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Original Research

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

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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.

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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.

However, between the initial third and the latter third of pregnancy there were no significant variations. Mean values for AP in allantoic fluid did not differ significantly during gestation. Williams et al [2] evaluated the levels of AP in the latter third of pregnancy and obtained similar values for allantoic fluid. AP values in the amniotic fluid were higher than those in the allantoic fluid, which is, incidentally, similar to findings of Kochhar et al [6] for the equine.

In the present study, the mean glucose concentration in amniotic fluid was significantly lower in the latter third of pregnancy, whereas the values in allantoic fluid for the same stage of pregnancy were significantly increased. However, the comparison between mean glucose levels in allantoic and amniotic fluids showed that the former presents with significantly higher levels of glucose during the latter third of pregnancy. These results are opposite to those in the study by Kochhar et al [6], who found higher values in the amniotic fluid.

Prestes et al [12] found ovine glucose values that were statistically different in the amniotic fluid at 70, 100, and 145 days of pregnancy. This was also observed in the present study, and it occurs because of the ingestion of glucose via fetal swallowing reflex. Nonetheless, glucose concentrations might differ among species, as Barreto et al [13] measured glucose levels in fetal fluids of 30- and 40-day pregnant bitches and found no differences between allantoic and amniotic fluid glucose values.

In the present study, the mean concentration of TP in amniotic fluid revealed a significant increase during the mid-third of pregnancy. As for the allantoic fluid, TP values increased during the mid-third of pregnancy and remained high until the latter third phase. Hence, the values found for allantoic and amniotic fluids in the present study greatly differed from those in the study by Kochhar et al [6] in the latter third of pregnancy. TP values in the amniotic fluid during pregnancy differed from those resported by Prestes et al [12] in the ovine, as it increased at 100-day pregnancy and remained practically steady. Notwithstanding, the low quantity of protein found in fetal fluids of the present study is similar to the ones found in other species [12-14].

Mean urea concentration in the amniotic fluid were significantly higher in the initial phase of pregnancy. The same was observed by Prestes et al [12] in the ovine amniotic fluid. As for the mid and latter third phases of pregnancy, values did not show any differences. The average value for the latter third of pregnancy in the present study was similar to the one reported by Kochhar et al [6]. Mean urea concentrations in allantoic fluid was similar during the initial and mid-third pregnancy phases. However, there was a decrease during the latter third, which might have occurred due to the great amount of urine in the allantoic sac [15]. The urea concentration in the latter third of pregnancy in the present study was similar to the one found by Kochhar et al [6].

Mean creatinine concentrations in the amniotic fluid were increased during pregnancy as well as those in the allantoic fluid. Amniotic fluid values were higher than the ones for allantoic fluid during the mid-third and latter third of pregnancy. These results go along with findings by Kochhar et al [6]. The increase in creatinine is correlated with not only kidney maturity [12] but also fetal muscular activity [6]. According to Vaala [1], equine fetuses that suffer from perinatal asphyxia syndrome will have an increase in creatinine values because of diminished kidney perfusion blood flow.

The amniotic fluid showed a significant increase in Ca concentration during the mid-third of pregnancy compared to the initial and latter third phases. As for allantoic fluid, there was an increase in Ca concentration throughout pregnancy. However, if values for allantoic and amniotic fluids are compared, the initial third of pregnancy had similar values, whereas during the mid- and latter third phases, there was a higher level of Ca in the allantoic fluid. These results were similar to those of Kochhar et al [6], despite the fact that they used milli-equivalents per liter in their assessments. These authors assigned the higher concentration of Ca ion in allantoic fluid to renal activity.

The average Cl ion concentration in the amniotic fluid was higher during the mid-third pregnancy than during the initial and latter third of pregnancy. This was not observed by Prestes et al [12] in the amniotic fluid of ewes as their values were statistically different throughout pregnancy. The allantoic fluid showed a decrease in Cl concentration from the initial third to the mid-third of pregnancy, but values remained steady from the mid-third to the latter third. Nevertheless, the amniotic fluid presented a higher Cl concentration than the allantoic fluid in all three time periods.

The average Na ion concentration in the amniotic fluid was higher in the mid-third of pregnancy. As for the latter third of pregnancy, values were lower than the ones observed by Kochhar et al [6]. However, this was not observed by Prestes et al [12] in the ovine as they reported a significant decrease in Na concentration during pregnancy. On the other hand, the allantoic fluid showed no significant differences in Na concentration during pregnancy. Nevertheless, the amniotic fluid presented higher Na concentration than those found in the allantoic fluid in all pregnancy phases, and the greatest difference was seen in the mid-third of pregnancy.

