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The relationship between fetal and maternal selenium concentrations in sheep and goats

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Abstract

In this study, biological samples (slaughterhouse material) were collected from 30 sheep and 36 goats and classified according to gestational stage into either early or late gestation. Samples consisted of allantoic fluid, amniotic fluid, fetal liver, fetal kidney, fetal thyroid gland, maternal plasma and liver to determine selenium (Se) concentrations throughout gestation. The Se concentrations in the allantoic fluid, fetal liver and kidney increased significantly ($p < 0.01$) during late gestation. Concurrently, the Se concentrations in amniotic fluid, maternal plasma and liver decreased significantly ($p < 0.01$) over time. Significant ($p < 0.01$) positive relationships were recorded between the age of the fetus and Se concentrations in the allantoic fluid ($r = 0.57$–0.75), fetal liver ($r = 0.43$–0.59) and kidney ($r = 0.80$–0.81) in both sheep and goats. A significant ($p < 0.05$) positive relationships were also recorded between the Se concentrations in the allantoic fluid and fetal liver ($r = 0.35$–0.37), the maternal plasma and liver Se concentrations ($r = 0.37$–0.57) between sheep and goats. A significant ($p < 0.05$) negative correlation was recorded between the Se concentrations in the allantoic fluid with maternal plasma of sheep ($r = -0.41$) as well as between the fetal liver and maternal liver Se ($r = -0.22$ to 0.50) and a negative correlation ($r = -0.42$ to 0.43) ($p < 0.01$) between Se concentrations in the fetal liver and amniotic fluid in both sheep and goats, respectively. Se concentration in the fetal liver was significantly ($p < 0.01$) higher than that of the kidney and thyroid. In the thyroid gland no morphological differences were noted. Strong fetal–maternal relationships in Se concentration were evident throughout the gestational period and dams seem to sacrifice Se levels in order to maintain that in the fetus. Se concentrations in the amniotic and allantoic fluids could be used as a possible indicator of the Se status of the fetus throughout gestation.

Keywords: Selenium (Se); Sheep; Goat; Fetus; Allantoic; Amniotic

1. Introduction

Evidence indicates that Se deficiency leads to several economically important livestock diseases. Se-responsive problems include impaired fertility, abortion, retained placenta (Gabryszuk and Klewiec, 2002) and neonatal fatality or weakness, especially among growing animals (Norton and McCarthy, 1986; Spears et al., 1986). Immune and endocrine disorders may also occur, especially thyroid dysfunction, as Se is essential for thyroid function and thyroid hormone homeostasis, and apoptotic situations have also been observed (Beckett and Arthur, 2005). A positive correlation also exists between the Se content of the feed and the tissues in

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animals which have ingested the feed. The diagnosis of Se deficiency diseases depends on the analysis of animal tissues and the response trials to supplementation (Sheppard, 1984; Stowe and Herdet, 1992). Transfer of nutrients from the dam to the offspring occurs via two pathways, placental transfer and colostrum milk ingestion. The amount of nutrients transferred to the offspring depends on the maternal nutrient status and the efficiency of the transplacental and mammary transport mechanisms. Se is efficiently transmitted via the placenta to the fetus, even in cases of low maternal concentration of Se (Koller et al., 1984). In the pig, the liver is considered to be the major body storage site for Se and perhaps the organ best to reflect the Se status of the animal (Mahan et al., 1977; Mahan and Kim, 1996).

Langlands et al. (1982) reported Se concentration in all fetal tissues to decline during the third trimester of pregnancy between 100 and 148 days of gestation. Grace et al. (1986) recorded the Se concentration in fetal lamb tissue to remain similar during late pregnancy in monotoccous ewes. Abd Elrahman and Kincaid (1993) reported that the Se concentration in the bovine fetal kidney did not change significantly with the age of the fetus, but the fetal liver Se concentration increased from day 145 to195 and decreased again from day 195 to 245 of gestation. It was suggested that a lower Se concentration in the fetal liver possibly reflected a demand for Se for the synthesis of glutathione peroxidase and other selenoproteins on the part of the terminal fetus. Van Saun et al. (1989) suggested that maternal Se declined during late pregnancy, as a result of the mobilization of Se to colostrum and milk or for rapid fetal growth during the last period of gestation.

