BIOLOGY OF REPRODUCTION **72,** 842–850 (2005)
Published online before print 1 December 2004.
DOI 10.1095/biolreprod.104.036293

Polyamine Synthesis from Proline in the Developing Porcine Placenta[1]

Guoyao Wu,[2,3] Fuller W. Bazer,[3,4] Jianbo Hu,[3,4] Greg A. Johnson,[3,4] and Thomas E. Spencer[3,4]

Departments of Animal Science[3] and of Veterinary Integrative Biosciences,[4] Texas A&M University, College Station, Texas 77843-2471

ABSTRACT

Polyamines (putrescine, spermidine, and spermine) are essential for placental growth and angiogenesis. However, little is known about polyamine synthesis in the porcine placenta during conceptus development. The present study was conducted to test the hypothesis that arginine and proline are the major sources of ornithine for placental polyamine production in pigs. Placentae, amniotic fluid, and allantoic fluid were obtained from gilts on Days 20, 30, 35, 40, 45, 50, 60, 90, and 110 of the 114-day gestation (n = 6 per day). Placentae as well as amniotic and allantoic fluids were analyzed for arginase, proline oxidase, ornithine aminotransferase (OAT), ornithine decarboxylase (ODC), proline transport, concentrations of amino acids and polyamines, and polyamine synthesis using established radiochemical and chromatographic methods. Neither arginase activity nor conversion of arginine into polyamines was detected in the porcine placenta. In contrast, both proline and ornithine were converted into putrescine, spermidine, and spermine in placental tissue throughout pregnancy. The activities of proline oxidase, OAT, and ODC as well as proline transport, polyamine synthesis from proline, and polyamine concentrations increased markedly between Days 20 and 40 of gestation, declined between Days 40 and 90 of gestation, and remained at the reduced level through Day 110 of gestation. Proline oxidase and OAT, but not arginase, were present in allantoic and amniotic fluids for the production of ornithine (the immediate substrate for polyamine synthesis). The activities of these two enzymes as well as the concentrations of ornithine and total polyamines in fetal fluids were highest at Day 40 but lowest at Days 20, 90, and 110 of gestation. These results indicate that proline is the major amino acid for polyamine synthesis in the porcine placenta and that the activity of this synthetic pathway is maximal during early pregnancy, when placental growth is most rapid. Our novel findings provide a new base of information for future studies to define the role of proline in fetoplacental growth and development.

conceptus, placenta, pregnancy

INTRODUCTION

The placentae of all mammalian species undergo marked growth and rapid formation of new blood vessels (angiogenesis) during the first half of pregnancy [1, 2]. In the pig, which possesses a noninvasive, diffuse type of epitheliochorial placentation, the placenta grows most rapidly between Days 20 and 60 of gestation, and placental development is maximal by Day 70 of gestation [3]. Placental angiogenesis is necessary to increase fetoplacental blood flow and, therefore, the supply of nutrients from maternal to fetal blood [4]. Thus, placental growth is a critical factor for controlling the survival, growth, and development of fetal pigs. Despite extensive research in this area of reproductive biology, a 20–50% prenatal mortality rate still remains in swine [5, 6]. Placental insufficiency also is a major factor contributing to low birth weight of piglets (<1.1 kg), which occurs in 15–20% of newborn piglets [7], which in turn have a particularly high postnatal mortality rate (20–56%) [8]. Therefore, a better understanding of factors that regulate placental growth and development is essential to improve the reproductive efficiency of pigs.

Polyamines (putrescine, spermidine, and spermine) play a crucial role in regulating gene expression, signal transduction, ion-channel function, DNA and protein synthesis, as well as cell proliferation and differentiation [9]. Polyamines also are scavengers of reactive oxygen species, thereby protecting DNA, proteins, and lipids from oxidative damage [10]. Available evidence shows that polyamines are key regulators of angiogenesis, early mammalian embryogenesis, placental trophoblast growth, and embryonic development [7]. Thus, knowledge about placental polyamine synthesis will aid in developing new means to enhance placental and fetal growth.

