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Original Research

Biochemical Profile of Amniotic and Allantoic Fluid During Different Gestational Phases in Mares

Luiz Francisco Zanella PhD [a], Regina Kiomi Takahira PhD [b], Cely Marini Melo e Oña PhD [c,d], Luis Carlos Oña Magalhães MSc [d], Nereu Carlos Prestes PhD [d,*]

[a] UEL, Londrina State University, Londrina/PR, Brazil
[b] Department of Clinics, São Paulo State University, School of Veterinary Medicine and Animal Science, Botucatu, São Paulo, Brazil
[c] Department of Animal Production, Goiás Federal University, Goiânia/GO, Brazil
[d] Department of Animal Reproduction and Veterinary Radiology, School of Veterinary Medicine and Animal Science, Botucatu, São Paulo, Brazil

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ABSTRACT

Fetal fluids have different vital functions that sustain both pregnancy and normal parturition. The biochemical composition of amniotic fluid during gestation is not well established; thus the purpose of the present study was to determine the biochemical profile of both amniotic and allantoic fluids from mares during initial, mid, and latter third phases of pregnancy. Samples were collected after slaughter, using allantocentesis and amniocentesis. Sixty samples of fetal fluids were analyzed. Alkaline phosphatase (AP), glucose, total protein (TP), urea, creatinine, Ca, chloride (Cl), Na, and K concentrations were measured using commercially available kits. The AP concentration in amniotic fluid was higher than that in allantoic fluid during the three gestational phases ($P < .05$). There were no differences between glucose mean values of allantoic and those of amniotic fluids ($P < .05$). However, glucose values were higher in the allantoic fluid in the last trimester of pregnancy. TP was higher in the amniotic fluid than in allantoic fluid ($P < .05$). Urea values varied among the phases; however, there were no differences between the amniotic and allantoic fluid values ($P > .05$). Creatinine values were higher in allantoic fluid ($P < .05$). Na and Cl concentrations were higher in amniotic fluid ($P < .05$). However, Ca and K concentrations were higher in the allantoic fluid.

1. Introduction

The biochemical compounds of amniotic fluid during the different phases of pregnancy in mares are not well established [1,2].

Fetal fluids are important for the fetus because: 1) they provide mechanical cushioning, and protection against temperature variations and dehydration [2-4]; 2) they avoid skin adherence to the amniotic membrane; 3.) they promote lubrication and enlargement of the birth canal during stage II of labor (expulsion) [2-4]; 4) they allow fetal movement and development inside the uterus; and 5) they inhibit bacterial growth [5].

The viscous amniotic fluid is formed by secretions coming from skin, mucus, amniotic epithelium fetal saliva, and nasopharyngeal secretions, whereas allantoic fluid consists primarily of excretion of fetal kidneys. These fluids contain various a1tal health, even ante partum (reviewed in reference [6]).

The present study aimed to evaluate the biochemical profiles of amniotic and allantoic fluids of mares in the initial, mid, and latter third phases of pregnancy.

* Corresponding author at: Nereu Carlos Prestes, PhD, Departamento de Reprodução Animal Radiologia Veterinária, FMVZ, UNESP, Distrito de Rubião Junior, 18618-970 Botucatu, São Paulo, Brazil.
E-mail address: nereu@fmvz.unesp.br (N.C. Prestes).

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2. Material and Methods

2.1. Animals

A slaughterhouse, which specializes in processing and commercialization of equine meat provided 60 mares that were randomized into 3 groups of 20 animals each. Mares presented different stages of pregnancy, so the initial third of pregnancy was considered the gestational period of up to 90 days of pregnancy. The mid-third of pregnancy covered the period between 90 and 240 days of pregnancy, and the latter third was the period from 240 days of pregnancy onward. The gestational phase of the mare was determined using of Roberts' methodology [7].

2.2. Sampling

Immediately after slaughter, the uteruses were dissected in order to perform allantocentesis and amniocentesis. Samples were collected at the slaughterhouse by using a fine needle (30 × 0.8 mm) and sterile (20-ml) syringes. Samples were inserted in 20- mL sterile test tubes and then frozen and stored in a conventional freezer at −20°C for later analysis [8].

2.3. Biochemical Analysis of Fetal Fluids

The following items were assessed: alkaline phosphatase (AP), total protein (TP), urea, creatinine, lactate dehydrogenase, Ca, Cl, Na, and K. Quantification was performed with the CELM SB-190 model spectrophotometer (Companhia Equipadora de Laboratórios Modernos, Barueri, São Paulo/SP- Brazil), for all elements except Na and K, for which the CELM FC-280 flame photometer (Companhia Equipadora de Laboratórios Modernos) was used. A CELM kit (Companhia Equipadora de Laboratórios Modernos) was used to quantify glucose, and catalytic activity (katal) kits were used to quantify creatinine, urea, Ca, and Cl, according to the manufacturer's instructions.

