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Plasma Levels of Vitamin D Metabolites in Fetal and Pregnant Ewes[1] (42543)

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Abstract. The plasma concentrations of calcium; inorganic phosphorus; 25-hydroxyvitamin D; 24,25-dihydroxyvitamin D; and 1,25-dihydroxyvitamin D were determined in sheep maternal and fetal arterial circulations. In addition, plasma concentrations of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D were determined simultaneously across the uterine and umbilical circulations. Fetal arterial levels of calcium ($r = 0.560$); inorganic phosphorus ($r = -0.095$); and 1,25-dihydroxyvitamin D ($r = 0.040$) were significantly higher than and did not correlate with maternal arterial levels. Maternal levels of 25-hydroxyvitamin D were significantly higher than and correlated ($r = 0.693$) with fetal 25-hydroxyvitamin D levels. No significant difference existed between maternal and fetal arterial levels of 24,25-dihydroxyvitamin D. No significant difference was detected in the concentrations of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D across the uterine or umbilical circulations.

Measurements of plasma mineral and vitamin D metabolite levels have been used to investigate the fetomaternal interrelationships of these substances. The fetomaternal relationship of mineral and vitamin D metabolites have been studied in the human (1–4), cow (5), pig (6), and rabbit (7). Plasma 25-hydroxyvitamin D (25-OH-D) concentrations in the fetus have been found to correlate significantly with the metabolite levels in the mother (1, 2, 5, 6). Fetal plasma 24,25-dihydroxyvitamin D (24,25-$(OH)_2$D) levels are generally lower than but correlate positively with those of the mother (8). From these data, it has been suggested that the mother makes a significant contribution to fetal levels of 25-OH-D and 24,25-$(OH)_2$D. Most studies have shown that maternal levels of 1,25-dihydroxyvitamin D (1,25-$(OH)_2$D), the hormonal form of vitamin D, do not correlate with fetal levels of the hormone (3–6, 9). These data suggest that the mother does not make a significant contribution to fetal 1,25-$(OH)_2$D levels.

The sheep is an important species for the study of nutrient and mineral metabolism during pregnancy (10). Limited information exists concerning the metabolism of vitamin D in the pregnant ewe. The interrelationship of the mineral and vitamin D status of the pregnant ewe and its fetus is examined in the present paper. We report simultaneous measurement of calcium, phosphorus, 25-OH-D, and 1,25-$(OH)_2$D in the pregnant ewe and its fetus during the third trimester of pregnancy. In addition, for the first time, arterial and venous 25-OH-D and 1,25-$(OH)_2$D concentrations in uterine and umbilical circulations are measured.

Materials and Methods. *Animals and blood sampling.* Sixteen pregnant "western" breed ewes with gestational ages between 115 and 140 days were sedated with phenobarbital and anesthetized by spinal anesthesia with 10 mg Pontocaine. Polyvinyl catheters were then placed in maternal and fetal circulations as previously described (11). Animals were allowed at least 1 week to recuperate from surgery prior to blood sampling. The sheep were kept in wooden carts and allowed water and a diet consisting of hay, Omolene (oats, corn, wheat, and molasses mix; Farmers Market Assoc., Denver, CO), and alfalfa pellets *ad libitum*. All experiments were performed in the summer, fall, or winter. In the study described in Table II, 4- to 10-ml blood samples were removed simultaneously from each of the fol-

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TABLE I. MATERNAL AND FETAL ARTERIAL PLASMA MINERAL AND VITAMIN D METABOLITE CONCENTRATIONS OF SHEEP DURING THIRD TRIMESTER OF PREGNANCY

Substance measured	Maternal artery concentration ($u \pm SE$)	Fetal artery concentration ($u \pm SE$)	Difference (P value)	Correlation coefficient
Calcium (mg/dl)	8.7 ± 0.2 (8)[a]	11.1 ± 0.6 (8)	$P < 0.05$	0.560 NS
Phosphorus (mg/dl)	5.0 ± 0.5 (6)	6.4 ± 0.4 (6)	NS[b]	−0.095 NS
25-OH-D (ng/ml)	14.7 ± 1.6 (9)	7.4 ± 1.4 (9)	$P < 0.001$	0.693 $P < 0.05$
24,25-$(OH)_2$D (ng/ml)	1.6 ± 0.3 (6)	2.7 ± 1.0 (6)	NS	0.035 NS
1,25-$(OH)_2$D (pg/ml)	31.4 ± 4.0 (13)	60.6 ± 5.0 (13)	$P < 0.001$	0.040 NS