The average K concentrations did not vary throughout pregnancy in the amniotic fluid. The latter third of pregnancy showed values that were similar to the ones described by Kochhar et al [6], who found the concentration of 9.2 mEq/L. Taking into account the K concentration average in the allantoic fluid, values were higher in the mid-third of pregnancy. The latter third of pregnancy showed a concentration average of 8.13mEq/L, which is very close to the values found by Kochhar et al [6]. The allantoic fluid had shown higher K concentration than the amniotic fluid in the mid-third of pregnancy, and this is correlated with the increase in the production of fetal urine as well as the increase of fetal metabolic activity during pregnancy [6,12].

5. Conclusions

By comparing the biochemical profiles of fetal fluids, we observed that the concentrations of AP, TP, Cl, and Na were higher in the amniotic fluid in all three thirds of pregnancy. As for glucose and urea concentrations, they were similar throughout pregnancy. On the other hand, the Ca and K concentrations were higher in allantoic fluid.

Based on the results of the present study, the biochemical differences between the amniotic and allantoic fluids of mares as well as their variations during pregnancy can be seen. Hence, we believe that the study of the biochemical profile of fetal fluids will contribute to establishing a fetal health indicator.

References

[1] Vaala WE. Peripartum asphyxia syndrome in foals. Proc Am Assoc Eq Pract 1999;45:247–53.
[2] Williams MA, Wallace SS, Tyler JW, McCall CA, Gutierrez A, Spano JS. Biochemical characteristics of amniotic and allantoic fluid in late gestational mares. Theriogenology 1993;40:1251–7.
[3] Grunert E, Birgel EH. Obstetrícia veterinária [Veterinary Obstetrics]. 3rd ed. Porto Alegre: Editora Sulina; 1989 [in Portuguese].
[4] Asbury AC, LeBlanc MM. The placenta. In: McKinnon AO, Voss JL, editors. Equine reproduction. Philadelphia: Lea & Febiger; 1993. p. 509–16.
[5] Landim-Alvarenga FC. Reconhecimento materno do concepto e início da placentação [Maternal recognition of both conceptus and early placentation]. In: Prestes NC, Landim-Avarenga FC, editors. Obstetrícia veterinária [Veterinary Obstetrics]. Rio de Janeiro: Guanabara-Koogan; 2006. p. 22–40 [in Portuguese].
[6] Kochhar HPS, Simran PS, Nanda AS, Kaur R. Comparative biochemical indices of fetal fluids in normal foaling and stressful delivery in Indian Thoroughbred. J Equine Vet Sci 1997;17:206–10.
[7] Roberts SJ. Gestation and pregnancy diagnosis in the mare. Philadelphia: WB Saunders; 1986. p. 670–8.
[8] Williams MA, Schmidt AR, Carleton CL, Darien BJ, Goyert GL, Sokol RJ, et al. Amniotic fluid analysis for ante-partum foetal assessment in the horse. Equine Vet J 1992;24:236–8.
[9] Ayres M, Ayres Jr M, Ayres DL, Santos AS. BioEstat 4.0: aplicações estatísticas nas áreas das ciências biológicas e médicas [BioEstat 4.0: statistical applications in the field of Biological Sciences and Medicine]. Belém: Sociedade Civil Mamirauá, CNPq 2005 [in Portuguese].
[10] Zar JH. Biostatistical analysis. 4th ed. Upper Saddle River, NJ: Prentice Hall; 1998.
[11] Hahnemann N, Sorenson SA. Studies on alkaline phosphatase in amniotic in fluid. Acta Obstet Gynaecol Scand 1974;53:15–22.
[12] Prestes NC, Chalhoub MCL, Lopes MD, Takahira RK. Amniocentesis and biochemical evaluation of amniotic fluid in ewes at 70, 100 and 145 days of pregnancy. Small Rumin Res 2001;39:277–81.
[13] Barreto CS, Souza FF, Prestes NC. Avaliação bioquímica dos fluidos amniótico e alantoideano de cadelas entre 30 a 40 dias de gestação [Biochemical evaluation of amniotic and allantoic fluids from bitches at 30 to 40 days of pregnancy]. Rev Portuguesa Cienc Vet 2006;101:215–7 [in Portuguese].
[14] Reddy AP, Reddy VSC, Rao AS, Sharma GP, Reddy GUN, Reddy TJ, et al. Biochemical studies in the ewe (*Ovis aries*) amniotic fluid during different phases of gestation. Indian J Anim Sci 1995;10:321–3.
[15] Mellor DJ, Slater JS. Variation in the distribution of glucose and fructose within chronically catheterized sheep conceptuses during the last two months of pregnancy. Res Vet Sci 1973;15:317–21.