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Developmental Changes in Polyamine Levels and Synthesis in the Ovine Conceptus[1]

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ABSTRACT

Polyamines (putrescine, spermidine, and spermine) are essential for placental growth and angiogenesis. However, little is known about changes in polyamine synthesis associated with development of the ovine conceptus (embryo/fetus and associated placental membranes). We hypothesized that rates of placental polyamine synthesis were maximal during the rapid placental growth that occurs in the first half of pregnancy. This hypothesis was tested using ewes between Days 30 and 140 of gestation. Columbia cross-bred ewes were hysterectomized on Days 30, 40, 60, 80, 100, 120, or 140 of gestation (Day 0 = mating; n = 4 ewes/day) to obtain placentomes, intercotyledonary placenta, intercaruncular endometrium, and allantoic as well as amniotic fluids. The tissues were analyzed for ornithine decarboxylase (ODC) and arginase activities; arginine, ornithine, and polyamine concentrations; and polyamine synthesis using radiochemical and chromatographic methods. Maximal ODC and arginase activities and the highest rates of polyamine synthesis were observed in all tissues on Day 40 of gestation. Concentrations of ornithine and polyamines in placentomes and intercaruncular endometrium also peaked on Day 40 of gestation. In ovine allantoic and amniotic fluids, polyamines were most abundant during early (Days 40–60) and late (Days 100–140) gestation, respectively. Amniotic fluid spermine increased progressively with advancing gestation. Results of the present study indicate metabolic coordination among the several integrated pathways that support high rates of polyamine synthesis in the placenta and endometrium during early pregnancy. Our findings may have important implications for both intrauterine growth retardation and fetal origins of diseases in adults.

developmental biology, placenta, pregnancy, uterus

INTRODUCTION

The placentae of all mammalian species undergo rapid formation of new blood vessels (angiogenesis) and marked growth during pregnancy [1, 2]. Placental angiogenesis is necessary to increase placental-fetal blood flow and the supply of nutrients for transfer from maternal to fetal blood. Therefore, placental growth is a critical factor for controlling the survival, growth, and development of the fetus, and a better understanding of factors that regulate placental growth is essential to improve the reproductive efficiency of domestic animals and humans. The sheep has a synepitheliochorial placenta, the growth of which is maximal between Days 20 and 60 of gestation (term is 147 days) [3, 4]. The ovine placenta has 60–100 individual cotyledons formed by the attachment of fetal trophoblast cells at predetermined sites (caruncles) in the uterine endometrium as well as the intercotyledonary chorioallantoic placenta [3].

Polyamines (putrescine, spermidine, and spermine) are key regulators of angiogenesis, early mammalian embryogenesis, placental trophoblast growth, and embryonic development in the uterus [5, 6]. Through binding intracellular RNA, DNA, nucleotide triphosphates, proteins, and other negatively charged molecules, polyamines (polycationic molecules) regulate gene expression, signal transduction, and ion-channel function, as well as DNA and protein synthesis [7]. Polyamines are also endogenous scavengers of reactive oxygen species, thereby protecting DNA, proteins, and lipids from oxidative damage [8]. Thus, polyamines are essential for cell proliferation, differentiation, and function.

Ornithine decarboxylase (ODC) is a rate-controlling enzyme of the polyamine-synthetic pathway in mammalian cells [9]. It converts ornithine (a product of arginine hydrolysis by arginase) into putrescine, which is subsequently converted into spermidine and spermine by spermidine synthase and spermine synthase, respectively. Despite previous studies regarding the role of polyamines in placental and fetal development [5, 6], little is known about changes in placental polyamine synthesis associated with conceptus development in any species. Although placental ODC activity has been reported for some mammals, including rats, mice, and pigs [5, 6, 10], it is necessary to determine rates of polyamine synthesis in intact tissues or cells, because a change in enzyme (e.g., ODC) activity measured under in vitro assay conditions does not necessarily indicate a change in metabolic flux or product formation [11, 12]. Information about polyamine synthesis is crucial for understanding the molecular regulation of placental and fetal growth and for elucidating the mechanisms responsible for intrauterine growth retardation and fetal origin of adult-onset diseases.

We recently reported marked increases in the concentrations of both ornithine and arginine (substrates for polyamine synthesis) in ovine allantoic fluids (a reservoir for nutrients in the fetus) between Days 30 and 60 of gestation [13]. Such changes coincide with the period of most rapid growth of the ovine placenta [3, 4]. On the basis of this observation, we hypothesized that placental ODC and arginase activities and polyamine synthesis were maximal during the first half of pregnancy. This hypothesis was tested using ewes between Days 30 and 140 of gestation. Endometrium was analyzed in the same manner, because it is closely associated with placental development and function [14–16]. Because amniotic and allantoic compartments are integral parts of the ovine conceptus essential for fetal

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growth [17], we also determined changes in the concentrations of polyamines in amniotic and allantoic fluids during pregnancy.

MATERIALS AND METHODS

Chemicals

Putrescine, spermidine, spermine, and amino acids were purchased from Sigma Chemicals (St. Louis, MO). L-[^{14}C]ornithine and L-[U-^{14}C]arginine were obtained from American Radiolabeled Chemicals (St. Louis, MO). High-performance liquid chromatography (HPLC)-grade water and methanol were purchased from Fisher Scientific (Fair Lawn, NJ).

Experimental Animals

Suffolk rams and Columbia cross-bred ewes were mated when estrus was detected in ewes (Day 0) and at 12 and 24 h later. Ewes were then assigned randomly to be hysterectomized (n = 4 ewes/day) on Day 30, 40, 60, 80, 100, 120, or 140 of gestation to allow collection of placental and endometrial tissues as well as amniotic and allantoic fluids. Because marked changes in physiological parameters were observed in the ovine conceptus during pregnancy (e.g., see [13, 14]) and because coefficients of variations for all measured parameters were relatively small (<3%–7%), we used four ewes per day of gestation on the basis of statistical power calculation. In preliminary studies, we determined that concentrations of polyamines in placental and endometrial tissues, amniotic fluid, or allantoic fluid were not affected by the number of fetuses (data not shown). Thus, samples were obtained from one randomly selected fetus per ewe for polyamine analysis when twin fetal lambs were available. None of the ewes in the present study had more than twin fetuses. Throughout gestation, ewes had free access to water and were fed individually 1.4 kg/day of an alfalfa-based diet containing 90.9% dry matter, 58.7% total digestible nutrients, 15.8% crude protein, 3.7% fat, 27.0% acid detergent fiber, 35.0% neutral detergent fiber, 0.065% vitamin mixture, and 0.15% salt mixture that met National Research Council (NRC) requirements [13]. Ewes consumed all of the feed provided daily. The present study was approved by the Texas A&M University Institutional Agricultural Animal Care and Use Committee.

