Zealand White x Chinchilla rabbits on day 28 post-service: maternal plasma and the amniotic fluid concentrations of sodium (Na), potassium (K), chloride (Cl), bicarbonate (HCO_3), calcium (Ca), inorganic phosphate (PO_4), creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), amylase, total proteins, albumin, globulins, albumin-globulin ratio, cholesterol, triglycerides, creatinine, urea, and uric acid. The maternal plasma concentrations of Ca, CK, ALT, amylase, total proteins, albumin, globulins, cholesterol and triglycerides were significantly ($P<0.05$) higher than those found in the amniotic fluid. The AP level was significantly higher in the amniotic fluid than in the maternal plasma ($P<0.05$). There was no cholesterol in the amniotic fluid. These findings might be relevant in prenatal detection of disease states in both the dam and the foetus.

Key words: maternal plasma, amniotic fluid, biochemical parameters, doe

Introduction

In all species of mammals amniotic fluid accumulates early and subsequently diminishes as the embryo itself enlarges (ADOLPH, 1967). The amniotic fluid may be formed, partly or entirely, by the amnion, embryonic skin, foetal kidney, foetal lung, buccal cavity and nasal cavity (QUEENAN, 1978; MOORE, 1982; HAMMER et al., 1997).

Earlier investigations on the composition of the amniotic fluid of rat and humans have involved only few electrolytes, total proteins, metabolites and enzymes (BARKER, 1961; MULIVOR et al., 1979; GULBIS et al., 1998). Most of these studies did not compare maternal plasma and amniotic fluid. Recently, BIELECKI et al. (2000) and KANENISHI et al. (2001) compared the maternal plasma and foetal amniotic fluid concentrations of adrenomedullin and interleukin-6 in women.

* Contact address:
Peter Chuka Ozegbe, Department of Veterinary Anatomy, University of Ibadan, Ibadan, Nigeria, E-mail: pcozegbe@yahoo.com

ISSN 0372-5480
Printed in Croatia

The purpose of the present investigation is to examine the composition of the amniotic fluid relative to the maternal plasma. The baseline data generated may contribute to the current efforts aimed at prenatal detection of inborn errors of metabolism.

Materials and methods

Six adult crosses of New Zealand White and Chinchilla rabbits of known gestational age were used. The animals were selected from a colony at the Experimental Animal Unit, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria and were fed with commercial rabbit pellets (Ladokun ® Feeds Nigerian Limited). Water was supplied *ad libitum*.

Each rabbit was anaesthetized with 25% urethane (1mg/kg) injected intraperitoneally, on day 28 post-copulation. Maternal blood sample, obtained by cardiac puncture, was drawn into a lithium heparin-coated bottle and centrifuged at 3000g for 10 minutes to obtain plasma. Amniotic fluid was obtained by puncturing the foetal membrane and sucking out the fluid with a Pasteur pipette. Collected amniotic fluids from the same doe were pooled for analysis.

The concentrations of the various maternal plasma and amniotic fluid components were determined, using commercially available kits, on an auto-analyser (Hitachi 704, Boehringer Mannheim Hitachi, Japan). The parameters were subjected to statistical analysis using Statistical Analysis Software (SAS) of Statistical Analysis System Institute, Cary, N.C. U.S.A. (1988 version). The relationship between uric acid, cholesterol and triglycerides was established using Pearson's correlation coefficient of Statistical Analysis System Institute, Cary, N.C. U.S.A. (1988 version).

Results

The mean, standard error of mean, and range of the various biochemical parameters in the maternal plasma and amniotic fluid, are shown in Tables 1, 2 and 3. The concentrations of mono-valent cations (sodium and potassium), mono-valent anions (chloride and bicarbonate), some metabolites (urea and creatinine) and alkaline phosphatase were higher in the amniotic fluid than in the maternal plasma. The levels of bivalent anion and cation (inorganic phosphate and calcium, respectively), as well as cholesterol, triglycerides, creatine kinase, lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, amylase, uric acid, total proteins, albumin, globulin and albumin to globulin ratio, were lower in the amniotic fluid relative to the maternal plasma. There were significant differences ($P<0.05$) between the concentrations of proteins, lipids, calcium and enzymes, excepting lactate dehydrogenase and alanine aminotransferase, in the maternal plasma and the amniotic fluid.