[a] The number in parentheses represents the number of animals sampled.
[b] NS = not significant.

lowing vessels: a maternal femoral artery, uterine vein, the common umbilical vein, and a pedal artery (advanced to the abdominal aorta). In the study described in Table I and in Figs. 1 and 2, blood was removed only from the maternal femoral artery and fetal aorta.

TABLE II. CONCENTRATIONS OF 25-OH-D AND 1,25-$(OH)_2$D IN THE UTERINE AND UMBILICAL CIRCULATIONS OF THE PREGNANT EWE[a]

Animal number	Uterine circulation		Umbilical circulation	
	Maternal artery	Uterine vein	Umbilical vein	Fetal artery
		Plasma 1,25-$(OH)_2$D (pg/ml)		
1	13.8	8.2	32.5	22.2
2	28.5	43.7	—	—
3	34.3	26.5	105.0	73.5
4	32.0	60.2	40.5	38.5
5	7.8	7.3	36.8	34.8
6	31.5	20.5	75.4	53.8
7	37.0	49.1	74.5	80.1
$u \pm SE$	26.4 ± 4.2	30.8 ± 7.8	60.8 ± 11.7	50.5 ± 9.3
	NS[b]		NS	
		Plasma 25-OH-D (ng/ml)		
1	7.4	6.4	—	—
2	—	—	—	—
3	19.9	30.3	—	—
4	13.7	15.4	6.0	2.4
5	21.0	36.5	8.1	13.6
6	7.5	7.3	—	—
7	12.0	11.3	4.1	4.2
$u \pm SE$	13.6 ± 2.4	17.9 ± 5.1	6.1 ± 1.2	6.7 ± 3.5
	NS		NS	

[a] All data were compared by paired Student's t test. Comparison of maternal arterial and umbilical venous 1,25-$(OH)_2$D levels yields a t value of 3.91, significant at $P < 0.02$.
[b] NS = values are not significant.

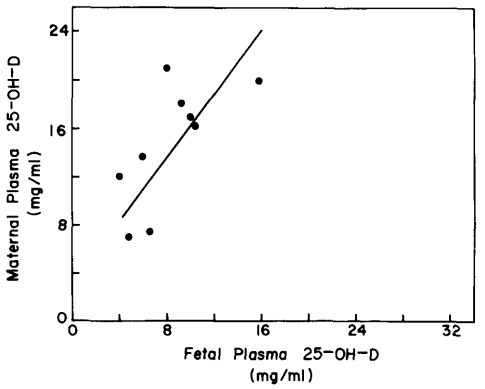

FIG. 1. Regression analysis of maternal plasma 25-OH-D on fetal plasma 25-OH-D. The correlation coefficient was 0.693. The regression was significant at $P < 0.05$.

Plasma was separated from cells by centrifugation and frozen at −20°C until analysis.

Plasma analysis. Plasma calcium was determined in the presence of 0.1% lanthanum chloride by atomic absorption spectrophotometry. Plasma inorganic phosphorus was determined colorimetrically according to the method of Chen (12). Plasma concentrations of the vitamin D metabolites were determined according to the established procedure of Shepard *et al.* (13). The limits of detection of the assay for 25-OH-D; 24,25-$(OH)_2$D; and 1,25-$(OH)_2$D are 1 ng/ml, 0.2 ng/ml, and 7 pg/ml, respectively. The interassay variations as reported by Shepard *et al.* (13) for 25-OH-D; 24,25-$(OH)_2$D; and 1,25-$(OH)_2$D were 10, 13, and 26%, respectively.

Statistical analysis. Correlations were determined by regression analysis. Comparisons between two groups were done with Student's *t* test. Data comparing concentrations of 25-OH-D and 1,25-$(OH)_2$D across umbilical and uterine circulations were analyzed by paired Student's *t* test.