Hysterectomy and Sample Collection

Hysterectomies were performed between 0800 and 0900 h at 24 h after the last feeding [13]. All ewes were administered isofluorane (5%) via an inhalation mask to induce anesthesia, which was maintained with isofluorane (1%–5%). A midventral laparotomy was performed to expose the reproductive tract. Amniotic and allantoic fluids were collected through the amniochorion and chorioallantoic membranes, respectively. Placentomes, intercotyledonary placenta, and intercaruncular endometrium were obtained on all days of gestation. A portion of these tissues was used immediately for ODC assays and metabolic studies; the remaining tissues were stored at −80°C for determination of polyamines, amino acids, and arginase activity within 1 wk. In the present study, we did not separate the placentomes into maternal and fetal components, because such a procedure would require a prolonged period of time to complete, which might compromise the biochemical viability of the tissues for metabolic studies.

Determination of ODC Activity

The ODC activity in placental and endometrial tissues was measured using L-[1-^{14}C]ornithine as we described previously for porcine tissues [10]. Briefly, tissues (~200 mg) were homogenized, using a glass homogenizer, in 0.5 ml of 50 mM sodium phosphate buffer (pH 7.2) containing 0.2 mM pyridoxal-5-phosphate, 1 mM EDTA, 2.5 mM dithiothreitol, 150 mM sucrose, and protease inhibitors (5 μg/ml of phenylmethylsulfonyl fluoride, 5 μg/ml of aprotinin, 5 μg/ml of chymostatin, and 5 μg/ml of pepstatin A). The homogenizer was rinsed with 0.5 ml of the buffer, and the combined homogenates were centrifuged at 13 000 × *g* for 15 min at 4°C. The supernatants (free of mitochondria) were used for ODC assays to prevent the potential production of $^{14}CO_2$ from [1-^{14}C]ornithine via mitochondrial ornithine aminotransferase and Krebs-cycle enzymes [11, 18]. Pyrroline-5-carboxylate (P5C) reductase activity was measured as a marker for cytosolic enzymes as previously described [18]. The assay mixture (0.5 ml) for ODC consisted of 0.2 mM L-[1-^{14}C]ornithine (5500 dpm/nmol), 0.2 mM pyridoxal-5-phosphate, 0.2 mM EDTA, 0.5 mM dithiothreitol, enzyme preparations (equivalent to ~20 mg of tissue), and 50 mM sodium phosphate buffer (pH 7.2). On Days 40 and 140 of gestation, ODC activity was also measured in the presence of 2 mM ornithine. Radioactivity blanks containing [1-^{14}C]ornithine but no enzyme preparations were run. After incubation at 37°C for 1 h, $^{14}CO_2$ was collected in 0.2 ml of NCS (Amersham, Arlington Heights, IL), and its radioactivity was measured in a liquid scintillation counter (Packard, Meriden, CT). Recovery of $^{14}CO_2$ from the incubation medium was 96%, as determined using a known amount of [^{14}C]$NaHCO_3$. Rates of ornithine decarboxylation by ODC were calculated by dividing the radioactivity of collected $^{14}CO_2$ (dpm) by the specific activity of [1-^{14}C]ornithine (dpm/nmol) in the assay solution. Enzyme activity was expressed on the basis of the amount of tissue in the assay mixture. Coefficients of variation for intra- and interassays of ODC were less than 4% and 7%, respectively.

Determination of Arginase Activity

Arginase activity in placental and endometrial tissues was measured by quantifying ornithine production from arginine as we described previously for porcine tissues [18]. Briefly, placental or endometrial tissues (~200 mg) were homogenized, using a glass homogenizer, in 1 ml of homogenization buffer (pH 7.4) containing 300 mM sucrose, 1 mM EDTA, 3 mM dithiothreitol, 5 mM Hepes, protease inhibitors (5 μg/ml of phenylmethylsulfonyl fluoride, 5 μg/ml of aprotinin, 5 μg/ml of chymostatin, and 5 μg/ml of pepstatin A), and 0.5% Triton X-100. The homogenizer was rinsed with 1 ml of the buffer, and the combined homogenates were used for arginase assays. The assay mixture (1 ml), which consisted of 50 mM potassium phosphate buffer (pH 7.5), 10 mM arginine, 10 mM $MnCl_2$, and tissue homogenates (equivalent to ~10 mg of tissue), was incubated at 37°C for 0 or 15 min. On Days 40 and 140 of gestation, arginase activity was also measured in the presence of 2 mM arginine. The reaction was terminated by the addition of 0.2 ml of 1.5 M $HClO_4$, and the ornithine produced was analyzed by HPLC [10]. Rates of arginine hydrolysis by arginase were calculated on the basis of ornithine production during a 15-min assay period. Enzyme activity was expressed on the basis of the amount of tissue in the assay mixture. Coefficients of variation for intra- and interassays of arginase were less than 3% and 5%, respectively.

Determination of Polyamines and Amino Acids

Placental and endometrial tissues (~200 mg) were homogenized at 4°C in 1 ml of 1.5 mM $HClO_4$ using a glass homogenizer, and the homogenizer was rinsed with 1 ml of 1.5 mM $HClO_4$. The combined homogenates were transferred to 12- × 75-mm polypropylene tubes and neutralized with 1 ml of 2 mM K_2CO_3. The homogenates were centrifuged at 3000 × *g* and 4°C for 15 min to obtain the supernatant fluid for polyamine analysis. Fetal fluid samples were centrifuged at 3000 × *g* at 4°C for 10 min. An aliquot of the supernatant (0.5 ml) was deproteinized with 0.5 ml of 1.5 M $HClO_4$, followed by neutralization with 0.25 ml of 2 M K_2CO_3. Recovery of putrescine, spermidine, spermine, ornithine, and arginine from placental and endometrial samples as well as fetal fluids was determined by adding known amounts of polyamine and amino acid standards [11] and was found to be greater than 93% for polyamines and greater than 96% for amino acids.