Ornithine decarboxylase (ODC) catalyzes the decarboxylation of ornithine to yield putrescine, which subsequently is converted to spermidine and spermine (Fig. 1). Arginine and proline are potentially major substrates for ornithine production in mammalian cells [11], whereas S-adenosylmethionine (a metabolite of methionine) provides the methyl group for spermidine and spermine synthesis [12]. Despite recent studies of polyamine synthesis in the ovine placenta [13] and ODC activity in the porcine placenta at Days 40 and 60 of gestation [14], little is known about changes in placental polyamine synthesis associated with porcine conceptus development. Of note, we recently reported substantial increases (up to 50-fold) in concentrations of the arginine-family amino acids (arginine, ornithine, glutamine, and glutamate) in porcine allantoic fluid (a reservoir for nutrients) between Days 20 and 40 of gestation [15]. Such changes coincide with the period of most rapid growth of the porcine placenta [3]. Based on these findings, we hypothesized that arginine and proline are the major amino acids for polyamine production in pig placentae and that placental polyamine synthesis is maximum during early gestation. This hypothesis was tested using gilts between Days 20 and 110 of gestation (term = 114 days). Because amniotic and allantoic compartments are integral parts of the porcine conceptus essential for fetal growth [16], we also determined concentrations of ornithine and polyamines in amniotic and allantoic fluids.

[1]Supported, in part, by USDA/NRI grants 2000-2290, 2001-02259, and 2001-02166.
[2]Correspondence: Guoyao Wu, Department of Animal Science, 212 Kleberg Center, 2471 TAMU, Texas A&M University, College Station, TX 77843-2471. FAX: 979 845 6057; e-mail: g-wu@tamu.edu

Received: 15 September 2004.
First decision: 19 October 2004.
Accepted: 9 November 2004.

ISSN: 0006-3363. http://www.biolreprod.org

MATERIALS AND METHODS

Chemicals

The L-[U-^{14}C]proline, L-[U-^{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). Soluene 350 and UltimaGold were purchased from PerkinElmer (Boston, MA), and NCS-II was obtained from Amersham Biosciences (Piscataway, NJ). All other chemicals, including putrescine, spermidine, spermine, amino acids, and dithiothreitol (DTT), were purchased from Sigma (St. Louis, MO).

Experimental Animals and Collection of Placentae and Fetal Fluids

Sexually mature crossbred gilts (Yorkshire × Landrace dams and Duroc × Hampshire sires) of approximately 8 mo of age and weighing 95–100 kg were observed daily (0700 h) for estrous behavior through direct exposure to intact boars. Gilts exhibiting at least two estrous cycles of normal duration (18–21 days) were bred to crossbred boars (Yorkshire × Landrace dams and Duroc × Hampshire sires). A total of eight boars, ranging in age between 8 mo and 1.5 yr and in weight between 135 and 205 kg, were randomly distributed across all treatments for the present study. Throughout gestation, gilts had free access to drinking water and were individually fed once daily with 2.3 kg of a sorghum- and soybean meal-based diet (consisting of 72.35% milo, 10.0% wheat middlings, 7.55% soybean meal [47.5% grade], 5.0% soy hulls, 3.5% meat and bone, 0.58% limestone, 0.50% salt, 0.34% monocalcium phosphate, 0.08% trace mineral premix, 0.05% choline chloride, and 0.05% vitamin premix) that met the recommended National Research Council nutrient requirements [17]. The major protein source of the diet is soybean meal (47.5% grade). The diet provided the following nutrients: 89.8% dry matter, 14.2% crude protein, 13 372 kJ/kg of metabolizable energy, 0.56% lysine, 0.83% arginine, 1.26% proline, 0.71% calcium, and 0.61% phosphorus. The weight gain of pregnant gilts averaged 0.36 kg/day. Pregnant gilts were hysterectomized on either Day 20, 30, 35, 40, 45, 50, 60, 90, or 110 of gestation (n = 6 per day) [18]. Briefly, gilts received an i.m. injection of Telazol (1 mg/kg) to induce anesthesia, followed by administration of isofluorane (1–5%) via inhalation during surgery. The uterus was removed by midventral laparotomy. Placentae (the chorioallantois) were exposed and isolated by dissection as recently described [18]. We collected the entire chorioallantois from each fetoplacental unit and took great care not to include the amnion or necrotic tips. The placenta of the first fetus located near the uterotubal junction of the left uterus horn was weighed and used for metabolic and enzymatic assays. A portion of placental tissue was used immediately for proline transport, proline degradation, and polyamine synthesis; the remaining placental tissue was stored at −80°C for analyses of metabolites and enzyme assays within 1 wk. Allantoic and amniotic fluids were obtained directly by aspiration into a syringe via an 18-gauge needle [15], and total volume of each fluid was measured in a graduated cylinder. No measurable amniotic fluid was present at Day 20 of gestation. The present study was approved by the Texas A&M University Institutional Agricultural Animal Care and Use Committee.