Many studies have focused on the composition of electrolytes and the dynamics in amniotic and allantoic fluid, considering for example, K, Cl and Na (Thomsen, 1976; Jang and Brace, 1992) or thyroid hormone in the fetus (Baetz et al., 1976), but there are very few studies on the Se concentrations in specific fetal fluids of sheep. William et al. (1994) determined the Se concentration in the fetal fluid of sheep, as identified in mixed amniotic and allantoic fluids and the intra-membranous surface of the placenta was shown to be a route for rapid exchange of water, ions, and molecules between the amniotic fluid and fetal blood. Gilbert (1999) concluded that solutes, dissolved in water, must leave the allantoic cavity exclusively through the intra-membranous pathway. Furthermore, the movement of solutes through the intra-membranous pathway may explain the ability of the fetus to maintain molecular and solute gradients between fluid compartments and fetal blood and may also play an important role in amniotic and allantoic fluid volume regulation and composition.

In this study, Se concentration in amniotic and allantoic fetal fluids was measured in sheep and goats throughout gestation. The relationship between Se concentration in fetal fluids and tissues was established and related to concentration in the plasma and tissues in the dam and the fetal thyroid studied to indicate the Se status of the fetus throughout gestation.

2. Materials and methods

Samples were taken from 30 pregnant singleton sheep and 36 pregnant singleton goats 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, kidney, and thyroid gland (10 samples from sheep and 11 from goats in the late gestation). During early pregnancy the size of the thyroid gland was not large enough. All samples used for Se determination and for studying the fetal–maternal relationships and between the state of thyroid development and Se status. 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—with 10 sheep and 16 goats were at this stage; while the late stage of gestation was defined as after day 90 (which included 20 sheep and 20 goats).

2.1. Preparation and analysis of the samples

Maternal blood samples were centrifuged ($2000 \times g$; 15 min) to obtain plasma (−20 °C) for later analyses. Amniotic and allantoic fluid, maternal liver, fetal liver, kidney and thyroid gland were frozen (−20 °C) for Se determinations. A part of the thyroid gland was processed in 10% buffered formalin solution for later histopathological evaluation.

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, NJ)—keeping the solution at room temperature for 30 min in sealed Teflon vessels (Koening et al., 1997). Subsequently, the samples were placed in a microwave digester (Mars 5 CEM Corporation, USA) with an increasing temperature slope for 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 7 M HCl and left overnight (4 °C) to be analyzed the following day. Se concentrations were determined with the aid of atomic absorption spectrophotometry (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

Table 1
Mean (±S.E.) Se concentrations (ppb) in maternal plasma, liver, amniotic fluid, allantoic fluid, fetal liver, kidney and thyroid tissue in early and late stage of pregnancy in sheep and goats

Items	Sheep		Goat	
	Early pregnancy	Late pregnancy	Early pregnancy	Late pregnancy
Maternal plasma	226.2 ± 35.3**	158.3 ± 22.6	137.2 ± 16.4	113.1 ± 14.6
Maternal liver	1029.8 ± 15.1**	631.9 ± 53.4	950.2 ± 42.1**	654.4 ± 37.7
Amniotic fluid	101.3 ± 11.9**	29.7 ± 3.2	60.3 ± 3.1**	18.5 ± 2.8
Allantoic fluid	29.8 ± 1.6**	118.5 ± 6.4	35.5 ± 2.5**	152.1 ± 19.3
Fetal liver	374.7 ± 32.2**	600 ± 38.7	320.4 ± 45.7**	590.2 ± 40.9
Fetal kidney	28.9 ± 2.8**	198.2 ± 34.3	46.3 ± 10.6**	169.6 ± 26.3
Fetal thyroid glands		279.7 ± 21.7		239.2 ± 29.4

** $p<0.01$.

distilled water in a Teflon vessel (Rowntree et al., 2004; Shaw et al., 2002). Samples were then allowed to digest for approximately 1 h 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, NJ) was added to each tissue sample. The digested samples were transferred to volumetric flasks and brought to a uniform volume of 25 ml with 7 M HCl and then stored until analyses. Se concentrations were determined with the aid of an atomic absorption spectrophotometer (Varian, model Spectra AA-800) (Galan and Frank, 1993).

2.2. 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 Institute, Raleigh, NC, 1985).