Polyamines were analyzed by an ion-pairing HPLC method involving precolumn derivatization with *o*-phthaldialdehyde [11]. Briefly, 100 μl of sample were mixed with 100 μl of 1.2% benzoic acid (in 40 mM sodium borate, pH 9.5) and 1.4 ml of HPLC-grade H_2O. An aliquot (100 μl) of the assay mixture was derivatized, in an autosampler (Model 712 WISP; Waters, Inc., Milford, MA), with 100 μl of 30 mM *o*-phthaldialdehyde (in 3.1% Brij-35, 50 mM 2-mercaptoethanol, and 40 mM sodium borate, [pH 9.5]). An aliquot of the derivatized mixture (25 μl) was injected into a Supelco 3-μm reversed-phase C_{18} column (150 × 4.6 mm [inner diameter]; Sigma) guarded by a Supelco 40-μm reversed-phase C_{18} column (50 × 4.6 mm [inner diameter]). Polyamines were separated using a solvent gradient consisting of solution A (0.1 M sodium acetate, 2 mM sodium dodecyl sulfate, 0.5% tetrahydrofuran, and 9% methanol [pH 7.2]) and solution B (100% methanol and 2 mM sodium dodecyl sulfate). Putrescine, spermidine, and spermine in samples were quantified on the basis of authentic standards using Millenium-32 Software (Waters).

Amino acids were analyzed using the HPLC system as described above, except that the mobile-phase solutions did not contain the ion-pairing agent sodium dodecyl sulfate [18]. Amino acids in samples were quantified on the basis of authentic standards using Millenium-32 Software [11].

Determination of Polyamine Synthesis

Polyamine synthesis was determined in placental and endometrial tissues using L-[U-^{14}C]arginine and L-[U-^{14}C]ornithine, as we described for

TABLE 1. Ornithine decarboxylase activity (nmol g tissue^{-1} h^{-1}) in ovine placenta and endometrium.

	Day of gestation							
	30	40	60	80	100	120	140	SEM
Intercotyledonary placenta	0.58[a]	0.57[a]	0.36[b]	0.32[b]	0.34[b]	0.32[b]	0.35[b]	0.04
Placentome	1.79[b]	3.54[a]	1.50[bc]	1.44[c]	0.56[d]	0.49[d]	0.47[d]	0.13
Intercaruncular endometrium	0.60[b]	0.82[a]	0.56[b]	0.54[b]	0.34[c]	0.32[c]	0.35[c]	0.05

[a–d] Data are the mean with pooled SEM values for four ewes per gestational age. Means sharing different superscript letters within a row are different ($P < 0.01$). Enzyme activity was measured in the presence of 0.2 mM ornithine.

porcine tissues [11]. Briefly, placental or endometrial tissues (~500 mg) were rinsed twice with oxygenated (95% O_2/5% CO_2) Basal Eagle Medium (BEM; Gibco BRL, Grand Island, NY) and then incubated at 37°C for 3 h in 2 ml of oxygenated (95% O_2/5% CO_2) BEM containing 5 mM glucose, 0.5 mM L-methionine, and either 1 mM L-arginine plus 2 μCi L-[U-^{14}C]arginine (1 μCi/μmol) or 1 mM L-ornithine plus 2 μCi L-[U-^{14}C]ornithine (1 μCi/μmol). Incubations, in which medium contained all of the above components but no tissues, were run as blanks. The ^{14}C-labeled substrates were used to improve the sensitivity of detecting polyamine synthesis in placental and endometrial tissues. Incubations were terminated by the addition of 0.2 ml of 1.5 M $HClO_4$. The acidified tissues plus medium were homogenized using a glass homogenizer, and the homogenates were neutralized with 0.1 ml of 2 M K_2CO_3. The neutralized extracts were dried in a Model RC10.10 centrifugal evaporator (Jouan, Inc., Winchester, VA) and suspended in 0.3 ml of H_2O for separation of [^{14}C]putrescine, [^{14}C]spermidine, and [^{14}C]spermine by HPLC. The fractions containing [^{14}C]putrescine, [^{14}C]spermidine, and [^{14}C]spermine were collected from the HPLC column for measuring radioactivities in a Packard liquid scintillation counter. Recovery of [^{14}C]putrescine, [^{14}C]spermidine, and [^{14}C]spermine from the HPLC column was determined using known amounts of [^{14}C]polyamine standards and was found to be greater than 91%. Blank radioactivities were subtracted from sample values. Rates of production of putrescine, spermidine, and spermine were calculated on the basis of intracellular specific activities of [^{14}C]ornithine, which were measured as described by Wu [19].

Calculations and Statistical Analysis

Concentrations of polyamines and amino acids in placental and endometrial tissues as well as fetal fluids were calculated on the basis of the recovery rates of putrescine, spermidine, spermine, ornithine, and arginine from the ovine placental and uterine tissues and fetal fluids. For each gestational age, total content of polyamines in amniotic and allantoic fluids was calculated by multiplying polyamine concentrations by amniotic and allantoic fluid volumes, respectively. Concentrations of total polyamines were a mathematical sum of putrescine, spermidine, and spermine. Data obtained between Days 30 and 140 of gestation were subjected to least-squares analyses of variance and one-way analysis of variance [20] using the PROC GLM, PROC REG, and PROC CORR procedures of the SAS software (SAS Institute, Inc., Cary, NC). Differences between means were determined by the Student-Newman-Keuls multiple-comparison test following one-way analysis of variance [20]. Differences between ODC and arginase activities in the same tissue on Day 40 or 140 of gestation were determined by paired *t*-test. Statistical significance was set at $P \leq 0.05$.

RESULTS

ODC Activity in Placental and Endometrial Tissues

As summarized in Table 1, marked changes ($P < 0.01$) in ODC activity occurred in ovine placental and endometrial tissues during conceptus development. In intercotyledonary placenta, ODC activity was the highest on Days 30–40 of gestation and then decreased ($P < 0.01$) on Day 60 of gestation. No change in intercotyledonary placental ODC activity was observed between Days 60 and 140 of gestation. In intercaruncular endometrium, ODC activity increased ($P < 0.01$) by 35% between Days 30 and 40 of gestation, declined ($P < 0.01$) progressively between Days 40 and 100, and remained at low values on Days 100 to 140. Placentomes exhibited the greatest ODC activity among the placental and endometrial tissues examined between Days 30 and 140 of gestation ($P < 0.01$). Placentomal ODC activity increased ($P < 0.01$) approximately 2-fold between Days 30 and 40 of gestation and then decreased ($P < 0.01$) progressively between Days 40 and 100 of gestation. Placentomal ODC activity did not differ ($P > 0.05$) between Days 100 and 140 of gestation.