Determination of Amino Acids, Pyrroline-5-Carboxylate, and Polyamines in Pig Placentae and Fetal Fluids

Placentae (~200 mg) were homogenized at 4°C in 2 ml of 1.5 mM $HClO_4$ using a glass homogenizer. The solution was transferred to a 12- × 75-mm polypropylene tube and neutralized with 1 ml of 2 M K_2CO_3. The homogenates were centrifuged at 3000 × *g* and 4°C for 15 min to obtain the supernatant fluid, which was stored at −80°C. Samples of fetal allantoic and amniotic fluids (10 ml) were centrifuged at 3000 × *g* at 4°C for 10 min; an aliquot of the supernatant fluid (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. Polyamines and amino acids (including S-adenosylmethionine) were analyzed using HPLC methods involving precolumn derivatization with *o*-phthaldialdehyde as described previously [18, 19]. The retention times of S-adenosylmethionine, methionine, and ornithine were 21.4, 35.5, and 44.1 min, respectively. Pyrroline-5-carboxylate (P5C) was determined through its reduction to proline [20], followed by HPLC analysis of proline [19]. Briefly, 0.5 ml of neutralized samples were loaded into an AG 1-X8 resin (Bio-Rad Laboratories, Hercules, CA) column (0.6 × 6.5 cm), followed sequentially by elution with 8 ml of water and 6 ml of 25 mM acetic acid. The column was finally eluted with 4 ml of 25 mM acetic acid, and the effluent solution (containing 99% P5C but no proline) was mixed with 1 ml of 50 mM sodium borohydride. The resultant solution (containing proline) was dried in a Model RC10.10 centrifugal evaporator (Jouan, Inc., Winchester, MA), and the residue was suspended in 0.3 ml of HPLC-water for HPLC analysis of proline [19].

Proline Transport in Placentae

Proline transport in pig placentae was determined using L-[U-^{14}C]proline as described previously for branched-chain amino acids [18]. Briefly, samples of placentae (~200 mg) were washed three times in oxygenated (95% O_2/5% CO_2, v/v) Krebs-Henseleit bicarbonate (KHB) buffer containing 20 mM Hepes (pH 7.4) and 5 mM glucose. Samples were then incubated at 37°C for 5 min in 1 ml of oxygenated KHB buffer consisting of 20 mM Hepes, 2 mM glutamate, 5 mM glucose, 0.5 or 2 mM proline, 0.05 μCi L-[U-^{14}C]proline, and 0.05 μCi [^{3}H]inulin (an extracellular marker). After the 5-min incubation, the tissues were rinsed thoroughly with fresh KHB buffer and then solubilized in 0.5 ml of Soluene 350. The solution was measured for ^{14}C and ^{3}H radioactivities using a dual-channel counting program in a Packard 1900 liquid scintillation counter (Meriden, CT). The specific activity of [^{14}C]proline in the medium was used to calculate proline uptake by placentae. Results from preliminary experiments established that proline uptake was linear over a 5-min period.

Proline and Arginine Degradation in Placentae

Proline degradation in placentae was quantified using L-[U-^{14}C]proline as described previously for pig enterocytes [21]. Briefly, samples of placentae (~200 mg) were preincubated at 37°C for 30 min in 2 ml of oxygenated (95% O_2/5% CO_2, v/v) KHB buffer and then incubated at 37°C for 2 h in 2 ml of oxygenated KHB containing 20 mM Hepes (pH 7.4), 5 mM glucose, 2 mM glutamate, 0.5 or 2 mM proline, and 0.5 μCi L-[U-^{14}C]proline. After a 2-h incubation at 37°C, 0.2 ml of Soluene 350 was injected through the rubber cap into suspended center-wells, and 0.2 ml of 1.5 M $HClO_4$ acid was injected into the incubation medium to liberate $^{14}CO_2$. After a 1-h incubation period, suspended wells were transferred to scintillation vials containing 15 ml of cocktail for measurement of $^{14}CO_2$ using a Packard liquid scintillation counter [21]. The neutralized medium was analyzed for [^{14}C]ornithine and other amino acids using HPLC and liquid scintillation spectrometry [21]. Arginine degradation in placentae was determined as described above for proline degradation except that 0.5 and 2 mM arginine plus 0.5 μCi L-[U-^{14}C]arginine were included instead of proline in the incubation medium.