Results. *Maternal and fetal plasma Ca and P concentrations.* Maternal and fetal arterial plasma calcium and phosphorus concentrations are shown in Table I. All mothers were slightly hypocalcemic (8.7 ± 0.2) during the third trimester of pregnancy. Fetal arterial levels of calcium (11.1 ± 0.6) and phosphorus (6.4 ± 0.4) were significantly higher than and did not correlate with ($r = 0.560$ and −0.095, respectively) those of the mother.

Maternal and fetal arterial vitamin D metabolite concentrations. The data concerning maternal and fetal arterial vitamin D metabolite levels are in Table I. Maternal arterial 25-OH-D concentrations which ranged from 7 to 21 ng/ml (Fig. 1) were significantly higher than and correlated significantly with arterial levels of the fetus (Fig. 1). The concentrations of 24,25-$(OH)_2$D in the arterial plasma of mothers and fetuses did not differ significantly and did not correlate ($r = 0.035$). Arterial levels of 1,25-$(OH)_2$D were significantly higher in the fetus (60.6 ± 5.0 pg/ml) than in the mother (31.4 ± 0.4 pg/ml). Maternal and fetal levels of 1,25-$(OH)_2$D did not correlate ($r = 0.040$) (Fig. 2).

There was no significant correlation between maternal arterial levels of 1,25-$(OH)_2$D and fetal arterial levels of calcium ($r = 0.242$) or inorganic phosphorus ($r = -0.031$). Similarly, no correlation existed between fetal arterial levels of 1,25-$(OH)_2$D and fetal arterial levels of calcium ($r = -0.221$) or phosphorus ($r = 0.491$).

A-V differences of 25-OH-D and 1,25-$(OH)_2$D across uterine and umbilical circulations. Plasma concentrations of 25-OH-D and 1,25-$(OH)_2$D determined simultaneously across the uterine (maternal femoral artery and uterine vein) and umbilical (umbilical vein and fetal abdominal aorta) circulations are presented in Table II. No significant difference was detected in the concentrations of 25-OH-D and 1,25-$(OH)_2$D across the uterine or um-

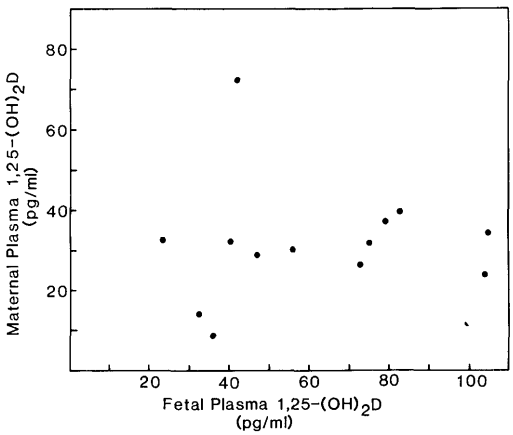

FIG. 2. Regression analysis of maternal plasma 1,25-$(OH)_2$D on fetal plasma 1,25-$(OH)_2$D. The correlation coefficient was 0.297. The regression was not significant.

bilical circulations. As observed in Table I, the plasma concentrations in the fetal circulation of 1,25-$(OH)_2D$ were greater and those of 25-OH-D were lower than those in the mother.

Discussion. We report the plasma calcium; phosphorus; 25-OH-D; 24,25-$(OH)_2D$; and 1,25-$(OH)_2D$ levels in the pregnant ewe and its fetus during the third trimester of pregnancy. From these data the interrelationships between maternal and fetal vitamin D metabolites were examined. Also, we measured for the first time in the sheep 25-OH-D and 1,25-$(OH)_2D$ concentrations in both the uterine and the umbilical circulations. Using these techniques we were unable to detect net fluxes of these compounds across the placenta.

Similar to observations in laboratory animals (5–7, 14) but not in humans (1, 2), we found maternal arterial plasma levels of 25-OH-D to be significantly higher than those of the fetus (Tables I and II). In addition, a linear correlation was found between maternal and fetal arterial levels of 25-OH-D. These findings confirm a previous preliminary report in the sheep (14). Similar observations have been made in the human (1, 2), cow (5), pig (6), and rabbit (7). A linear correlation between maternal and fetal 25-OH-D concentrations is evidence that the mother makes a significant contribution to fetal levels of the metabolite. In support of this hypothesis radiolabeled 25-OH-D has been shown to cross the placenta in the rat (15) and sheep (16). It is not known whether the vitamin D 25-hydroxylase is active in the fetal liver (17) and further study is needed to determine the contribution of this organ to fetal 25-OH-D levels.

