



Comparison between Biochemical Analysis of Cattle Amniotic Fluid and Maternal Serum Components during Pregnancy

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ABSTRACT

The present study aimed to compare the biochemical components including Total Protein (TP), albumin, globulins, cholesterol, triglycerides, High and Low-Density Lipoproteins (HDL and LDL), creatinine, urea, sodium (Na), potassium (K), chloride (Cl), calcium (Ca) and inorganic phosphorus (P), of Amniotic Fluid (AF) with those of Maternal Serum (MS) during the first, second and third trimesters of pregnancy in cattle and Fetal Serum (FS) at birth. At birth AF, MS and FS were collected. Maternal blood samples and gravid uteri were collected after accidental slaughter. The actual data recorded during three trimesters according to the curved crown-anus length of the fetus. The MS concentrations of globulins, cholesterol, triglycerides, lipoproteins, creatinine, Na, K, Cl, Ca and inorganic-P were significantly higher than the AF during the first trimester. At delivery, the concentrations of cholesterol, triglycerides, and creatinine in the AF were lower than those in the MS or FS. The concentrations of Ca and inorganic-P in the FS were higher than those in the MS or AF. The levels of TP, creatinine, urea in the AF and urea in the MS increased as the gestation stages advanced. The levels of Na and Ca in the AF decreased as the gestation stage advanced while the K concentration increased. In conclusion, our results indicated an active placental transport for Ca and P. The TP, albumin, globulins, cholesterol, triglycerides, HDL and LDL, creatinine, urea, Na, K, Cl, Ca and P in AF and MS during the first, second and third trimesters of pregnancy in cattle might be changed with progressing the gestation.

Keywords: Amniotic fluid, Cattle, Fetal serum, Gestation, Maternal blood

INTRODUCTION

The amnion formation occurs on days 13-14 of pregnancy in cattle, and then, the Amniotic Fluid (AF) fills the amniotic sac (Robert, 1986). Fetal membranes are extra-embryonic in nature (Minazaki et al., 2008). Amniotic fluid (AF), the protective liquid contained in the amniotic sac, is essential for fetal development and growth during gestation (Underwood et al., 2005, Fitzsimmons and Bajaj, 2019). AF is formed partly or entirely by the amnion, secretion from the respiratory tract, buccal cavity, nasal cavity, and embryonic skin before keratinization occurs (Moore 1982, Brace 1994, Hammer et al., 1997). AF accumulates early and subsequently diminishes as the embryo itself grows and this occurs in all species of mammals (Adolph, 1967). The fixed exchange of water and fluid component between the fetal compartments and the mother circulation by the fetoplacental unit shows the changes in the physical, chemical, and biochemical constituents of fetal fluids (Aidasani et al., 1993).

The fetal fluids are important for the handling of the fetal waste products and protect the fetus from the mechanical shock that has developed throughout pregnancy (Amle et al., 1992); they prevent adherence between fetal skin and the amniotic membrane (Williams et al., 1993); and during the expulsive stage they lubricated and widened the birth canal (Asbury and Blanc, 1993); they allow fetal development and movement inside the uterus (Zanella et al., 2014); and also inhibit bacterial and fungal growth (Zare-Bidaki et al., 2017). Biochemical analysis of AF is significantly important for the evaluation of fetal metabolism and pathological conditions during gestation (Prestes et al., 2001).

The purpose of the present study was to evaluate the biochemical components of AF in relation to maternal blood serum during the first, second and third trimesters in addition to those of Fetal Serum (FS) at birth to support our assumption. Cattle AF was a simple MS or FS dialysate, and the fetus played an important role in the final biochemical composition of the amniotic fluid during pregnancy.

ORIGINAL ARTICLE
pII: S232245682000009-10
Received: 13 Jan 2020
Accepted: 15 Feb 2020

MATERIAL AND METHODS

Ethical approval

This experiment was conducted according to the rules of the Research Ethics Committee of the Veterinary faculty of Aswan University in Egypt.