Determination of Polyamine Synthesis in Placentae

Polyamine synthesis was determined in porcine placentae using L-[U-^{14}C]proline, L-[U-^{14}C]ornithine, and L-[U-^{14}C]arginine as described previously for ovine placentae [13]. Briefly, placental tissues (~500 mg) were rinsed twice with oxygenated (95% O_2/5% CO_2, v/v) Basal Eagle medium (BEM; Gibco RL, 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 one of the following: 0.5 or 2 mM L-proline plus 2 μCi L-[U-^{14}C]proline and 2 mM glutamate, 0.5 or 2 mM L-ornithine plus 2 μCi L-[U-^{14}C]ornithine, or 0.5 or 2 mM L-arginine plus 2 μCi L-[U-^{14}C]arginine. Blank incubations were run using medium containing all the above components but no tissues. The ^{14}C-labeled substrates were used to improve the sensitivity of detecting polyamine synthesis in placental tissue. Incubations were terminated by addition of 0.2 ml of 1.5 M $HClO_4$. The acidified tissues plus medium were analyzed for [^{14}C]putrescine, [^{14}C]spermidine, and [^{14}C]spermine by HPLC and liquid scintillation spectrometry [19]. 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 [21].

Measurement of Proline Oxidase Activity in Placentae and Fetal Fluids

Placentae (~200 mg) were homogenized in 2 ml of homogenization buffer (pH 7.2) containing 250 mM sucrose, 1 mM EDTA, 2.5 mM DTT, protease inhibitors (5 μg/ml of phenylmethylsulfonyl fluoride, 5 μg/ml of aprotinin, 5 μg/ml of chymostatin, 5 μg/ml of pepstatin A), and 50 mM potassium phosphate buffer with the use of a glass pestle. The homogenate was centrifuged at 600 × *g* and 4°C for 10 min, and the supernatant fluid was centrifuged at 12 000 × *g* and 4°C for 10 min. The resultant mitochondrial pellets were suspended in 1.5 ml of 50 mM potassium phosphate

buffer (pH 7.5), stored at −80°C, and used for enzyme assay within 3 days. Fetal allantoic and amniotic fluids were centrifuged at 3000 × *g* for 15 min, and the supernatant was used directly for enzyme assays. Proline oxidase activity was determined as described previously [19]. Briefly, the enzyme assay mixture (1.0 ml), which consisted of 15 mM proline, 20 μM ferricytochrome C, mitochondrial pellet (~0.5 and 1 mg of protein), and 50 mM potassium phosphate buffer (pH 7.5), was incubated at 37°C for 0, 15, or 30 min. The reaction was terminated by addition of 0.5 ml of 10% TCA, followed by addition of 0.1 ml of 100 mM *o*-aminobenzaldehyde. The mixture was allowed to stand at room temperature for 30 min before centrifugation at 600 × *g* for 5 min. Absorbance of the supernatant was measured at 440 nm. Blanks (0-min incubation) were subtracted from sample values before calculating the formation of P5C from proline based on the molar extinction coefficient of P5C (2.7×10^3 M^{-1} cm^{-1}).

Measurement of Arginase, Ornithine Aminotransferase, and P5C Dehydrogenase Activities in Placentae and Fetal Fluids

Placentae (~500 mg) were homogenized at 4°C in 2 ml of homogenization buffer (pH 7.4) containing 300 mM D-mannitol, 5 mM Hepes, 0.2 mM EDTA, 3 mM DTT, 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) with the use of a glass pestle. The homogenate was centrifuged at 600 × *g* for 10 min at 4°C, and the supernatant fluid was centrifuged at 12 000 × *g* for 15 min at 4°C. The resultant supernatant (cytosol) was used for assays of arginase I. The pellet was resuspended in 1.5 ml of fractionation buffer (300 mM D-mannitol, 5 mM Hepes, 5 mM EDTA, and 3 mM DTT; pH 7.4) and centrifuged at 600 × *g* for 4 min at 4°C. The resulting supernatant was centrifuged at 4000 × *g* for 10 min at 4°C, and the pellet (the mitochondrial fraction) was suspended in 0.5 ml of the homogenization buffer containing 0.5% Triton X-100 for assays of arginase II, ornithine aminotransferase (OAT), and P5C dehydrogenase. Fetal allantoic and amniotic fluids were centrifuged at 3000 × *g* for 15 min, and the supernatant was used directly for enzyme assays.