2.3.1. Alkaline Phosphatase

The kinetic method was used to quantify AP. This method uses hydrolysis of *p*-nitrophenyl phosphate by AP in either serum or plasma in pH 9.8. The formation velocity (in a 450-nm wave length) is proportional to the present enzyme activity.

2.3.2. Total Protein

Total protein was quantified by the colorimetric method (modified Bradford method) that uses Coomassie Blue stain (Coomassie Brilliant Blue G-250; Biorad Laboratories, CA) to detect even minimal amount of protein. This stain binds to albumin and different varieties of globulin that are present in the liquor and urine. Readings were performed with a spectrophotometer (610 nm).

2.3.3. Sodium and Potassium

Na and K were quantified by flame photometry in order to detect Na, K, and lithium ions in samples of "hemolysis-free" serum. This process is based on the thermal excitation of alkaline metals that can be isolated by an optical filter. Its emission is proportional to the excited atoms, thus determining the ion concentration in the sample.

2.4. Statistical Analysis

Data were analyzed by BioEstat version 4.0 software [9], and the class values (variables) that presented normal distribution and similar variances (protein and urea in amniotic fluid) used analysis of variance test, and differences among the three pregnancy phases were analyzed by the Tukey test. Classes that did not fit in the normal distribution or had similar variances (further classes in the amniotic fluid and all classes in the allantoic fluid) or both were assayed using the Kruskal-Wallis nonparametric test [10], and comparisons were made with the Student-Newman-Keuls test. Minimal significance was set at a P value of $<.05$.

3. Results

Mean values for AP, glucose, TP, urea, creatinine, Ca, Cl, Na, and K for each gestational phase is shown in Table 1.

4. Discussion

AP has been correlated with fetal maturity, fetal bone formation, and kidney function, especially in humans. In the early stages of human pregnancy, the increase in AP is associated with intestinal epithelial peeling [11]. Mean AP activity in amniotic fluid increased in the mid-third of pregnancy.

Table 1
Mean protein values of amniotic and allantoic fluids in pregnant mares for each gestational phase[a]

Biochemical Analyzed	Amniotic Fluid			Allantoic Liquid		
	Initial Third	Mid-third	Latter Third	Initial Third	Mid-third	Latter Third
AP, U/L	69.6 ± 44.40^{aB}	104.2 ± 44.71^{bB}	51.4 ± 15.40^{aB}	18.7 ± 13.32^{aA}	19.2 ± 9.25^{aA}	19.9 ± 4.79^{aA}
Glucose, mg/dL	7.5 ± 6.06^{aA}	12.2 ± 8.59^{aA}	4.0 ± 4.94^{bB}	7.1 ± 5.60^{aA}	16.4 ± 8.23^{bA}	5.1 ± 3.84^{aA}
TP, mg/dL	35.8 ± 9.79^{aB}	51.9 ± 11.0^{bB}	30.7 ± 10.93^{aA}	10.8 ± 8.72^{bA}	40.5 ± 19.26^{aA}	32.1 ± 7.94^{aA}
Urea, mg/dL	59.9 ± 15.71^{bA}	49.3 ± 11.63^{aB}	43.4 ± 11.59^{aA}	65.7 ± 19.78^{aA}	61.84 ± 17.63^{aA}	41.7 ± 9.76^{bA}
Creatinine, mg/dL	0.3 ± 0.28^{aA}	1.9 ± 2.31^{bB}	4.2 ± 2.12^{cB}	0.4 ± 0.42^{aA}	7.6 ± 5.07^{bA}	13.9 ± 3.13^{cA}
Ca, mg/dL	5.4 ± 2.07^{aA}	8.3 ± 4.55^{bB}	5.6 ± 2.30^{aB}	4.1 ± 2.98^{bA}	17.6 ± 4.21^{aA}	20.1 ± 8.93^{aA}
Cl, mEq/L	19.5 ± 4.42^{a}	33.8 ± 9.38^{b}	21.2 ± 4.41^{a}	7.5 ± 3.29^{b}	4.8 ± 3.42^{a}	2.9 ± 1.49^{a}
Na, mEq/L	96.9 ± 31.20^{a}	130.1 ± 12.89^{b}	92.1 ± 26.94^{a}	18.1 ± 10.39^{b}	41.0 ± 27.55^{a}	48.4 ± 21.46^{a}
K, mEq/L	8.1 ± 5.19^{a}	5.9 ± 1.49^{a}	6.6 ± 3.44^{a}	7.6 ± 5.61^{a}	12.0 ± 4.32^{b}	8.1 ± 10.52^{a}

AP, alkaline phosphatase; TP, total protein.
[a] Lower case letters: comparison in different time periods. Capital letters: comparison between amniotic and allantoic fluids.