Arginase Activity in Placental and Endometrial Tissues

Relatively high arginase activity was present in ovine placentomes, intercotyledonary placenta, and intercaruncular endometrium (Table 2). Placentomal arginase activity was highest ($P < 0.01$) among the tissues examined between Days 30 and 140 of gestation. In intercotyledonary placenta, arginase activity increased ($P < 0.01$) between Days 30 and 80 of gestation and declined ($P < 0.01$) thereafter. In intercaruncular endometrium, arginase activity increased ($P < 0.01$) between Days 30 and 60 of gestation and then declined ($P < 0.01$). In placentomes, arginase activity peaked ($P < 0.01$) on Day 40 of gestation and declined ($P < 0.01$) thereafter. In each of these tissues, arginase activity was the lowest ($P < 0.01$) on Days 120–140 of gestation.

Polyamine Synthesis in Placental and Endometrial Tissues

Data regarding polyamine synthesis from [^{14}C]ornithine and [^{14}C]arginine are summarized in Tables 3 and 4, respectively. Rates of polyamine synthesis from [^{14}C]ornithine or [^{14}C]arginine were greatest in placentomes compared with other sites on all days of gestation ($P < 0.01$). Polyamine synthesis from ornithine peaked ($P < 0.01$) on Day 40 of gestation in placentomes, intercaruncular endometrium, and intercotyledonary placenta. In each of these tissues, polyamine synthesis from arginine peaked ($P < 0.01$) on Day 40 of gestation and declined ($P < 0.01$)

TABLE 2. Arginase activity (nmol g tissue^{-1} h^{-1}) in ovine placenta and endometrium.

	Day of gestation							
	30	40	60	80	100	120	140	SEM
Intercotyledonary placenta	172[b]	225[a]	232[a]	238[a]	166[b]	122[c]	114[c]	7
Placentome	425[e]	953[a]	760[b]	687[c]	515[d]	441[e]	420[e]	17
Intercaruncular endometrium	257[b]	441[a]	435[a]	246[b]	239[b]	177[c]	158[c]	7

[a–e] Data are the mean with pooled SEM values for four ewes per gestational age. Means sharing different superscript letters within a row are different ($P < 0.01$). Enzyme activity was measured in the presence of 10 mM arginine.

TABLE 3. Polyamine synthesis (pmol g tissue^{-1} 3 h^{-1}) from ornithine in ovine placenta and endometrium.

		Day of gestation							
	Polyamine	30	40	60	80	100	120	140	SEM
Intercotyledonary placenta	Putrescine	170^{a}	190^{a}	121^{b}	127^{b}	82^{c}	87^{c}	75^{c}	5
	Spermidine	223^{a}	206^{a}	135^{b}	113^{b}	129^{b}	120^{b}	124^{b}	7
	Spermine	130^{b}	204^{a}	103^{c}	106^{c}	126^{b}	142^{b}	128^{b}	6
	Total	523^{a}	598^{a}	359^{b}	345^{b}	337^{b}	349^{b}	327^{b}	16
Placentome	Putrescine	363^{bc}	694^{a}	424^{b}	343^{c}	133^{d}	117^{d}	124^{d}	15
	Spermidine	624^{b}	1044^{a}	508^{c}	441^{d}	248^{e}	255^{e}	221^{e}	12
	Spermine	648^{b}	1171^{a}	487^{c}	449^{c}	227^{d}	214^{d}	202^{d}	29
	Total	1635^{b}	2909^{a}	1419^{bc}	1233^{c}	608^{d}	586^{d}	546^{d}	56
Intercaruncular endometrium	Putrescine	150^{b}	202^{a}	146^{b}	135^{b}	87^{c}	89^{c}	77^{c}	7
	Spermidine	226^{b}	369^{a}	218^{bc}	197^{c}	152^{d}	101^{d}	113^{d}	8
	Spermine	221^{b}	281^{a}	241^{b}	236^{b}	146^{c}	152^{c}	162^{c}	8
	Total	598^{b}	853^{a}	603^{b}	567^{b}	385^{c}	341^{c}	352^{c}	20

$^{a–e}$ Data are the mean with pooled SEM values for four ewes per gestational age. Means sharing different superscript letters within a row are different ($P < 0.01$).

thereafter, with the lowest values on Days 120–140. Rates of putrescine, spermidine, and spermine synthesized from ornithine or arginine varied greatly with placental and endometrial tissues as well as gestational ages. In placentomes, spermidine and spermine were the major polyamines synthesized from ornithine between Days 30 and 40 and between Days 80 and 140 of gestation, whereas rates of putrescine, spermidine, and spermine synthesis were similar on Day 60 of gestation. In intercaruncular endometrium, spermidine and spermine were the major polyamines synthesized from ornithine and arginine between Days 30 and 100 of gestation, whereas rates of spermine synthesis were highest on Days 120–140. In intercotyledonary placenta, rates of putrescine, spermidine, and spermine synthesis were similar between Days 40 and 80 of gestation, whereas spermidine and spermine were the major polyamines synthesized between Days 100 and 140 of gestation.

Polyamine Concentrations in Placental and Endometrial Tissues

Data regarding polyamine concentrations in placental and endometrial tissues are summarized in Table 5. Biphasic changes were observed in total polyamine concentrations in intercotyledonary placenta during pregnancy: Values were similar ($P > 0.05$) between Days 30 and 40 of gestation, increased ($P < 0.01$) approximately 2-fold on Days 60–80, declined ($P < 0.01$) on Day 100, and increased again ($P < 0.01$) on Days 120–140 to values similar to those on Days 60–80 of gestation. Concentrations of total polyamines in intercaruncular endometrium were 41%–45% higher ($P < 0.01$) on Days 40–60 compared with Day 30 of gestation, and they progressively declined ($P < 0.01$) between Days 60 and 100 of gestation. Placentomes exhibited the highest ($P < 0.01$) concentrations of polyamines among the ovine placental and endometrial tissues examined between Days 30 and 120 of gestation. Placentomal concentrations of total polyamines increased ($P < 0.01$) by 113% between Days 30 and 40 of gestation and decreased ($P < 0.01$) markedly between Days 40 and 80 of gestation. No changes ($P > 0.05$) were observed in placentomal concentrations of total polyamines between Days 80 and 120 of gestation. Concentrations of polyamines were similar ($P > 0.05$) among intercotyledonary placenta, intercaruncular endometrium, and placentomes on Day 140 of gestation.