Activities of arginase I and II, OAT, and P5C dehydrogenase were determined as described previously [22–24]. The assay mixture (0.15 ml) for arginase I (a cytosolic enzyme) consisted of 50 mM Tris-HCl buffer (pH 7.5), 3 mM $MnCl_2$, 10 mM arginine, and cytosolic extracts (0.5 and 1 mg of protein). The enzyme and $MnCl_2$ mixture was preheated at 55°C before addition of arginine. After cooling to room temperature, the solution was incubated at 37°C for 0, 10, and 20 min. At the end of the predetermined period of incubations, the reaction was terminated by addition of 50 μl of 1.5 M $HClO_4$, and the neutralized solution was analyzed for ornithine by HPLC. The activity of arginase II (a mitochondrial enzyme) was determined as described for arginase I except that the enzyme and $MnCl_2$ mixture was not preheated at 55°C before addition of arginine, because such a procedure resulted in a substantial reduction of arginase II activity. The assay mixture (2.0 ml) for OAT contained 75 mM potassium phosphate buffer (pH 7.5), 20 mM ornithine, 0.45 mM pyridoxal phosphate, 5 mM *o*-aminobenzaldedhyde, 0 (blank) or 3.75 mM α-ketoglutarate, and mitochrondria (0.1 and 0.2 mg of protein). At the end of the 7.5- and 15-min incubation periods at 37°C, the colorimetric complex resulting from the reaction of P5C with *o*-aminobenzaldehyde was determined at 440 nm. The assay mixture (1.2 ml) for P5C dehydrogenase contained 4 mM NAD^+, 2 mM DL-P5C, mitochondria (0.5 and 1 mg), and 100 mM potassium phosphate buffer (pH 7.5). The increase in NADH over a 5-min period after addition of 0 or 2 mM DL-P5C was measured at 37°C by a fluorometer (excitation wavelength, 340 nm; emission wavelength, 460 nm). Assay mixtures without P5C was used as blanks.

Determination of ODC Activity in Placentae

The ODC activity in porcine placentae was measured using L-[1-^{14}C]ornithine as described for ovine placentae [13]. 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 DTT, 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; the assay mixture (0.5 ml) consisted of 2 mM L-[1-^{14}C]ornithine (2500 dpm/nmol), 0.2 mM pyridoxal-5-phosphate, 0.2 mM EDTA, 0.5 mM DTT, enzyme preparations (equivalent to ~10 and 20 mg of tissue), and 50 mM sodium phosphate buffer (pH 7.2). Radioactivity blanks containing [1-^{14}C]ornithine but no enzyme preparations were included. After incubation at 37°C for 1 h, $^{14}CO_2$ was collected in 0.2 ml of NCS-II, and its radioactivity was measured in a liquid scintillation counter. 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.

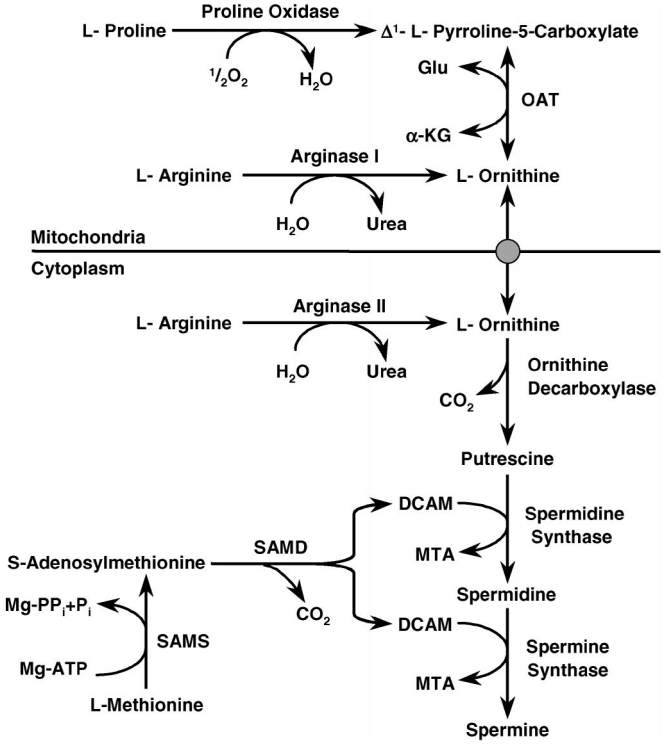

FIG. 1. Polyamine synthesis in animals. Arginine and proline are potentially major substrates for the production of ornithine (the immediate precursor for putrescine, spermine, and spermine synthesis) in mammalian cells. Results of the present study indicate that arginase I and II are absent from the porcine placenta, in which proline is the major amino acid for polyamine synthesis throughout pregnancy. DCMA, Decarboxylated 5-adenosylmethionine; Glu, glutamate; α-KG, α-ketoglutarate; MTA, methylthioadenosine; PPi, inorganic pyrophosphate; SAMD, S-adenosylmethionine decarboxylase.

Calculations and Statistical Analyses

Concentrations of polyamines and amino acids in placentae, allantoic fluid, and amniotic fluid were calculated based on the recovery rates (93–98%) of these substances from the samples as described previously [13]. Concentrations of total polyamines were a mathematical sum of putrescine, spermidine, and spermine. Data were subject to least-squares analysis of variance and correlation analysis [25] using the PROC GLM and PROC CORR procedures of the Statistical Analysis System software (SAS Institute, Cary, NC). Probability values of 0.05 or less were taken to indicate statistical significance. Data are presented as the mean ± pooled SEM.