Sampling

A field survey planned on using data collected from different abattoirs located in Darwa, Aswan, Egypt during the period between 2018 and 2019. Maternal serum and amniotic fluid were harvested from 40 emergency slaughtered animals at different stages of gestation. The age of these animals varied from 2 and 12 years old and the breeding history was unknown. 20 animals were enrolled during normal labor.

Maternal serum

Maternal blood samples were obtained via jugular venipuncture and collected in sterile glass tubes. The blood samples were allowed to clot, and centrifuged at 3000 round per minute (rpm) for 15 minutes; the serum was separated and stored at 20°C for further analysis.

Amniotic fluid

The gravid uteri were removed immediately after slaughter to collect the AF. The gravid uteri were incised through greater curvature with a sharp scalpel to locate the fetal sacs, and then carefully separated from the endometrium and slowly enclosed outside the horn. The AF was collected by puncturing the amniotic sac and 10 ml of AF was aspirated from the amniotic sac using 10 ml disposable syringes, or amniotic fluid samples were obtained transcervically during delivery. The aspirated fluid was stored in labelled tubes and frozen at -20°C until biochemical analysis.

Fetal serum

Fetal blood was obtained from the umbilical vessels. The blood samples were allowed to clot, and centrifuged at 3000 rpm for 15 minute; the serum was separated and stored at -20°C for further analysis.

Fetal age and detection of the gestation period

The fetuses were expelled from enclosing membranes and the fetal ages were determined by applying the age estimation formula, $X = 2.5(y+21)$, presented by Richardson et al. (1990), where X equaled to developmental age in days and y was the crown-anus length in centimeters. The cattle presented different stages of pregnancy and were divided into first, second and third trimesters of pregnancy.

Biochemical studies

Serum urea and creatinine levels were determined calorimetrically using diagnostic kits according to Tietz et al. (1995). The serum Ca level was determined according to Connell (2012). The inorganic phosphorus (inorganic P) level in the serum content was determined according to Berti et al. (1988). The Na and potassium (K) levels in the serum were estimated with a flame-photometer according to Bauer (1982) and serum chloride (Cl) level was determined according to Chirife and Resnik (1984). The Total Protein (TP) content was determined according to Henry et al. (1974). Albumin content was determined according to Doumas and Biggs (1972). The serum globulin level was calculated by subtracting the albumin level obtained from the TP content. Immunoglobulin electrophoresis was performed as described by Henry et al. (1974). The total serum cholesterol was measured according to Mamoru et al. (1977). The serum triglyceride concentrations were measured according to Izzo et al. (1981). The serum High-Density Lipoprotein Cholesterol (HDL-C) and Low-Density Lipoprotein Cholesterol (LDL-C) levels were estimated according to Nauck et al. (2002) and Friedewald et al. (1972) respectively.

Statistical analysis

The data obtained were statistically analyzed by F-test according to Tamhane and Dunlop (2000) using the computer program MSTAT-C. Means values in the same row with different letters are statistically significant and the highest values are represented with the letter (a). Statistical significance was declared at the $p \leq 0.05$ level and the data are presented as the mean \pm SE.

RESULTS

The TP, albumin, globulin, α_1 globulin, α_2 globulin, β globulin, γ globulin, cholesterol, triglycerides, HDL, LDL, some metabolites such as urea and creatinine, monovalent cations (Na and K) and monovalent anion (Cl), as well as Ca and phosphorus levels of the MS and AF are given in tables 1 and 2.

Table 1. Lipids and proteins levels in amniotic fluid and maternal serum of pregnant cattle slaughtered in Darwa and Aswan abattoirs, of Egypt during first, second and third trimesters of gestation.