The relationship between the maternal plasma cholesterol and triglycerides, as well as that between maternal plasma uric acid and triglycerides, were non-significantly negative (r =-0.8131, P>0.05 and r =-0.6745; P>0.05, respectively). However, the correlation between the plasma uric acid and cholesterol were significantly positive (r = 0.9117; P<0.05). The amniotic fluid uric acid and triglycerides were positively but non-significantly correlated (r = 0.6132; P>0.05).

Table 1. Maternal plasma and amniotic fluid proteins, lipids and metabolites

Parameter	Fluid	Plasma	P value
Total proteins (g/L)	18.8 ± 2.5 (10-25)	52. ± 4.1 (46-68)	***
Albumin (g/L)	11.4 ± 1.2 (9-15)	33.8 ±1.4 (31-39)	***
Globulins (g/L)	7.4 ± 2.1) (1-13)	19 ± 4.3 (12-36)	*
Albumin-globulin ratio	1.48 ± 0.06 (0.69-2.17)	1.86 ± 0.06 (0.89-2.83)	ns
Cholesterol (mmol/L)	0.0 ± 0.0 (0-0)	0.83 ± 0.113 (0.44-1.09)	***
Triglycerides (mmol/L)	0.426 ± 0.064 (0.262-0.627)	0.812 ± 0.066 (0.65-0.97)	**
Urea (mmol/l)	10.5 ± 1.45 (7.14-15.71)	10 ± 1.66 (6.78-15.71)	ns
Creatinine (μmol/L)	137.9 ± 21.22 (97.24-221)	125.53 ± 8.84 (106.1-159.12)	ns
Uric acid (μmol/L)	60.69 ± 16.1 (0.0-89.3)	67.83 ± 19.04 (0.0-107.1)	ns

ns = not significant; *P<0.05; **P<0.01; ***P<0.001; Values are given as mean ± SEM (range)

Table 2. Maternal plasma and amniotic fluid levels of some enzymes (μ/L) in rabbits

Parameter	Fluid	Plasma	P value
Creatine kinase	85.6 ± 11.6 (43-112)	1033.2 ± 204.8 (538-1559)	***
Lactate dehydrogenase	301.2 ± 51.09 (197-486)	411.4 ± 41.9 (298-502)	ns
Alanine aminotransferase	36.2 ± 7.65 (10-53)	81 ± 21.45 (41-163)	ns
Aspartate aminotransferase	11.8 ± 1.66 (8-17)	41.8 ± 3.6 (30-51)	***
Alkaline phosphatase	445.8 ± 155.6 (189-1057)	68.8 ± 19.8 (28-129)	**
Amylase	506.2 ± 89.6 (295-756)	1463.2 ± 219.7 (1051-2317)	**

ns = not significant; **P<0.01; ***P<0.001; Values are given as Mean ± SEM (range)

Table 3. Concentrations of some electrolytes (μmol/L) in the maternal plasma and amniotic fluid

Parameter	Fluid	Plasma	P values
Sodium	140.2 ± 3.2 (130-150)	138 ± 1.7 (132-142)	ns
Potassium	5.52 ± .28 (4.8-6.5)	5.48 ± .25 (4.9-6.3)	ns
Chloride	103.2 ± 1.74 (97-107)	102 ± 1.8 (95-105)	ns
Bicarbonate	20.2 ± .66 (18-21)	18.8 ± .49 (17-20)	ns
Calcium	2.47 ± 0.17 (2.13-2.88)	3.08 ± 0.2 (2.5-3.6)	*
Inorganic phosphate	1.563 ± 0.132 (1.292-1.97)	1.634 ± 0.132 (1.26-2.067)	ns

Values are given as: Mean ± SEM (range). * P<0.05; ns = not significant

Discussion

Generally, the observed differentials in the concentrations of some biochemical parameters in the maternal plasma and foetal amniotic fluid in this study may be attributed to the epithelia of the amnion which, as reported by KLEFLIN et al. (1988a) and SHANDLEY

et al. (1997), display changes related to increased capacity for the solute and fluid transport regulation as it matures.

The concentration gradients of calcium, potassium and sodium observed in the maternal plasma and amniotic fluid of rabbits in the present study are similar to those reported in humans by NUSBAUM and ZETTNER (1973), who consequently concluded that amniotic fluid is a simple dialysate of maternal or foetal serum. Calcium and phosphorus are important in the development of foetal skeleton. Thus, it would be expected that the foetus in conserving them would excrete very little into the amniotic fluid. The observed distribution trend of chloride and bicarbonate in the maternal plasma and amniotic fluid is a usual phenomenon in chloride shift.