Ornithine and Arginine Concentrations in Placental and Endometrial Tissues

Concentrations of arginine were higher ($P < 0.01$) than those of ornithine in all placental and endometrial tissues examined between Days 30 and 140 of gestation (Table 6). Placentomal arginine and ornithine concentrations increased ($P < 0.01$) by 437% and 840%, respectively, between Days 30 and 40 of gestation and declined ($P < 0.01$) thereafter. In intercotyledonary placenta, concentrations of ornithine increased ($P < 0.01$) progressively between Days 30 and Days 60–80 of gestation and declined ($P < 0.01$) thereafter, whereas concentrations of arginine increased

TABLE 4. Polyamine synthesis (pmol g tissue^{-1} 3 h^{-1}) from arginine in ovine placenta and endometrium.

		Day of gestation							
	Polyamine	30	40	60	80	100	120	140	SEM
Intercotyledonary placenta	Putrescine	21^{b}	27^{a}	20^{bc}	18^{bc}	17^{c}	12^{d}	12^{d}	1
	Spermidine	26^{b}	36^{a}	23^{b}	25^{b}	20^{c}	16^{d}	15^{d}	1
	Spermine	15^{c}	32^{a}	24^{b}	22^{b}	16^{c}	14^{c}	13^{c}	1
	Total	62^{b}	95^{a}	67^{b}	65^{c}	53^{d}	42^{e}	40^{e}	3
Placentome	Putrescine	113^{b}	224^{a}	108^{b}	107^{b}	56^{c}	42^{d}	38^{d}	3
	Spermidine	132^{c}	326^{a}	176^{b}	129^{c}	89^{d}	63^{e}	60^{e}	6
	Spermine	120^{c}	365^{a}	192^{b}	171^{b}	93^{d}	66^{e}	68^{e}	6
	Total	365^{c}	915^{a}	477^{b}	407^{c}	237^{d}	182^{e}	166^{e}	11
Intercaruncular endometrium	Putrescine	23^{c}	41^{a}	32^{b}	21^{c}	15^{d}	14^{d}	13^{d}	1
	Spermidine	31^{c}	69^{a}	44^{b}	26^{d}	21^{e}	17^{f}	16^{f}	1
	Spermine	25^{e}	57^{a}	45^{b}	39^{c}	30^{d}	24^{e}	23^{e}	1
	Total	78^{c}	167^{a}	121^{b}	86^{c}	65^{d}	54^{e}	52^{e}	2

$^{a–e}$ Data are the mean with pooled SEM values for four ewes per gestational age. Means sharing different superscript letters within a row are different ($P < 0.01$).

TABLE 5. Concentration (nmol/g tissue) of polyamines in ovine placenta and endometrium.

		Day of gestation							
	Polyamine	30	40	60	80	100	120	140	SEM
Intercotyledonary placenta	Putrescine	91[b]	85[b]	154[a]	170[a]	81[b]	93[b]	95[b]	8
	Spermidine	90[c]	93[c]	192[a]	182[a]	92[c]	145[b]	185[a]	9
	Spermine	73[c]	82[c]	135[b]	171[a]	84[c]	170[a]	196[a]	8
	Total	254[c]	260[c]	481[a]	523[a]	257[c]	408[b]	476[a]	23
Placentome	Putrescine	314[b]	665[a]	387[b]	152[c]	169[c]	144[c]	115[d]	25
	Spermidine	477[c]	1039[a]	682[b]	274[d]	349[d]	288[d]	231[d]	32
	Spermine	506[c]	1064[a]	710[b]	353[d]	387[d]	367[d]	191[e]	51
	Total	1298[c]	2767[a]	1779[b]	778[d]	904[d]	799[d]	537[e]	96
Intercaruncular endometrium	Putrescine	226[c]	298[b]	356[a]	180[d]	116[e]	124[e]	127[e]	7
	Spermidine	355[c]	554[a]	447[b]	314[c]	244[d]	229[d]	244[d]	16
	Spermine	329[c]	505[a]	512[a]	442[b]	260[d]	258[d]	239[d]	20
	Total	910[b]	1357[a]	1316[a]	936[b]	620[c]	611[c]	594[c]	47

[a–e] Data are the mean with pooled SEM values for four ewes per gestational age. Means sharing different superscript letters within a row are different ($P < 0.01$).

progressively between Days 30 and 100 of gestation and then decreased ($P < 0.01$) progressively between Days 100 and 140 of gestation. In intercaruncular endometrium, concentrations of ornithine increased ($P < 0.01$) between Days 30 and 40 of gestation and declined ($P < 0.01$) thereafter, whereas concentrations of arginine increased ($P < 0.01$) between Days 30 and 100 of gestation and decreased ($P < 0.01$) progressively during late gestation. Interestingly, arginine concentrations peaked ($P < 0.01$) on Day 100 of gestation in both intercotyledonary placenta and intercaruncular endometrium. Also, placentomal arginine concentrations were higher ($P < 0.01$) on Day 100 of gestation compared with earlier (Days 30 and 60–80) and later (Days 120–140) stages of gestation.

Polyamine Concentrations in Allantoic and Amniotic Fluids

Data regarding polyamine concentration in allantoic and amniotic fluids are summarized in Table 7. Concentrations of total polyamines in ovine allantoic fluid were higher than ($P < 0.01$), similar to ($P > 0.05$), and lower than ($P < 0.01$) those in amniotic fluid on Days 30–80, 100, and 120–140, respectively. Concentrations of total polyamines in allantoic fluid increased ($P < 0.01$) progressively between Days 30 and 60 of gestation and declined ($P < 0.01$) during the remainder of pregnancy, with the lowest values being observed on Days 120–140 of gestation. Concentrations of total polyamines in amniotic fluid increased ($P < 0.01$) progressively between Days 30 and 100 of gestation and then declined ($P < 0.01$) during late gestation (Days 120–140). In allantoic or amniotic fluids, concentrations of polyamines did not differ ($P > 0.05$) between Days 120 and 140 of gestation. Concentrations of putrescine, spermidine, and spermine in allantoic and amniotic fluids varied greatly with day of gestation. For example, concentrations of putrescine in allantoic fluid were greater than ($P < 0.01$), similar to ($P > 0.05$), and lower than ($P < 0.01$) those of spermidine and spermine on Days 30–40, 60–80, and 100–140 of gestation, respectively. In addition, concentrations of putrescine in amniotic fluid were greater than ($P < 0.01$) those of spermine but similar to ($P > 0.05$) and lower than ($P < 0.01$) those of spermidine and spermine on Days 30, 40–60, and 100 of gestation, respectively.