RESULTS

Placental Growth, Fetal Growth, and Fluid Volume

Rate of placental growth (expressed as percentage increase) was highest ($P < 0.01$) between Days 20 and 60 and declined ($P < 0.01$) thereafter (Table 1). Placental weight of gilts increased ($P < 0.01$) 267-, 3.1-, and 1.3-fold between Days 20 and 40, Days 40 and 60, and Days 60 and 110 of gestation, respectively (Table 1). Placental growth was nearly completed by Day 60 of gestation. Fetal weights increased ($P < 0.01$) with gestational age (Table 1). The weight gain (130 g) of the fetal pig was small between Days 20 and 60 of gestation but increased rapidly thereafter (1045 g between Days 60 and 110 of gestation).

TABLE 1. Porcine fetal weights, volumes of allantoic and amniotic fluids, and placental weights.[a]

	Day of gestation									Pooled
	20	30	35	40	45	50	60	90	110	SEM
Fetal weight (g)	0.063[j]	1.7[i]	4.5[h]	10.8[g]	22.6[f]	48.3[e]	130[d]	596[c]	1176[b]	8.6
ALF (ml)	4.1[i]	227[c]	107[f]	74.1[g]	132[e]	186[d]	347[b]	82.7[g]	55.8[h]	5.1
AMF (ml)	ND	2.2[h]	6.2[g]	12.5[f]	31.7[e]	46.2[d]	119[b]	127[b]	81.4[c]	2.3
Placental weight (g)	0.22[j]	33[i]	46[h]	59[g]	81[f]	125[e]	182[d]	208[c]	237[b]	3.0

[a] Data are means for six gilts on each day of gestation. ALF, Allantoic fluid; AMF, amniotic fluid; ND, not determined.
[b–j] Means with different superscript letters within the same row are different ($P < 0.01$).

Indeed, the weight gain (589 g) of the fetal pig between Days 90 and 110 of gestation was similar to that (596 g) between Days 20 and 90 of gestation. The volume of allantoic fluid increased ($P < 0.01$) 55-fold between Days 20 and 30 of gestation, declined ($P < 0.01$) progressively between Days 30 and 40 of gestation, and then increased ($P < 0.01$) fivefold between Days 40 and 60 of gestation. The volume of allantoic fluid decreased ($P < 0.01$) progressively between Days 60 and 110 of gestation. The volume of amniotic fluid increased ($P < 0.01$) progressively between Days 30 and 60 of gestation, remained at the elevated level between Days 60 and 90 of gestation, and then declined ($P < 0.01$) thereafter.

Concentrations of Proline, Ornithine, and P5C in Placentae and Fetal Fluids

Concentrations of proline, ornithine, and P5C in placentae increased ($P < 0.05$) by 51%, 46%, and 79%, respectively, between Days 20 and 40 of gestation, remained constant between Days 40 and 60, and declined ($P < 0.05$) thereafter (Table 2). In allantoic fluid, concentrations of proline increased ($P < 0.01$) 130% between Days 20 and 30 of gestation, declined ($P < 0.01$) progressively between Days 30 and 60, and then increased ($P < 0.01$) progressively between Days 60 and 110. A very different pattern of developmental change was observed for ornithine and P5C in allantoic fluid (Table 2). Concentrations of ornithine and P5C in allantoic fluid increased ($P < 0.01$) 34- and 8-fold, respectively, between Days 20 and 40 of gestation, declined ($P < 0.01$) progressively between Days 40 and 90, and remained at the reduced level through Day 110. Allantoic fluid concentrations of proline were greater ($P < 0.01$) than those of ornithine during early (Days 20–30) and late (Days 90–110) gestation, whereas the opposite was determined between Days 35 and 60. In amniotic fluid, concentrations of proline increased ($P < 0.01$) twofold between Days 30 and 45 of gestation, remained constant between Days 45 and 60, and declined ($P < 0.01$) thereafter. In contrast, amniotic fluid concentrations of ornithine or P5C increased ($P < 0.01$) progressively between Days 30 and 40 of gestation and then decreased ($P < 0.01$) progressively between Days 40 and 90 of gestation.

Concentrations of Methionine and S-Adenosylmethionine in Placentae

Concentrations of methionine were 10- to 14-fold greater than those of S-adenosylmethionine in porcine placentae throughout pregnancy (Table 3). Concentrations of methionine increased ($P < 0.01$) by 29% between Days 20 and 40 of gestation, remained at the elevated level between Days 40 and 60, and declined ($P < 0.01$) thereafter. Concentrations of S-adenosylmethionine doubled ($P < 0.01$) between Days 20 and 40 of gestation, declined ($P < 0.01$) progressively between Days 40 and 90, and remained at the reduced level through Day 110.