3. Results

Se concentrations in the maternal plasma, liver and amniotic fluid increased significantly ($p<0.01$) during the early stage of pregnancy compared to that of the late gestation, in both sheep and goats. In the sheep, the values in the early stage of pregnancy were 226.2 ± 35.3 ppb, 1029.8 ± 151.1 ppb and 101.3 ± 11.9 ppb, compared to the late stage pregnancy levels of 158.3 ± 22.6 ppb, 631.9 ± 53.4 ppb and 29.7 ± 3.2 ppb in the maternal plasma, liver and amniotic fluid, respectively. Similarly in goats, the levels during early pregnancy were 137.2 ± 16.4 ppb, 950.2 ± 42.1 ppb and 60.3 ± 3.1 ppb compared to late pregnancy levels of 113.1 ± 14.6 ppb, 654.4 ± 37.7 ppb and 18.5 ± 2.8 ppb in the maternal plasma, liver and amniotic fluid, respectively (Table 1). In contrast, the Se concentrations in the allantoic fluid, fetal liver and kidney increased significantly ($p<0.01$) during the late stage of pregnancy, compared to that of the early stage in both sheep and goats. Se concentration in the fetal liver was significantly ($p<0.01$) higher than that of the fetal thyroid and kidney in both sheep and goats (Fig. 1).

Histopathological examination of the fetal thyroid demonstrated two types of follicles—the first type contained colloidal material and a flat epithelium with a diameter ranging from 80 to 900 μm and the second type consisted of embryonic follicles, mainly at the periphery

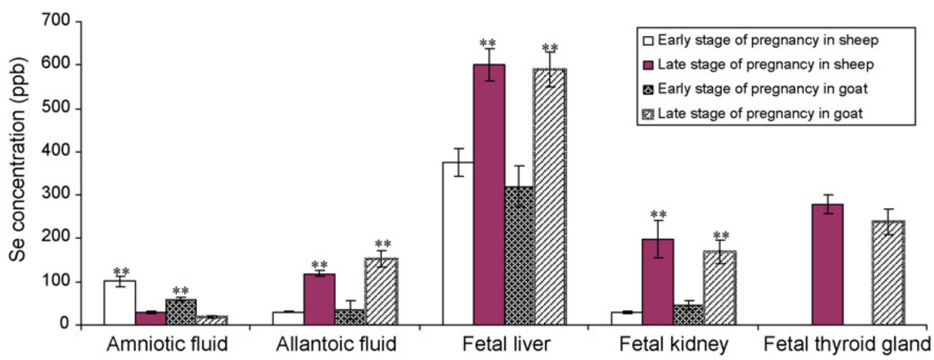

Fig. 1. Mean (±S.E.) Se concentrations (ppb) in the amniotic and allantoic fluid, fetal liver, kidney and thyroid gland in early and late stage of pregnancy in sheep and goats.

Table 2
Correlation between age of the fetus, Se concentration of the fetal liver and maternal plasma with Se concentration in the allantoic fluid, amniotic fluid, fetal kidney and maternal liver in sheep and goats

	Age of the fetus		Fetal liver		Maternal plasma	
	Sheep	Goats	Sheep	Goats	Sheep	Goats
Allantoic fluid	0.75**	0.57**	0.37*	0.35*	−0.41*	0.14 NS
Amniotic fluid	−0.64**	−0.73**	−0.43**	−0.42**	0.06 NS	0.2 NS
Maternal liver	−0.61**	−0.69**	−0.22**	−0.5**	0.57**	0.37*
Fetal liver	0.43**	0.59**				
Fetal kidney	0.81**	0.8**				

NS, non-significant.
* $p<0.05$.
** $p<0.01$.

of the gland which were empty and devoid of colloid, with a cubic epithelium which had a diameter of less than 30 μm. Only three samples presented the second type of follicles. Images suggestive of apoptosis were not seen. No relationships were established between thyroid development and Se concentration in the fetus or the dam.

Among the sheep and goats there were significant ($p<0.01$) negative correlations (Table 2) between the age of the fetus and the Se concentrations in the amniotic fluid (Fig. 2A and B) and maternal liver. A significant ($p<0.01$) positive correlation was recorded between the fetal age and Se concentrations in allantoic fluid (Fig. 2A and B), fetal liver and kidney.