Data regarding allantoic and amniotic fluid volumes as well as fetal weights of the same sheep as used for the present study have been published previously [13]. The volumes of ovine allantoic fluid averaged 31, 11, 31, 46, 199, 491, and 366 ml on Days 30, 40, 60, 80, 100, 120, and 140 of gestation, respectively. On the same days, the volumes of ovine amniotic fluid averaged 2.2, 23.8, 190, 543, 595, 383, and 449 ml, respectively. These data, along with concentrations of polyamines in allantoic and amniotic fluids (Table 7), were used to calculate the total content of polyamines in these two fetal fluid compartments (Table 8). Total content of polyamines in allantoic fluid was greater than ($P < 0.01$), similar to ($P > 0.05$), and lower than ($P < 0.01$) that in amniotic fluid on Days 30, 40, and 60–140 of gestation, respectively. Total content of polyamines in allantoic and amniotic fluids peaked ($P < 0.01$) on Day 100 of gestation. Total content of spermine in amniotic fluid increased ($P < 0.01$) progressively with advancing gestation. On the basis of ovine fetal weights, which averaged

TABLE 6. Concentrations (nmol/g tissue) of ornithine and arginine in ovine placenta and endometrium.

	Day of gestation							
	30	40	60	80	100	120	140	SEM
Intercotyledonary placenta								
Ornithine	316[c]	537[b]	852[a]	906[a]	328[c]	322[c]	340[c]	34
Arginine	1003[e]	1217[d]	1618[b]	1773[b]	1994[a]	1407[c]	1025[e]	72
Placentome								
Ornithine	183[e]	1719[a]	964[b]	613[c]	336[d]	324[d]	378[d]	78
Arginine	564[e]	3027[a]	1797[c]	1848[c]	2357[b]	1325[d]	1244[d]	104
Intercaruncular endometrium								
Ornithine	422[c]	897[a]	577[b]	395[c]	296[d]	288[d]	301[d]	36
Arginine	813[c]	1055[b]	807[c]	886[bc]	1569[a]	1051[b]	799[c]	59

[a–e] Data are the mean with pooled SEM values for four ewes per gestational age. Means sharing different superscript letters within a row are different ($P < 0.01$).

TABLE 7. Concentrations (nmol/L) of polyamines in ovine allantoic and amniotic fluids.

		Day of gestation							
	Polyamine	30	40	60	80	100	120	140	SEM
Allantoic fluid	Putrescine	2088[b]	3090[a]	3468[a]	1348[c]	545[d]	433[d]	423[d]	203
	Spermidine	1055[d]	1898[b]	3063[a]	1445[c]	2748[a]	795[e]	618[e]	167
	Spermine	923[d]	2155[b]	3260[a]	1643[c]	1195[d]	758[e]	865[de]	106
	Total	4065[c]	7143[b]	9790[a]	4435[c]	4488[c]	1985[d]	1905[d]	285
Amniotic fluid	Putrescine	480[e]	813[bc]	995[a]	773[cd]	698[d]	905[ab]	278[f]	50
	Spermidine	853[bc]	1060[b]	823[bc]	1745[a]	1978[a]	740[c]	545[d]	103
	Spermine	335[e]	1000[d]	1153[d]	1035[d]	1800[c]	2297[b]	2993[a]	132
	Total	1668[d]	2873[c]	2970[c]	3553[b]	4475[a]	3942[b]	3815[ab]	161

[a–f] Data are the mean with pooled SEM values for four ewes per gestational age. Means sharing different superscript letters within a row are different ($P < 0.01$).

0.88, 5.0, 61, 312, 1005, 2322, 4125 g on Days 30, 40, 60, 80, 100, 120, and 140 of gestation, respectively [13], total content of spermine in amniotic fluid was positively correlated with ovine fetal weights between Days 30 and 140 of gestation ($r = 0.88$, $P < 0.01$).

Placental and Endometrial ODC and Arginase Activities Measured with the Same Substrate Concentration (2 mM)

Intercotyledonary placenta, placentomes, and intercaruncular endometrium obtained on Days 40 and 140 of gestation were analyzed for ODC and arginase activities in the presence of the same substrate concentration (2 mM) (Table 9). Placental and endometrial ODC activities measured in the presence of 2 mM ornithine were approximately 1.4-fold greater than those obtained in the presence of 0.2 mM ornithine (Table 1), whereas arginase activities measured in the presence of 2 mM arginine were approximately 30% of the values obtained in the presence of 10 mM arginine (Table 2). At the same substrate concentration, arginase activity was 60- to 180-fold ODC activity in ovine placental and endometrial tissues ($P < 0.01$).

Correlations Between ODC Activity and Polyamine Synthesis or Polyamine Concentrations in Placental and Endometrial Tissues

Data regarding correlations between ODC activity and polyamine synthesis or polyamine concentration in placental and endometrial tissues are summarized in Table 10. In placentomes and intercaruncular endometrium, ODC activities were positively correlated ($P < 0.01$) with both rates of polyamine synthesis and concentrations of polyamines. In both tissues, concentrations of polyamines were positively correlated with rates of polyamine synthesis. Likewise, in intercotyledonary placenta, ODC activities were positively correlated ($P < 0.01$) with rates of putrescine, spermidine, and total polyamine synthesis. Interestingly, ODC activities were negatively correlated ($P < 0.05$) with concentrations of spermine, whereas rates of spermidine, spermine, and total polyamine synthesis were also negatively correlated ($P < 0.05$) with their concentrations in intercotyledonary placenta.

DISCUSSION

To our knowledge, this is the first report of changes in polyamine synthesis and concentrations in ovine placental and endometrial tissues during pregnancy. The present study has four major findings. First, maximal activities of ODC and arginase as well as the highest concentrations of polyamines were expressed in ovine placentomes, intercotyledonary placenta, and intercaruncular endometrium during the first half of pregnancy. Second, the highest concentrations of polyamines in ovine allantoic and amniotic fluids occurred during early and late gestation, respectively. Third, concentrations of putrescine, spermidine, and spermine varied greatly among ovine placental and endometrial tissues, fetal fluids, and day of gestation. Fourth, arginine was an important precursor for polyamine synthesis in the ovine placenta and endometrium.