The significantly higher level of total protein (as well as albumin and globulin) in the maternal plasma than in the amniotic fluid is confirmed by the earlier findings in women (BRZENZINSKI et al., 1964; NUSBAUM and ZETTNER, 1973). Although BRZENZINSKI et al. (1964) concluded that foetal circulation is the main source of amniotic fluid proteins, SUTCLIFFE and BROCK (1973) stated that most of the proteins in the amniotic fluid are of maternal origin. The data obtained from the present study support the conclusion of SUTCLIFFE and BROCK (1973).

Creatinine is an indicator of muscle mass as well as renal function. The foetus produces more creatinine than the placenta (ROOPNARINESINGH et al., 1972). The observed higher concentration of creatinine in the amniotic fluid than maternal plasma may, according to GULBIS et al. (1996), be secondary to the maturation of the foetal renal glomerular function. The major pathway of nitrogen excretion in humans is in the form of urea, which is synthesized in the liver and cleared by the kidney. The increased concentration of urea, accompanied by lower total proteins and uric acid, in the amniotic fluid relative to the maternal plasma, might also have resulted from the metabolic activities and the maturation of the foetal renal glomerular function during the later stages of gestation, as reported by GULBIS et al. (1998).

The triglycerides are the storage lipids of animals in the plasma where the body uses it, mainly as fuel. The dam uses the plasma lipids during the second half of pregnancy when energy requirements increase tremendously. Tissue and plasma cholesterol exchange is generally reported to be very low and hence the absence of cholesterol from the amniotic fluid.

The presence of creatine kinase, lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and amylase in the amniotic fluid and maternal plasma of the pregnant doe is confirmed by the earlier reports of their presence in humans and animals (SUTCLIFFE and BROCK, 1972; SUTCLIFFE et al., 1972; MULIVOR et al., 1979; KLEFLIN, 1988; KLEFLIN et al., 1988a and 1988b). Although these enzymes were mainly drawn from the digestive and respiratory tracts into the amniotic cavity (GULBIS et

al., 1998), their activities in the amniotic fluid are not clearly defined or understood, other than maintaining the metabolic requirements of the amnion.

The significantly positive relationship between the concentrations of maternal plasma uric acid and cholesterol and the negative relationship between uric acid and triglycerides in this study is contrary to the observation in humans by BERKOWITZ (1964).

References

ADOLPH, E. F. (1967): Ontogeny of volume regulations in embryonic extracellular fluids. Quart. Rev. Biol. 42, 1-39.

BARKER, J. N. (1961): Rat amniotic and yolk fluids. Fed. Proc. 20, 418.

BERKOWITZ, D. (1964): Blood lipid and uric acid interrelationship. JAMA 190, 856-858.

BIELECKI, M., J. ZDRODOWSKA, D. A. BIELECKI, A. BODZENTA-LUKASZYK, S. JAROCKI (2000): Maternal plasma and amniotic fluid interleukin-6 levels in imminent preterm labour. Ginekol. Pol. 71, 719-723 (in Polish).

BRZENZINSKI, A.E., E. SADOVSKY, E. SHAFRIR (1964): Protein composition of early amniotic fluid and foetal serum with a case of bis-albuminemia. Am. J. Obstet. Gynecol. 89, 488-494.

GULBIS, B., C. GERVY, E. JAUNIAUX (1998): Amniotic fluid biochemistry in second-trimester trisomic pregnancies relationships to fetal organ maturation and dysfunction. Early Hum. Dev. 52, 211-219.

GULBIS, B., E. JAUNIAUX, D. JURKOVIC, C. GERVY, H. A. OOMS (1996): Biochemical investigation of renal maturation in early pregnancy. Paediatr. Res. 39, 1-5.

HAMMER, A., H. HUTTER, A. BLASCHITZ, W. MAHNERT, M. HARTMANN, B. UCHANSKA-ZIEGLER, A. ZIEGLER, G. DOR. (1997): Amnion epithelial cells, in contrast to trophoblast cells, express all classical HLA class I molecules together with HLA-G. Am. J. Reprod. Immunol. 37, 161-171.

KANENISHI, K., H. KUWABARA, M. UENO, C. SATO, H. SAKAMOTO, T. HATA (2001): Change of adrenomedullin concentrations in plasma and amniotic fluid, and human placental adrenomedullin expression with advancing gestation. Placenta 22, 244-250.