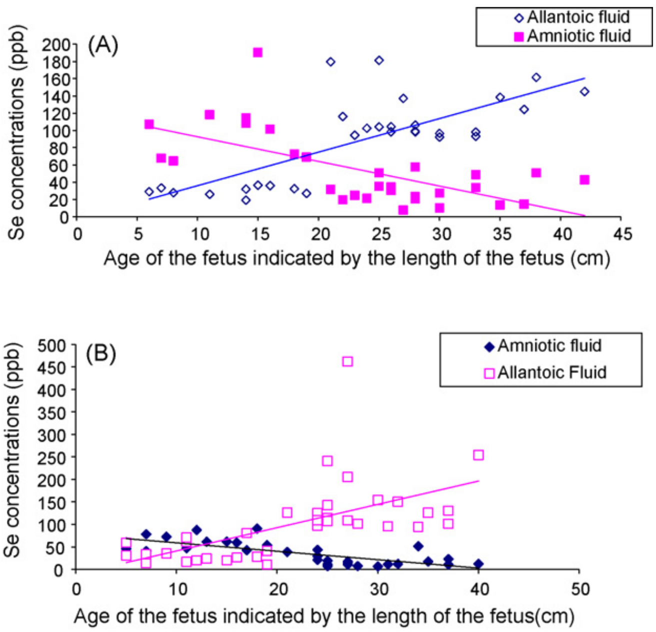

Fig. 2. (A) Correlation between age of the fetus and Se concentrations in amniotic fluid ($r=-0.64$, $p<0.01$) and allantoic fluid ($r=0.75$, $p<0.01$) in sheep. (B) Correlation between age of the fetus and Se concentrations in amniotic fluid ($r=-0.73$, $p<0.01$) and allantoic fluid ($r=0.57$, $p<0.01$) in goats.

For the sheep there was a significant negative relationship ($p<0.05$) between Se concentrations in the allantoic fluid and maternal plasma which was not evident among goats. For the sheep and goats a significant ($p<0.05$) positive correlation between Se concentrations in the allantoic fluid and fetal liver was recorded. A significant ($p<0.01$) negative relationship between the Se concentrations in the amniotic fluid and fetal liver was also recorded. A significant ($p<0.05$) negative relationship was also observed between the maternal and fetal liver Se concentrations, while significant ($p<0.05$) positive relationship between the maternal liver and plasma Se concentrations was also noted (Table 2).

4. Discussion

The absence of relationships between the amniotic fluid and maternal plasma Se concentrations suggests the fetus to obtain its Se requirements, regardless of the maternal plasma level. A significant ($p<0.01$) increase of Se concentration in the allantoic fluid with age of the fetus, indicates an increased transfer from the dam to the fetus and greater excretion of Se by the fetus. The significant ($p<0.05$) negative correlation ($r=-0.22$ to -0.50) between the level Se in the maternal and fetal liver and the significant ($p<0.05$) positive correlation ($r=0.37$–0.57) between the Se in the maternal liver and plasma (Table 2) indicates the withdrawal of a large quantity of maternal Se, which is then transferred to the fetus. The results obtained suggest that the drainage of maternal Se firstly occurs from the maternal plasma and then by means of liver mobilization—which may explain the low maternal Se status of the dam during late pregnancy.

In this study, Se concentrations in the allantoic fluid of sheep and goats increased significantly ($p<0.01$) together with the age of the fetus, while the concentrations in the amniotic fluid decreased significantly ($p < 0.01$). Grace et al. (1986) also found Se concentrations in mixed amniotic and allantoic fluid to increase in sheep as the fetus matured. Among monotocous ewes nearing the end of gestation, fetal Se accounted for about 66% of the total in the conceptus. Se concentration in the allantoic fluid increased during late pregnancy as was the case in cows. In cattle non-fetal conceptus, the Se level increased between190 and 270 days of gestation (Ferrell et al., 1982; William et al., 1994). The allantoic fluid Se concentration increased, indicating that the fetus excretes an elevated amount of this element at this late stage of pregnancy—which is probably associated with a high transplacental transfer from the dam (Young et al., 1961; Hamdy et al., 1963) or variations in fluid osmosis between amniotic and allantoic fluid (Thomsen, 1976; Gilbert, 1999).

