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Effect of Gestation and Maternal Copper on the Fetal Fluids and Tissues Copper Concentrations in Sheep

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Abstract: Problem statement: Samples of allantoic, amniotic fluid, fetal liver, kidney, maternal plasma and liver were collected from 30 ewes and classified into either early or late gestation and copper concentrations were measured. **Approach:** The Cu concentrations in the maternal plasma, allantoic, amniotic fluid, fetal liver and kidney increased significantly ($p<0.01$) during late gestation while maternal liver Cu decreased significantly ($p<0.01$). **Results:** Significant positive relationships were recorded between age of the fetus and Cu concentrations in the allantoic and amniotic fluid ($r = 0.71$-0.83, $p<0.001$), fetal liver ($r = 0.80$, $p<0.001$), kidney ($r = 0.59$, $p<0.01$) and maternal plasma ($r = 0.75$, $p<0.001$). Significant ($p<0.01$) positive relationships were also recorded between the Cu concentrations in the amniotic, allantoic fluid and maternal plasma with fetal liver Cu concentrations ($r = 0.36$-0.73), the maternal plasma and liver Cu concentrations were significantly negative correlated ($r = -42$, $p<0.05$). **Conclusion:** A significant negative correlation was recorded between the Cu concentrations in the maternal liver and fetal age ($r = -0.74$, $p<0.01$). Strong fetal-maternal relationships in Cu concentration were evident throughout the gestational period and dams seem to sacrifice Cu levels in order to maintain that in the fetus. Cu concentrations in the amniotic and allantoic fluids could be used as a possible indicator of the Cu status of the fetus throughout gestation.

Key words: Maternal copper, fetal fluids, amniotic fluid, fetal liver, maternal plasma, Cu concentration

INTRODUCTION

Pregnancy is a period of rapid growth and cell differentiation for both the mother and fetus. Consequently, it is a period when both are vulnerable to changes in dietary supply, especially of those nutrients that are marginal under normal circumstances (Gambling and McArdle, 2004). Each fetus is completely dependent on its dam via the placenta for its supply of essential trace elements (Abdelrahman and Kincaid, 1993). Copper is often one of the most limiting trace elements for the fetus and neonate for normal development. Deficiency of this element impairs fetal growth and can cause death (Mertz and Underwood, 1987). Calves normally are born with liver Cu concentrations of approximately 400 ppm, compared with adult concentrations of 200 ppm (Mertz and Underwood, 1987). When intakes of Cu are deficient, maternal transfer of Cu to the fetus is insufficient for normal development and abnormalities to the central nervous system, skeleton and metabolism result (Mertz, 1988; Widdowson *et al*., 1974). It is reported that there is extraordinary metabolic demands on both the mother and developing fetus associated with gestation because adequate maternal copper nutritive is essential for normal embryogenesis (Keen *et al*., 1998). Copper is an essential trace element that plays an important role in the biochemical reactions of the body; however, its requirement and interaction with other minerals is not

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clearly understood (Solaiman *et al*., 2001). Hepatic concentrations of trace elements are commonly used to estimate trace element storage pools because dietary intake is rarely available and nutrient interactions affect availability or retention (Hill and Matrone, 1970; Mertz, 1988).

Collection of fetal tissues from local slaughter houses may enable endemic deficiencies of minerals to be determined. However, if fetal tissues are to be used to assess the nutritional status of the dam, the effect of gestational age on the concentrations of minerals in fetal tissues must be known. Therefore, the present study was conducted to estimate and correlate maternal liver, plasma, fetal liver, fetal kidney and amniotic fluid and allantoic fluid copper concentrations and studying the effect of maternal copper on the fetal copper status through gestation in sheep.

MATERIAL AND METHODS

Samples were taken from 30 pregnant singleton Pelibuey sheep of 3-4 years old and their corresponding fetuses, at the time of slaughter. Maternal samples included blood and liver, while the fetal samples included amniotic fluid, allantoic fluid, liver and kidney. All samples were used for Cu determination and investigation the effect of the fetal age and maternal Cu on the Cu concentrations in the fetal fluids and tissue throughout the gestation. Samples were classified according to the estimated age of the fetus (Lyngest, 1971). The early stage of gestation was defined as before day 90 when fetal length was less than 20 cm which included 10 animals at this stage; while the late stage of gestation was defined as after day 90 which included 20 animals at this stage. Ewes have been kept on an adequate Cu diet.

Preparation and analysis of the samples: Maternal blood samples were centrifuged (2000 G; 15 min) to obtain plasma (-20°) for later analyses. Amniotic and allantoic fluid, maternal liver, fetal liver and kidney were frozen (-20°) for Cu determinations.