Proline Transport and Degradation in Placentae

Increasing extracellular concentrations of proline from 0.5 to 2 mM increased ($P < 0.01$) the rate of protein transport by porcine placentae on all days of gestation (Table 4). Proline transport increased ($P < 0.05$) threefold between Days 20 and 40 of gestation, decreased ($P < 0.01$) progressively between Days 40 and 50, remained constant between Days 50 and 60, and declined ($P < 0.01$) thereafter. Relatively large amounts of P5C and ornithine were produced from proline in porcine placentae (Table 5). Formation of P5C and ornithine from proline increased ($P < 0.05$) approximately 2.5-fold between Days 20 and 40 of gestation and decreased ($P < 0.01$) thereafter. No production of [^{14}C]glutamate, [^{14}C]aspartate, [^{14}C]alanine, or $^{14}CO_2$ from

TABLE 2. Concentrations of proline, ornithine, and P5C in porcine placentae and fetal fluids.[a]

Amino acid	Day of gestation									Pooled
	20	30	35	40	45	50	60	90	110	SEM
Placentae (nmol/g tissue)										
Proline	335[e]	372[d]	459[b]	477[b]	473[b]	466[b]	461[b]	410[c]	417[c]	9.5
Ornithine	155[e]	179[d]	202[c]	228[b]	226[b]	222[b]	224[b]	178[d]	173[d]	6.1
P5C	8.8[e]	11.5[d]	14.0[c]	19.2[b]	18.6[b]	14.3[c]	11.8[d]	9.0[e]	8.3[e]	0.53
Allantoic fluid (nmol/ml)										
Proline	120[f]	279[c]	206[d]	157[e]	169[e]	130[f]	58[g]	123[f]	364[b]	5.7
Ornithine	77[g]	134[f]	1135[d]	2648[b]	1602[c]	1067[d]	671[e]	83[g]	86[g]	49
P5C	3.4[g]	4.6[f]	18.5[d]	37.1[b]	26.5[c]	18.3[d]	13.4[e]	4.8[f]	5.1[f]	0.67
Amniotic fluid (nmol/ml)										
Proline	ND	92[d]	94[d]	97[d]	227[b]	219[b]	232[b]	138[c]	133[c]	3.5
Ornithine	ND	74[d]	124[c]	193[b]	118[c]	80[d]	53[e]	37[f]	34[f]	2.7
P5C	ND	6.3[e]	10.3[d]	19.0[b]	13.8[c]	10.8[d]	9.8[d]	6.6[e]	6.9[e]	0.44

[a] Data are means for six gilts on each day of gestation. ND, not determined.
[b–g] Means with different superscript letters within the same row are different ($P < 0.01$).

TABLE 3. Concentrations (nmol/g tissue) of methionine and S-adenosylmethionine in porcine placentae.[a]

Amino acid	Day of gestation									Pooled SEM
	20	30	35	40	45	50	60	90	110	
Methionine	215[e]	237[d]	271[b]	277[b]	274[b]	280[b]	272[b]	242[c]	235[c]	6.2
S-adenosylmethionine	13.8[f]	17.1[e]	20.4[d]	26.8[b]	23.5[c]	20.2[d]	19.8[d]	16.7[e]	16.2[e]	0.55

[a] Data are means for six gilts on each day of gestation.
[b–f] Means with different superscript letters within the same row are different ($P < 0.01$).

L-[U-^{14}C]proline was detectable in porcine placentae between Days 20 and 110 of gestation.

Polyamine Synthesis in Placentae

Synthesis of polyamines from proline or ornithine was detected in porcine placentae between Days 20 and 110 of gestation (Tables 6 and 7). Rates of placental polyamine synthesis from [^{14}C]proline or [^{14}C]ornithine increased ($P < 0.01$) by 150–160% between Days 20 and 40 of gestation and declined ($P < 0.05$) thereafter. Spermidine and spermine were the major polyamines synthesized from proline or ornithine at all days of gestation. No production of [^{14}C]ornithine, [^{14}C]glutamate, [^{14}C]putrescine, [^{14}C]spermidine, or [^{14}C]spermine from [U-^{14}C]arginine was detectable in porcine placentae between Days 20 and 110 of gestation. Between Days 20 and 40 of gestation, the synthesis of total polyamines was correlated positively with placental weight ($r = 0.916$, $P < 0.01$). Maximum placental polyamine synthesis occurred in advance of major changes in fetal growth.