KLEFLIN, A. (1988): Istraživanja aktivnosti kisele fostataze u amnionskoj i alantoisnoj tekućini svinje tijekom prve polovice gravidnosti. Vet. arhiv 58, 75-81.

KLEFLIN, A., M. ZOBUNDŽIJA, T. MARTINČIĆ, Z. SLADIĆ (1988a): Activity of nonspecific esterases in pigs' fetal fluids in the first half of gravidity. Vet. arhiv 58, 161-166.

KLEFLIN, A., M. ZOBUNDŽIJA, V. KOZUMPLIK, M. JAKOVAC (1988b): Prilog poznavanju aktivnosti alkalne fosfataze u amnionskoj i alantoisnoj tekucini svinja Vet. arhiv. 58, 41-48.

MOORE, K. L. (1982): The developing human: clinically oriented embryology. W.B. Saunders Co. Philadelphia.

MULIVOR, R. A., M. T. MENNUTI, H. HARRIS (1979): Origin of alkaline phosphatases in amniotic fluid. Am. J. Obstet. Gynecol. 135, 77-81.

NUSBAUM, M. J., A. ZETTNER (1973): The content of calcium, magnesium, copper, iron, sodium, and potassium in amniotic fluid from eleven to nineteen weeks' gestation. Am. J. Obstet. Gynecol. 115, 219-226.

QUEENAN, J. T. (1978): Amniotic fluid proteins, amniotic fluid amino acids and their clinical significance. In: Amniotic Fluid: Research and Clinical Application, 2nd ed., (Fairweather, D.V.I., T. A. K. B. Eskes, Eds.). Excerpta Medica. Amsterdam. pp. 187-208.

ROOPNARINESINGH, S. D., L. MORRIS, A. L. MATADI (1972): Amniotic fluid heat-stable alkaline phosphatase in normal pregnancy and in pre-eclampsia. J. Obstet. Gynaecol. Brit. Cmwlth. 79, 29-31.

SHANDLEY, L., D. ALCORN, E. M. WINTOUR (1997): Ovine amniotic and allantoic epithelia across gestation. Anat. Rec. 248, 542-553.

SUTCLIFFE, R. G., D. J. H. BROCK (1972): Observations on the origin of amniotic fluid enzymes. J. Obstet. Gynaecol. Brit. Cmwlth. 79, 902-910.

SUTCLIFFE, R. G., D. J. H. BROCK (1973): Immunological studies on the nature and origin of the major proteins in amniotic fluid. J. Obstet. Gynaecol. Brit. Cmwlth. 80, 721.

SUTCLIFFE, R. G., D. J. H. BROCK, J. G. ROBERTSON, J. B. SCRIMGEOU, J. M. MONAGHAN (1972): Enzymes in amniotic fluid: a study of specific activity patterns during pregnancy. J. Obstet. Gynaecol. Brit. Cmwlth. 79, 895-901.

Received: 28 May 2001
Accepted: 7 September 2005

OZEGBE, P. C.: Usporedno određivanje biokemijskog sastava amnionske tekućine i majčinske plazme u gravidnih kunica. Vet. arhiv 75, 431-437, 2005.

SAŽETAK

Koncentracija natrija (Na), kalija (K), klorida (Cl), bikarbonata (HCO_3) kalcija (Ca), anorganskog fosfata (PO_4), kreatin kinaze (KK), laktat dehidrogenaze (LDH), aspartat aminotransferaze (AST), alanin aminotransferaze (ALT), alkalne fostataze (AP), amilaze, ukupnih proteina, albumina, globulina, odnosa albumini/globulini, kolesterola, triglicerida, kreatinina, mokraćevine i mokraćne kiseline određena je u kunica križane pasmine (bijele×činčila) 28 dana nakon parenja. Koncentracija Ca, KK, ALT, amilaze, ukupnih proteina, albumina, globulina, kolesterola i triglicerida u plazmi majki bila je značajno veća ($P<0,05$) nego u amnionskoj tekućini. Razina AP bila je značajno veća u amnionskoj tekućini nego u majčinskoj plazmi ($P<0,05$). Kolesterol nije bio ustanovljen u amnionskoj tekućini. Ti nalazi mogu biti od važnosti u prenatalnom otkrivanju bolesti u gravidnih ženki i plodova.

Ključne riječi: amnionska tekućina, biokemijske pretrage, kunica, majčinska plazma