The plasma, amniotic and allantoic fluid samples were processed by mixing 1 mL of each sample with 10 ml of deionized water, 5 mL of concentrated nitric acid and 2 mL of hydrogen peroxide (30%) (J.T. Baker, Phillipsburg, N. J.)-keeping the solution at room temperature for 30 min in sealed Teflon vessels (Lyngest, 1971). Subsequently, the samples were placed in a microwave digester (Mars 5 CEM Corporation USA) with an increasing temperature slope of 5 min to reach 120°C and it was held in this temperature for 2 min. The temperature was then increased to 170°C within 5 min and maintained for 2 min with a maximum pressure of 350 psi (Ortman and Pehrson, 1997). The samples were allowed to cool for 5 min in an oven and then left to obtain room temperature (for 1 h). The samples were then transferred to 50 mL volumetric flasks and filled to the top with 7M HCl and left overnight (4°C) to be analyzed the following day. Cu concentrations were determined with the aid of atomic absorption spectrophotometer (Varian, model Spectra AA-800).

Tissue samples of 0.5 ± 0.1 g were digested using a microwave oven using 10 mL of nitric acid and 2 mL of double distilled water in a Teflon vessel (Rowntree *et al*., 2004; Shaw *et al*., 2002). Samples were then allowed to be digested for approximately 1h at room temperature. Vessels were then placed in a microwave digester (Mars 5 CEM Corporation USA), in order to gradually increase the pressure to 210 psi with the maximal vessel temperature being 190°C. The vessels were maintained at 210 psi for 10 min, allowed to cool for 10 min and then ventilated. Thereafter 2 mL of hydrogen peroxide (30%) (J.T. Baker, Phillipsburg, N.J) Was added to each tissue sample. The digested samples were transferred to volumetric flasks and brought to a uniform volume of 25 mL with 7M HCl and then stored until analyses. Cu concentrations were determined with the aid of atomic absorption spectrophotometer (Varian, model Spectra AA-800).

Statistical analysis: Means, Pearson correlation coefficient, Analysis Of Variance (ANOVA) by general linear model and regression analyses were performed using the Statistical Analysis System software (SAS, 1985).

RESULTS

In this study we found that, Cu concentrations in the fetal liver, kidney, amniotic fluid, allantoic fluid and maternal plasma were significantly ($p<0.05$-0.001) increased in late gestation than those of early gestation while there was significant ($p<0.001$) decrease in the maternal liver Cu concentration in late gestation than that of early gestation (Fig. 1). The relationship between age of the fetus and Cu concentration was significantly positive in amniotic fluid ($r = 0.83$, $p<0.001$), allantoic fluid ($r = 0.71$, $p< 0.001$), fetal liver (Fig. 2) ($r = 0.80$, $p<0.001$), fetal kidney ($r = 0.59$, $p<0.01$) and maternal plasma ($r = 0.75$, $p<0.001$) while the relationship was significantly negative between age of the fetus and Cu concentrations in the maternal liver (Fig. 2) ($r = -0.74$, $p<0.001$).

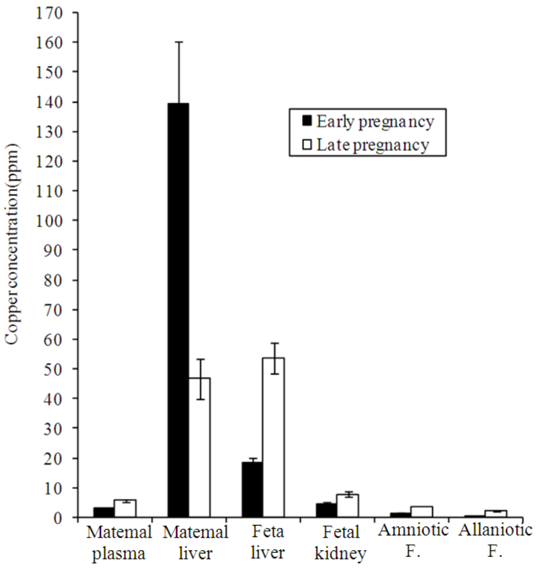

Fig. 1: Copper concentration (ppm) in maternal plasma, liver, fetal liver, kidney, amniotic fluid and allantoic fluid in the early and late stage of pregnancy in sheep

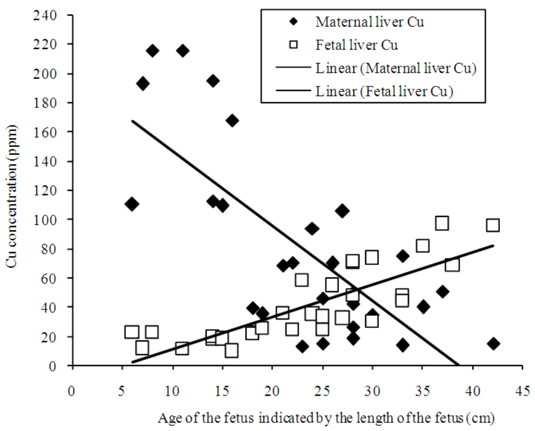

Fig. 2: The relationships between maternal liver Cu and fetal liver Cu concentrations with the fetal age in sheep