We found neither a significant difference nor a linear correlation between maternal and fetal arterial concentrations of 24,25-$(OH)_2D$. Our findings were in contrast with data of other species studied (1, 2, 5–7). Our observations indicate that the mother does not influence significantly the fetal levels of 24,25-$(OH)_2D$. Radiolabeled 24,25-$(OH)_2D$ has been shown to cross from mother to fetus in several species including the sheep (16, 18). However, 24,25-$(OH)_2D$ may cross the placenta in the sheep at such a rate as not to influence fetal levels of this vitamin D metabolite. In support of our findings the 25-OH-D-24-hydroxylase has been found in both placenta and fetal kidney (19, 20). It is possible that these organs produce enough 24,25-$(OH)_2D$ to dominate fetal concentrations of the 24-hydroxylated metabolite.

In most species studied, fetal or neonatal plasma levels of 1,25-$(OH)_2D$ have been found not to correlate with maternal plasma levels of 1,25-$(OH)_2D$ (1–6). Most investigators have interpreted these findings to mean that fetal 1,25-$(OH)_2D$ metabolism is not influenced by maternal 1,25-$(OH)_2D$ metabolism. Evidence that the placenta and fetal kidney produce 1,25-$(OH)_2D$ supports this interpretation (8). The ability of radiolabeled 1,25-$(OH)_2D$ to cross from mother to fetus (16, 21) shows that the mother can contribute to fetal 1,25-$(OH)_2D$ levels, but the rate that 1,25-$(OH)_2D$ crosses may not be sufficient to influence fetal 1,25-$(OH)_2D$ levels. Thus, maternal metabolism probably has little effect on fetal levels of 1,25-$(OH)_2D$. The present data support this conclusion.

Contrary to observations in other species (1–6), we have found sheep fetal plasma 1,25-$(OH)_2D$ levels to be higher than those of the mother. Our observation agrees with a previous study in sheep (22), but the reasons for this specific difference in sheep is not known. It is interesting that the fetal sheep plasma 1,25-$(OH)_2D$ levels are high even in the presence of elevated calcium and phosphorus levels. It is well established that hypocalcemia and hypophosphatemia will stimulate the renal 25-OH-D-1α-hydroxylase and raise plasma 1,25-$(OH)_2D$ levels. It is possible that such a regulatory system may not be functional in the sheep fetus.

In humans, Wieland and co-workers (9) found fetal arterial levels of 1,25-$(OH)_2D$ to be higher than umbilical vein levels of 1,25-$(OH)_2D$. Their data indicated that the 1,25-$(OH)_2D$ was produced *in vivo* by the fetal kidney. Although we were able to detect differences between maternal and fetal arterial levels of 25-OH-D and 1,25-$(OH)_2D$ with our assays, we were unable to find differences in the concentration of 1,25-$(OH)_2D$ or 25-OH-D across umbilical or uterine circulations in the sheep to support the observations of Wieland *et al.* (9). The lack of a significant difference between uterine and umbilical levels of these metabolites may be due to the variability in the data within each group (Table II). This variability may be a seasonal fluctuation in that animals

were sampled in winter, summer, and fall. Differences might have been found if larger numbers of animals were examined.

The present study reports the plasma levels of calcium; phosphorus; 25-OH-D; 24,25-$(OH)_2D$; and 1,25-$(OH)_2D$ in maternal and fetal arteries of the sheep. In addition, we report, for the first time in laboratory animals, plasma 25-OH-D and 1,25-$(OH)_2D$ levels in both uterine and umbilical arterial and venous blood. We conclude that in the sheep, fetal metabolism of 24,25-$(OH)_2D$ and 1,25-$(OH)_2D$ but not 25-OH-D is independent of maternal metabolism of these compounds. Furthermore, we were unable to detect any A–V differences of 25-OH-D or 1,25-$(OH)_2D$ across the umbilical or uterine circulations.

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