Increased excretion of Se to the allantoic fluid, especially during the late stage of pregnancy, is a factor that influences the loss of maternal Se and increases the risk of dam deficiency. High blood and tissue (kidney cortex, liver and spleen) Se concentrations have been reported in fetuses from Se-treated ewes—indicating transplacental transfer of Se from the dam (Ahmed et al., 1990). Thus, Se supplementation to pregnant animals during the last few weeks of gestation has been shown to be an important practical recommendation, even though the maternal plasma level may be normal, this precaution will help avoid the risk of Se deficiency caused by the rapid transplacental transfer of Se from the dam to the fetus. Se treatment in pregnant ewes has been proven to be successful in preventing nutritional muscular dystrophy in new born lambs, the therapy preventing a variation in the Se concentrations in both the plasma and the maternal liver (Young et al., 1961; Hamdy et al., 1963; Wright and Bell, 1966; Hidroglou et al., 1969).

In this study fetal liver Se concentration increased significantly ($p < 0.01$), together with the age of the fetus in both sheep and goats. Similar results have been previously reported by other authors for bovine (Goonerante and Christensen, 1989; Abd Elrahman and Kincaid, 1993). However, the fetal liver Se concentrations are not affected by the gestational stage in dairy cattle (Van Saun et al., 1989; William et al., 1994). Hidroglou et al. (1969) and Grace et al. (1986) found minimal changes in liver and other tissue Se concentrations of fetal lambs during late gestation.

In bovine, Koller et al. (1984), reported fetal liver Se to increase progressively as pregnancy advanced—reaching levels 3–6 times higher than those in the liver of the dam. These results do not reflect the results of the present study, where Se in the maternal liver was significantly ($p < 0.01$) higher than that found in the fetal liver. The results suggest that both the fetus and the dam depend on Se stored in the maternal liver to maintain their Se status. Quantification of Se in maternal liver may be considered the best method for determining the Se status in both the dams and fetuses. Changes in the maternal liver Se level may be attributed to the Se mobilization to colostrum and milk or to the rapid growth of the fetus during late pregnancy—causing an increase in fetal requirements and thus leading to a reduction of Se in the maternal blood and tissues (Hawkes et al., 2004).

Hostetler and Kincaid (2004) found the liver Se concentration to decrease in the fetus with advancement of gestation beyond day 45 in sows fed a diet low in Se. Mahan et al. (1975, 1977) and Mahan and Kim (1996) demonstrated maternal hepatic Se levels in sows to be greater than the hepatic concentration in the offspring. Variations in species regarding the relative concentrations of Se in the fetal and maternal liver have been recorded. These differences may be attributed to differences in maternal fetal transplacental transfer, liver–plasma mobilization, Se diet utilization and absorption, and metal hepatic metabolism.

In the current evaluation, the fetal kidney Se concentration also increased significantly ($p < 0.01$) as the fetus matured, which is in agreement with Abd Elrahman and Kincaid (1993). According to the findings of Ahmed et al. (1990), all the analyzed samples of fetal liver and kidney of sheep and goats in the present study were within the normal range. However, according to the reference values of Smith and Isopenko (1997) and Mustafa et al. (1998), 43.3% ($n = 13$) of the sheep and 52.7% ($n = 19$) of the goats and maternal plasma samples with an inadequate level of Se during late pregnancy. Normal fetal values recorded together with inadequate maternal levels, indicate that fetus values are independent from of the dam's Se status.

The fetal kidney and thyroid Se concentrations in both sheep and goats were significantly ($p < 0.01$) lower than that in the fetal liver. Similar results have been reported during the gestation period, with a median Se content in the kidney cortex and a thyroid level lower than that found in the liver (Tiran et al., 1995). Low Se thyroid content and histological examination of the gland suggested minor thyroid activity in the fetus, which is metabolically dependent on the dam during gestation. Other research report Se to be highest in the kidney, followed by the liver, pancreas, heart and finally in the skeletal muscle of adult sheep (Combs and Combs, 1986).

5. Conclusion

There is a strong relationship between the fetus and dam concerning Se metabolism during pregnancy. The dams seem to sacrifice their Se level in order to maintain the fetal Se disposition. Fetus Se which was transferred from the dam's system plays an important role in the incidence and occurrence of Se deficiency in the dam, especially in the final stage of pregnancy—when the fetal requirements are higher. Se concentration in the amniotic and allantoic fluids may be used as an indicator of Se status in the fetus throughout gestation. The fetal liver is considered to be the main storage organ of Se in the fetus and hepatic tissue has also been shown to be a good indicator of the Se status of the fetus.

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