There was significant (r = -0.50, p<0.01) negative relationship between the fetal and maternal liver Cu concentrations and maternal Cu tended to be significantly higher than fetal Cu in early gestation (p<0.001) while there was no significant changes between maternal and fetal liver Cu concentrations in late gestation. There were significant positive relationships between maternal plasma Cu concentrations with amniotic fluid (r = 0.42, p<0.01),

Table 1: The relationships between Cu concentrations in the fetal liver, kidney, amniotic fluid, allantoic fluid, maternal liver, plasma and age of the fetus in sheep

	Age of the fetus	Maternal plasma Cu	Maternal liver Cu	Fetal liver Cu
Amniotic fluid Cu	0.83**	0.42*	-0.63**	0.73**
Allantoic fluid Cu	0.71**	0.48**	-0.55**	0.56**
Fetal liver Cu	0.80**	0.36*	-0.5**	-
Fetal kidney Cu	0.59**	NS	-0.43*	-
Maternal liver Cu	-0.74**	-0.42*	-	-0.5**
Maternal plasma Cu	0.75**	-	-0.42*	0.36*

allantoic fluid (r = 0.48, p<0.01) and fetal liver (r = 0.36, p<0.01) Cu concentrations, while the relationship between maternal plasma and maternal liver Cu concentrations was significantly negative (r = -0.42, p<0.01). There were significant negative relationships between maternal liver Cu concentrations with amniotic fluid (r = -0.63, p<0.001) and allantoic fluid (r = -0.55, p<0.01), fetal liver (r = -0.50, p<0.01) and kidney (r = - 0.43, p<0.01) Cu concentrations. There were significant positive relationships between fetal liver Cu concentration with amniotic fluid (r = 0.73, p<0.001) and allantoic fluid (r = 0.56, p<0.01) Cu concentrations Table 1.

DISCUSSION

Maternal liver Cu was negatively correlated with fetal age, this results agree with the results obtained by (Gonneratne and Christensen, 1989b). While (Graham *et al*., 1994) found that maternal liver Cu was not correlated with fetal size. Fetal liver Cu increased as fetal age increased and was less than to maternal Cu in early gestation and there was no differences between maternal and fetal liver Cu in late gestation, while fetal liver Cu was significantly higher than that of the maternal liver through gestation (Gonneratne and Christensen, 1989a), as well as Cu concentration was significantly increased in early gestation than that of late gestation in the fetal liver (Abdelrahman and Kincaid, 1993) and kidney while (Richards, 1999) found that in fetal kidney, Cu concentration did not change significantly with gestation. An increase in fetal Cu with fetal size has previously been reported for cattle and sheep (Gonneratne and Christensen, 1989a; Williams *et al*., 1978; Williams and Bremn, 1976). Numerous studies have shown significant correlations between fetal and maternal tissue copper concentration. Because copper is essential for development of the central nervous system of the embryonic lamb, an acute maternal hypocuprosis can cause gross brain lesions in the fetal or neonate lamb (Hidiroglou and Knipfel, 1981).

In humans, fetal Cu concentrations reportedly increased or remained stable through gestation (Casey and Robinson, 1978; Widdowson *et al*., 1972). Ovine maternal and fetal liver Cu were negatively correlated in this and previous reports (Gonneratne and Christensen, 1989a). Presence of significant negative relationship between age of the fetus and maternal liver Cu concentration as well as the relationships between maternal liver and amniotic and allantoic fluid Cu concentrations were significantly negative, while the relationship between age of the fetus and maternal plasma, fetal liver, amniotic fluid, allantoic fluid and fetal kidney Cu concentrations were significantly positive may indicate that, the dam and fetus depend on the maternal liver Cu contents during gestation and it can be used as an indicator of the Cu status through gestation and fetuses have a capacity to sequester maternal Cu, even when the dam is Cu deficient (Graham *et al*., 1994). Parkinson *et al*. (1981) found that amniotic fluid copper concentration gradually increased during pregnancy.

CONCLUSION

From this study we can concluded that, there is a strong relationship between the fetus and dam concerning Cu metabolism through gestation. The dams seem to sacrifice their Cu level in order to maintain the fetal Cu disposition. Cu concentrations in the amniotic and allantoic fluids play a role in the metabolism and utilization of Cu through gestation and may be used as an indicator of Cu status in the fetus throughout gestation.

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