Despite the previous reports of changes in rat placental ODC activity during pregnancy [21, 22], little is known about polyamine synthesis or the concentrations of its physiological substrates in the placenta or endometrium of any species. The present results demonstrate clearly that ODC activity, polyamine synthesis from ornithine or arginine, and polyamine concentrations were maximal in ovine placentomes on Day 40 of gestation (Tables 1–6), when placental growth and placentomal development are most rapid [3, 4]. The ODC activity and polyamine synthesis in intercotyledonary placenta and intercaruncular endometrium, as well as endometrial concentrations of polyamines, also peaked on Day 40 of gestation, when these tissues undergo

TABLE 8. Total content (nmol) of polyamines in ovine allantoic and amniotic fluids.

		Day of gestation							
	Polyamine	30	40	60	80	100	120	140	SEM
Allantoic fluid	Putrescine	64[d]	34[e]	108[c]	62[d]	109[c]	214[a]	155[b]	11
	Spermidine	31[f]	23[f]	96[d]	67[e]	548[a]	393[b]	227[c]	18
	Spermine	30[e]	26[e]	102[d]	76[d]	240[c]	371[a]	316[b]	15
	Total	125[e]	83[f]	306[c]	205[d]	895[a]	978[a]	670[b]	66
Amniotic fluid	Putrescine	1[f]	19[e]	189[c]	420[a]	416[a]	345[b]	126[d]	24
	Spermidine	2[g]	25[f]	157[e]	949[b]	1178[a]	283[c]	245[d]	76
	Spermine	1[f]	24[e]	218[d]	563[c]	1072[b]	882[b]	1344[a]	129
	Total	4[f]	68[e]	564[d]	1931[b]	2667[a]	1510[c]	1715[bc]	195

[a–g] Data are the mean with pooled SEM values for four ewes per gestational age. Means sharing different superscript letters within a row are different ($P < 0.01$).

TABLE 9. ODC and arginase activities (nmol g tissue^{-1} h^{-1}) in ovine placenta and endometrium.[a]

	Day 40 of gestation		Day 140 of gestation	
	ODC	Arginase	ODC	Arginase
Intercotyledonary placenta	0.82 ± 0.06	64 ± 3[b]	0.48 ± 0.03	33 ± 2[b]
Placentome	4.97 ± 0.35	304 ± 12[b]	0.67 ± 0.04	120 ± 6[b]
Intercaruncular endometrium	1.16 ± 0.09	136 ± 7[b]	0.49 ± 0.03	51 ± 3[b]

[a] Data are the mean ± SEM, n = 4. The ODC and arginase activities were measured in the presence of 2 mM ornithine and 2 mM arginine, respectively.
[b] $P < 0.01$ vs. ODC activity.

marked morphological and functional changes [14–16]. On the basis of water content of the ovine placentome (~81%), intercotyledonary placenta (~87%), and intercaruncular endometrium (~74%) (unpublished data), concentrations of polyamines in these tissues were estimated to be 0.66–3.5 mM, 0.29–0.61 mM, and 0.82–1.8 mM, respectively. These values are substantially higher than the concentrations of polyamines in maternal or fetal plasma (2–5 μM; unpublished data), indicating the abundance of polyamines in ovine placental and endometrial tissues. Our results suggest an important role for polyamines in placental and endometrial growth and, therefore, in fetal growth. In support of this view, an inhibition of ODC activity during pregnancy markedly reduced the placental size and birth weights of rats [23].

A novel finding of the present study is the relatively high arginase activity in ovine placental and endometrial tissues (Table 2). In addition to ODC, the arginine-derived ornithine can be catabolized by ornithine aminotransferase to yield P5C, a substrate for the synthesis of glutamate and proline by mitochondrial P5C dehydrogenase and cytosolic P5C reductase, respectively [24]. Thus, through the formation of ornithine, arginase would be expected to play a regulatory role in polyamine synthesis from arginine in the ovine placenta and endometrium, as recently reported for endothelial cells, vascular smooth muscle cells, and activated macrophages [24–26]. Indeed, our results demonstrate that arginine was actively converted into polyamines in ovine placental and endometrial tissues (Table 4) and that rates of polyamine synthesis were closely correlated with arginase activity in these tissues (Table 2). Ovine allantoic fluid is rich in ornithine and arginine [13] and likely is a major source of these two amino acids for the placenta and endometrium. This view is supported by our findings that the highest concentrations of arginine in ovine allantoic fluid on Day 100 of gestation [13] were associated with the highest concentrations of arginine in intercotyledonary placenta and intercaruncular endometrium (Table 6) and with elevated concentrations of arginine in placentomes (Table 6). On the basis of water content of ovine placentomes, intercotyledonary placenta, and intercaruncular endometrium, concentrations of ornithine in these tissues were estimated to be 0.23–2.1 mM, 0.36–1.0 mM, and 0.39–1.2 mM, respectively, and concentrations of arginine in these tissues were estimated to be 0.70–3.7 mM, 1.2–2.3 mM, and 1.1–2.1 mM, respectively. These values are substantially higher than the concentrations of ornithine and arginine in ovine maternal plasma (0.04–0.2 mM) and fetal plasma (0.1–0.6 mM) [13]. Interestingly, between Days 30 and 80 of gestation, increases in substrate concentrations and polyamine synthesis in ovine placental and endometrial tissues (Tables 3–5) are associated with increases in ornithine and arginine concentrations in ovine allantoic fluid [13], further supporting the earlier suggestion that this fetal fluid is an important nutrient reservoir during gestation [17]. Collectively, our results indicate that arginine is a major source of the ornithine used for polyamine synthesis in ovine placental and endometrial tissues.

The ODC and arginase assay conditions, which involved 0.2 mM ornithine (~2-fold the K_m value of the mammalian ODC for ornithine [9]) and 10 mM arginine (~2-fold the K_m value of the mammalian arginase for arginine [24]), respectively, were commonly found in the literature (e.g., see [10, 21, 22, 26]). The higher arginase activity (Table 2) compared to ODC activity (Table 1) in ovine placental and endometrial tissues was not caused by the inclusion of higher substrate (arginine) concentrations in assay solutions. For example, when ODC and arginase activities were determined using the same substrate concentrations (2 mM), which were similar to the ornithine and arginine concentrations in the placentomes on Day 40 of gestation (Table 6), we found that arginase activity was also much higher than

TABLE 10. Correlations between ODC activity and polyamine synthesis (PAS) or polyamine concentrations ([PA]) in ovine placenta and endometrium.[a]

	Polyamine	ODC vs. PAS	ODC vs. [PA]	[PA] vs. PAS
Intercotyledonary placenta	Putrescine	0.480[b]	0.204	0.005
	Spermidine	0.527[b]	0.325	−0.608[b]
	Spermine	0.335	−0.406[c]	−0.390[c]
	Total	0.523[b]	0.357	−0.599[b]
Placentome	Putrescine	0.937[b]	0.904[b]	0.903[b]
	Spermidine	0.947[b]	0.823[b]	0.859[b]
	Spermine	0.940[b]	0.769[b]	0.819[b]
	Total	0.953[b]	0.834[b]	0.882[b]
Intercaruncular endometrium	Putrescine	0.669[b]	0.556[b]	0.754[b]
	Spermidine	0.697[b]	0.718[b]	0.890[b]
	Spermine	0.696[b]	0.423[c]	0.809[b]
	Total	0.708[b]	0.594[b]	0.854[b]

[a] Values are Pearson correlation coefficients, n = 28.
[b] $P < 0.01$.
[c] $P < 0.05$.