Parameter	Type of fluid	Trimester		
		First (n=20)	Second (n=10)	Third (n=10)
Total proteins, (g/dl)	Amniotic fluid	4.37 ± 0.08 ^b	6.16 ± 0.56 ^a	6.44 ± 0.39 ^a
	Maternal serum	4.66 ± 0.35 ^b	5.80 ± 0.44 ^a	5.81 ± 0.46 ^a
Albumin, (g/dl)	Amniotic fluid	3.1 ± 0.07 ^b	4.26 ± 0.28 ^a	4.48 ± 0.31 ^a
	Maternal serum	2.33 ± 0.06 ^c	3.59 ± 0.3 ^a	3.6 ± 0.81 ^a
Globulin, (g/dl)	Amniotic fluid	1.27 ± 0.02 ^c	1.9 ± 0.23 ^b	1.96 ± 0.32 ^b
	Maternal serum	2.33 ± 0.3 ^a	2.21 ± 0.15 ^a	2.2 ± 0.17 ^a
α1 globulin, (g/dl)	Amniotic fluid	0.13 ± 0.01 ^c	0.22 ± 0.009 ^{ab}	0.14 ± 0.006 ^c
	Maternal serum	0.25 ± 0.03 ^a	0.20 ± 0.009 ^b	0.18 ± 0.003 ^{bc}
α2 globulin, (mg/dl)	Amniotic fluid	0.28 ± 0.03 ^c	0.22 ± 0.08 ^c	0.32 ± 0.02 ^c
	Maternal serum	1.45 ± 0.19 ^a	1.17 ± 0.009 ^a	0.87 ± 0.06 ^b
β globulin, (g/dl)	Amniotic fluid	0.42 ± 0.04 ^d	1.89 ± 0.18 ^b	0.45 ± 0.029 ^d
	Maternal serum	2.9 ± 0.44 ^a	1.6 ± 0.057 ^{bc}	1.11 ± 0.015 ^c
γ globulin, (g/dl)	Amniotic fluid	0.42 ± 0.03 ^c	1.84 ± 0.12 ^c	0.55 ± 0.06 ^c
	Maternal serum	2.71 ± 0.12 ^a	2.12 ± 0.06 ^b	1.68 ± 0.08 ^d
Cholesterol, (mg/dl)	Amniotic fluid	17.32 ± 0.56 ^d	16.93 ± 0.97 ^d	16.08 ± 0.79 ^d
	Maternal serum	182 ± 6.47 ^a	136 ± 2.7 ^b	124.57 ± 5.85 ^c
Triglycerides, (mg/dl)	Amniotic fluid	20.41 ± 3.22 ^c	22.3 ± 4.3 ^c	18.7 ± 0.91 ^c
	Maternal serum	103.34 ± 4.1 ^a	95.83 ± 1.09 ^a	77.2 ± 6.1 ^b
HDL, (mg/dl)	Amniotic fluid	28.1 ± 2.7 ^b	25.38 ± 2.07 ^b	21.42 ± 0.6 ^b
	Maternal serum	49.24 ± 2.17 ^a	47.17 ± 1.92 ^a	43.72 ± 2.18 ^a
LDL, (mg/dl)	Amniotic fluid	5.67 ± 0.23 ^d	5.24 ± 0.42 ^d	5.13 ± 0.5 ^d
	Maternal serum	54.4 ± 1.69 ^c	62.33 ± 2.85 ^b	69.67 ± 0.96 ^a

Data are expressed as mean values ± Standard error; the number of studied samples in each trimester of gestation are shown in parentheses. Values with different superscripts are significantly different at $p \leq 0.05$. n = the number of studied samples, HDL= High Density Lipoprotein, LDL= Low Density Lipoprotein

Table 2. The levels of metabolites and ions in amniotic fluid and maternal serum during first, second and third trimesters of gestation in cattle slaughtered in Darwa and Aswan abattoirs of Egypt.

Parameters	Type of fluid	Trimesters		
		First (n = 20)	Second (n = 20)	Third (n = 20)
Creatinine (mg/dl)	Amniotic fluid	1.06 ± 0.17 ^b	1.26 ± 0.13 ^{ab}	1.62 ± 0.14 ^a
	Maternal serum	1.63 ± 0.4 ^a	1.70 ± 0.05 ^a	1.77 ± 0.06 ^a
Urea (mg/dl)	Amniotic fluid	22.87 ± 1.27 ^c	24.37 ± 2.01 ^c	26.3 ± 2.06 ^{ab}
	Maternal serum	24.11 ± 0.58 ^c	27 ± 1.5 ^{ab}	28.37 ± 1.68 ^a
Sodium (mEq/L)	Amniotic fluid	111.36 ± 1.43 ^b	107.3 ± 3.7 ^b	86.2 ± 5.47 ^c
	Maternal serum	135.8 ± 1.9 ^a	133.27 ± 0.93 ^a	131.33 ± 4.67 ^a
Potassium (mEq/L)	Amniotic fluid	1.76 ± 0.29 ^c	2.06 ± 0.17 ^c	2.7 ± 0.06 ^b
	Maternal serum	3.84 ± 0.12 ^a	3.5 ± 0.06 ^a	3.57 ± 0.22 ^a
Chloride (mEq/L)	Amniotic fluid	78.55 ± 2.45 ^b	80.29 ± 0.09 ^b	83.2 ± 0.97 ^b
	Maternal serum	95.81 ± 0.79 ^a	94.27 ± 0.38 ^a	93.33 ± 0.88 ^a
Calcium (mg/dl)	Amniotic fluid	7.23 ± 0.3 ^b	6.69 ± 0.22 ^b	6.13 ± 0.23 ^c
	Maternal serum	9.22 ± 0.71 ^a	9.24 ± 0.15 ^a	8.82 ± 0.18 ^a
Inorganic phosphorus (mg/dl)	Amniotic fluid	3.22 ± 0.21 ^b	3.9 ± 0.19 ^{ab}	4.04 ± 0.09 ^{ab}
	Maternal serum	5.16 ± 0.08 ^a	5.05 ± 0.11 ^a	4.77 ± 0.14 ^a

Data are expressed as mean values ± Standard error; the number of studied samples in each trimester of gestation is shown in parentheses. Values with different superscripts are significantly different at $p \leq 0.05$. n = the number of studied samples

Proteinogram

The AF proteinogram showed a significant ($p \leq 0.05$) increase in TP content in the 2nd and 3rd trimesters compared to the MS. The TP, α₂ globulin and γ globulin levels increased as the gestation increased. The MS TP content was not significantly higher than the AF TP content during the first trimester. The albumin level increased significantly in the first trimesters in the AF compared to the MS. The globulin, α₁ globulin, α₂ globulin, β globulin and γ globulin levels were significantly lower in the AF than in the MS.

Cholesterol, triglycerides, HDL and LDL

The cholesterol, triglycerides, HDL and LDL levels in the AF were not significantly different among the three trimesters of gestation but remained low compared to those in the MS throughout the pregnancy. The mean cholesterol, triglycerides, and HDL values in the MS were decreased significantly ($p \leq 0.05$) as the gestation stage advanced, but the LDL level increased with gestation progressed.

Creatinine

The AF creatinine increased with gestation stage. The AF creatinine level was lower than the MS creatinine level in the 1st trimester. The MS creatinine level did not change significantly ($p \leq 0.05$) throughout the pregnancy.

Urea

The concentration of urea in the AF gradually increased during gestation. The urea concentration in the AF was significantly lower than that in the MS in the 2nd trimester of gestation.

Sodium (Na)

The AF Na concentration decreased significantly ($p \leq 0.05$) as the pregnancy progressed and was lower than that in the MS, but the MS Na concentration did not change significantly during pregnancy.

Potassium (K)

The AF K level increased significantly ($p \leq 0.05$) with increasing the gestational stage and remained in lower concentrations than the MS K levels. However, the MS K levels did not differ significantly ($p \leq 0.05$) during the three trimesters of pregnancy.

Chloride (Cl)

The concentrations of Cl were significantly higher in MS than in the AF.

Calcium (Ca)

The AF showed a significant ($p \leq 0.05$) decrease in Ca concentration as the gestation stage advanced while there was no significant difference in Ca levels in the MS. The AF Ca levels were consistently lower than the MS Ca levels throughout the pregnancy.