ODC activity in ovine placental and endometrial tissues (Table 9). Importantly, the present results indicate that changes in ODC and arginase activities in ovine placental and endometrial tissues during pregnancy are consistent with changes in the rates of polyamine synthesis from ornithine and arginine, respectively.

Tissue concentrations of polyamines depend on the balance between rates of their synthesis and catabolism [24]. Polyamine synthesis is regulated by ODC and arginase activities as well as by the availability of ornithine and arginine [24–26]. Thus, ODC activity was positively correlated with polyamine concentrations in placentomes and endometrium (Table 10). In addition, concentrations of both ornithine and polyamines peaked between Days 60 and 80 of gestation in intercotyledonary placenta (Tables 4 and 5). These data further support an important role for polyamine synthesis in regulating polyamine concentrations. In mammalian cells, ODC degradation is catalyzed by the 26S proteasome via a ubiquitin-independent pathway and is greatly accelerated by its association with the polyamine-induced regulatory protein antizyme [27]. Spermidine/spermine N^1-acetyltransferase converts spermine and spermidine to acetylspermine and N^1-acetylspermidine, respectively. Acetylspermine and N^1-acetylspermidine are catalyzed by polyamine oxidase to form spermidine and putrescine, respectively. The latter is oxidized by diamine oxidase to form succinate plus ammonia. At present, little is known about developmental changes of the ODC antizyme or polyamine-degrading enzymes in ovine tissues. Future studies are necessary to obtain such information to fully explain the developmental changes in polyamine concentrations in ovine tissues (including intercotyledonary placenta) during pregnancy.

Amniotic and allantoic fluid derive, in part, from secretions and transport of water across the placenta and endometrium. Thus, increases in polyamine concentrations in allantoic fluid between Days 30 and 60 of gestation (Table 7) were closely correlated with increases in polyamine synthesis in ovine placentomes and endometrium (Tables 3 and 4). With the development of intestinal polyamine transport systems during gestation, the intake of amniotic fluid by the fetus provides a source of polyamines for supporting proliferation and differentiation of intestinal epithelial cells. This is consistent with the increasing content of total polyamines in amniotic fluid with advancing gestation (Table 8). The nutritional significance of amniotic fluid is graphically illustrated by the finding that esophageal ligation, which prevents the entry of this fluid into the small intestine, results in intrauterine growth retardation in fetal sheep [28]. Although early anatomical studies suggested that the allantoic sac served as a reservoir for fetal wastes, it is now clear that allantoic fluid nutrients may be absorbed by the allantoic epithelium into the fetal-placental circulation and be utilized by fetal tissues [17]. The increases in concentrations of polyamines (Table 7) and substrates (ornithine and arginine) for polyamine synthesis [13] in allantoic fluid during early gestation may play an important role in fetal growth and development.

Our findings indicate that patterns of polyamine synthesis and substrate availability vary greatly with ovine placental and endometrial tissues. Whatever the differences, however, the highest concentrations of polyamines in these tissues (Tables 1–5) occur when their growth is the most rapid during gestation [3, 4, 29–31]. Importantly, our results reveal metabolic coordination among the several integrated pathways that support high rates of polyamine synthesis in placental and endometrial tissues. For example, placentomal arginase activity and arginine concentrations peaked on Day 40 of gestation (Table 2), thus maximizing the hydrolysis of arginine to ornithine and markedly increasing intracellular concentrations of ornithine (Table 6). The latter was coupled with the highest ODC activity for polyamine synthesis (Table 1), thereby maximizing polyamine concentrations (Table 5) in ovine placentomes during early gestation. Similarly, arginase and ODC activities, intracellular ornithine concentrations, and polyamine synthesis from both ornithine and arginine were maximal in the intercaruncular endometrium on Day 40 of gestation, resulting in the highest concentrations of polyamines (Table 5). Furthermore, concentrations of glutamine, a major substrate for ornithine and arginine synthesis [24] and a stimulator of ODC activity [11], were highest in ovine allantoic fluid between Days 40 and 60 of gestation [13].

The present findings raise important questions regarding the physiologic significance of polyamine synthesis in fetal-placental nutrition and development. In this regard, it is noteworthy that maternal undernutrition decreases ornithine concentrations in porcine fetal plasma and allantoic fluid and impairs fetal growth [32, 33]. It also may program permanent structural, metabolic, and functional alterations [34, 35]. Maternal undernutrition in sheep (50% of NRC nutrient requirements) from Day 28 to Day 78 of gestation decreased concentrations of putrescine, spermidine, and spermine in amniotic and allantoic fluids by 38%–43% (unpublished data) and reduced fetal growth by 32% on Day 78 of gestation [36]. Because recent epidemiological studies in humans suggest that the existence of links between intrauterine growth retardation and development of chronic disease (e.g., diabetes, hypertension, and coronary heart disease) later in life [34, 35], placental synthesis of polyamines may have important implications for both intrauterine growth retardation and fetal origins of diseases in adults.

As noted previously, physiological concentrations of polyamines (putrescine, spermidine, and spermine) are key regulators of angiogenesis, early embryogenesis, placental trophoblast growth, and embryonic development in the uterus [5, 6]. Results of the present study indicate that polyamine synthesis and concentrations were the highest in ovine placentomes and endometrium on Day 40 of gestation, when their growth and morphological changes were most rapid. Relatively high levels of polyamine concentrations were present in ovine placental and endometrial tissues during the second half of pregnancy, when development of the placental vascular bed continues and total uterine blood flow increases to support fetal growth [2, 4]. Importantly, metabolic coordination occurs among the several integrated pathways that support high rates of polyamine synthesis in the placenta and endometrium. Our findings provide a new base of information for future studies to define the roles of polyamines in fetal-placental growth and development.

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