Inorganic P

The concentrations of inorganic P in the AF increased insignificantly ($p \leq 0.05$) as the pregnancy progressed. The MS inorganic P level did not change significantly throughout the pregnancy. At delivery state, the levels of all the examined biochemical components were determined simultaneously in the AF, MS and FS samples (Table 3). The mean concentrations of TP, albumin and globulin in the matched samples were insignificantly ($p \leq 0.05$) different among the AF, MS and FS, but the α_1 globulin, α_2 globulin, β globulin and γ globulin levels in AF were significantly ($p \leq 0.05$) lower than those in the MS or FS. The concentrations of cholesterol and triglycerides were significantly ($p \leq 0.05$) lower in the AF than in the MS or FS. The creatinine level in the AF was significantly lower than that in the MS and FS. The urea level was not significantly different in the matching samples; amniotic fluid (AF) and maternal serum (MS) samples collected during three trimesters of gestation in cattle. The concentrations of Na, K and Cl in the AF were not significantly higher than those in the MS and FS, and concentrations of other electrolytes including Ca and inorganic P were significantly lower in the AF and the MS than in the FS.

Table3. The levels of biochemical components in amniotic fluid, maternal serum and fetal serum at birth time of cattles slaughtered in Darwa and Aswan abattoirs of Egypt

Parameter	Amniotic fluid (n=20)	Maternal serum (n=20)	Fetal serum (n=20)
Total proteins (g/dl)	6.64 ± 0.21	7.22 ± 0.94	6.15 ± 0.43
Albumin (g/dl)	4.37 ± 0.41	4.91 ± 0.23	4.17 ± 0.17
Globulin (g/dl)	2.27 ± 0.14	2.31 ± 0.31	1.98 ± 0.31
α_1 globulin (g/dl)	0.05 ± 0.02 ^c	0.19 ± 0.003 ^b	0.21 ± 0.02 ^a
α_2 globulin (g/dl)	0.093 ± 0.04 ^c	1.22 ± 0.07 ^a	1.17 ± 0.12 ^b
β globulin (g/dl)	0.17 ± 0.07 ^c	1.5 ± 0.057 ^a	1.81 ± 0.23 ^a
γ globulin (g/dl)	0.22 ± 0.08 ^c	1.99 ± 0.05 ^a	1.02 ± 0.12 ^b
Cholesterol (mg/dl)	15.67 ± 0.67 ^c	103.5 ± 4.8 ^a	85.87 ± 4.5 ^b
Triglycerides (mg/dl)	18.32 ± 2.67 ^b	76.05 ± 4.8 ^a	75.57 ± 4.27 ^a
HDL (mg/dl)	10.43 ± 0.29 ^b	42.17 ± 2.35 ^a	39.62 ± 5.32 ^a
LDL (mg/dl)	5.82 ± 0.23 ^c	73.67 ± 2.9 ^a	34.39 ± 2.49 ^b
Creatinine (mg/dl)	1.75 ± 0.11 ^b	1.85 ± 0.07 ^{ab}	2.27 ± 0.28 ^a
Urea (mg/dl)	26.96 ± 2.28	28.47 ± 2.3	29.83 ± 2.08
Sodium (mEq/L)	125 ± 1.53	121.7 ± 4.36	117.00 ± 2.31
Potassium (mEq/L)	2.99 ± 0.05	2.85 ± 0.12	2.8 ± 0.02
Chloride (mg/dl)	91.12 ± 0.68	89.09 ± 1.37	88.29 ± 0.41
Calcium (mg/dl)	8.43 ± 0.22 ^b	8.77 ± 0.14 ^b	10.54 ± 0.25 ^a
Inorganic phosphorus (mg/dl)	4.64 ± 0.32 ^b	4.38 ± 0.17 ^b	7.35 ± 0.11 ^a

Data are expressed as mean values ± Standard error; rows with different letters (a, b, c) are significantly different at $p \leq 0.05$. The number of studied samples is shown in parentheses. n = the number of studied samples, HDL= High Density Lipoprotein, LDL= Low Density Lipoprotein.

DISCUSSION

The developing fetus is surrounded by AF. The AF volume and composition are affected by fetal urination, drinking and fetal membrane permeability. Hormones such as prolactin and cortisol, may play an important role by affecting membrane permeability (Wintour et al., 1986).

The Na concentrations in the AF decreased in the three trimesters studied compared to those in the MS. In ovine, a similar result was recorded by Prestes et al. (2001) who found that the minerals act on fetal kidneys, increasing the K and decreasing the Na concentration in fetal urine. At the end of pregnancy, the fetal kidney reabsorbed 85% to 95% of Na ion from the filtrate load. So, the hypo-tonicity of fetal urine compared to plasma, indicating the efficacy of the collecting duct (Robillard et al., 1988). The results of the present study were concurred with high Na reabsorption of and a relatively low AF concentration in the third trimester of gestation, as mentioned by Brenner (1990). According to Wintour et al. (1986) a classic Na pump could be responsible and the alteration in the relative permeability for Na and K might affect the transport. The K content increased in the AF as gestation progressed. These results incompetence with the maturation of distal and collecting tubules, that are responsible for K regulation by the fetal kidney (Satlin, 1991), confirming observations reported by Benzie et al. (1974). There was an insignificant decrease in Cl concentration as pregnancy progressed, and the concentration was significantly lower in the AF than in the MS. According to Mellor and Slatter (1971), fetal orosomucoid secretion could be a source of Cl (found in the AF).

The Ca content in the AF decreased as gestation advanced. This was contrariwise for the phosphorus content in AF. Ca and phosphorus are important for the development of the fetal skeleton. Thus, it would be expected that the fetus excreted very little into the AF when preserving these elements. Wales and Murdoch (1973) reported that the Ca concentration in the ovine fetus AF decreased very slightly between 31st and 44th day of pregnancy.

At delivery, there were no available references to compare the results of the present study with it. Comparing the biochemical components and electrolytes levels in AF, MS and FS in the cattle, had been found that first the levels of organic substances in the AF, including TP, cholesterol, and triglycerides were lower than in MS and FS; second insignificant change in the concentrations of metabolites including urea and creatinine in the AF and MS or FS except significant increase in the FS urea than in the AF, and third the levels of Na, K and Cl in the AF were almost the same as in the MS and FS and the levels of Ca and P in the FS were significantly higher than those in the AF and MS. These data suggested that the electrolytes in the AF might be derived from MS but the fetus was the main source of other organic components and metabolites present in the AF. The present study revealed that the Ca and P levels in fetal serum were higher than in MS, which could be caused by active transport of Ca and P from pregnant cattle to the fetus through the placenta and increased absorption in the fetus. The presence of metabolites, including urea and creatinine in the AF, represented the excretion of urine from the fetus (Anderer and Schinder, 1975). The content of TP in the AF and FS were similar in the present study, which could suggest that AF played a role in fetal development and that both electrolytes and proteins found in the AF were required to balance the osmotic pressure between AF and blood, as reported by Tong et al. (2009).

CONCLUSION

Cattle AF was a simple MS or FS dialysate, and the fetus played an important role in the final biochemical composition of the amniotic fluid during pregnancy. The concentration of cholesterol, triglycerides, TP, creatinine, urea, Na, K, Cl, Ca and P in cattle AF and MS might change as the pregnancy progressed. Studying the changes in the biochemical composition of the AF during the development of pregnancy was of great value, because it explained the mechanisms of AF formation and determined the physiological function of AF during the development of the fetus. In addition, this knowledge was of utmost importance in order to understanding fetal metabolism and pregnancy associated abnormalities. Further research is needed to better correlate changes in the AF biochemical composition changes with fetal organ development and maturation, and to investigate pregnancy-associated abnormalities.

DECLARATION

Competing interests

The authors declare that there is no conflict of interest in the present work. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sector. The protocol was approved accordance with National Regulations on Institutional Animal Care and Use Ethical Committee and animal welfare.

Consent to publish

All authors have given their consent before entering the study.

Authors' contributions

Walaa M. Essawi detected the stage of pregnancy, collected the samples and participated in the preparation of the manuscript, Doaa I.A. Mostafa and Amal I.A. El Shorbagy performed the biochemical investigations, data analysis and prepared the manuscript (writing and revision). All authors approved the final version of manuscript before publication.

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