Activities of Ornithine Metabolic Enzymes

Arginase I, arginase II, and P5C dehydrogenase activities were not detected in porcine placentae, allantoic fluid, or amniotic fluid between Days 20 and 110 of gestation. However, proline oxidase, OAT, and ODC activities were present in porcine placentae throughout pregnancy (Table 8). The activities of these three enzymes increased ($P < 0.01$) by 132%, 148% and 240%, respectively, between Days 20 and 40 of gestation and declined ($P < 0.01$) thereafter. At all days of gestation, OAT activity was higher ($P < 0.01$) than proline oxidase activity, whereas ODC activity was the lowest ($P < 0.01$). Both ODC and proline oxidase activities showed positive correlations with rates of polyamine synthesis from proline in porcine placentae ($r = 0.938$ and 0.925, respectively; $P < 0.01$). In allantoic fluid, proline oxidase and OAT activities increased ($P < 0.01$) 4- and 10-fold, respectively, between Days 20 and 40 of gestation and declined ($P < 0.01$) thereafter (Table 9). In amniotic fluid, the activities of both enzymes increased ($P < 0.01$) approximately threefold between Days 20 and 40 of gestation and declined ($P < 0.01$) thereafter (Table 9). Except at Days 40 and 45 of gestation, allantoic fluid activity of proline oxidase was similar to that of OAT at all other days of gestation ($P > 0.05$). In contrast, amniotic fluid activity of proline oxidase was higher ($P < 0.01$) than that of OAT at all days of gestation. No ODC activity was detected in porcine allantoic or amniotic fluid between Days 20 and 110 of gestation.

Polyamine Concentrations in Placentae and Fetal Fluids

Porcine placentae contained high levels of putrescine, spermidine, and spermine at all days of gestation (Table 10). Placental concentrations of spermidine were similar to ($P > 0.05$) and lower than ($P < 0.01$) those of spermine between Days 20 and 50 and between Days 60 and 110 of gestation, respectively. Placental concentrations of total polyamines increased ($P < 0.01$) 160% between Days 20 and 40 of gestation and declined ($P < 0.01$) between Days 40 and 90. Positive correlations were found between concentrations of total polyamines and polyamine synthesis in porcine placentae ($r = 0.946$, $P < 0.01$). Concentrations of polyamines in allantoic fluid were higher than ($P < 0.01$) and similar to ($P > 0.05$) those in amniotic fluid at Days 30–60 and Days 90–110 of gestation, respectively (Table 10). In both fluids, concentrations of polyamines increased ($P < 0.01$) progressively between Days 30 and 40 of gestation, declined ($P < 0.01$) progressively between Days 40 and 60, and then remained at the reduced level through Day 110. During late gestation (Days 90–110), spermine was the most abundant polyamine in fetal fluids, followed in decreasing order by spermidine and putrescine. Concentrations of total polyamines were positively correlated with placental weights between Days 20 and 40 of gestation ($r = 0.963$, $P < 0.01$).

DISCUSSION

To our knowledge, this is the first report concerning polyamine synthesis and concentrations in the porcine placenta during pregnancy. The present study had three major findings: First, proline oxidase and OAT, but not arginase, were present for the production of P5C and ornithine in the porcine placenta and fetal fluids. Second, the activities of proline oxidase, OAT, and ODC as well as polyamine synthesis and concentrations of polyamines were highest in the porcine placenta at Day 40 of gestation. Third, concentrations of polyamines were positively correlated with proline oxidase activity and polyamine synthesis in the placenta.

TABLE 4. Proline transport (nmol g tissue^{-1} min^{-1}) in porcine placentae.[a]

Medium [proline] (mM)	Day of gestation									Pooled SEM
	20	30	35	40	45	50	60	90	110	
0.5	6.2[f]	8.5[e]	13.9[c]	18.3[b]	14.2[c]	11.2[d]	11.6[d]	8.7[e]	8.1[e]	0.21
2	16.8[f]	22.4[e]	39.6[c]	51.0[b]	39.2[c]	31.1[d]	32.0[d]	21.9[e]	22.8[e]	0.65

[a] Data are means for six gilts on each day of gestation. On each day of gestation, the transport rate of proline at 2 mM was higher ($P < 0.01$) than that at 0.5 mM.
[b–f] Means with different superscript letters within the same row are different ($P < 0.01$).

TABLE 5. Proline degradation (nmol mg tissue^{-1} h^{-1}) in porcine placentae.[a]

Product	Day of gestation									Pooled SEM
	20	30	35	40	45	50	60	90	110	
0.5 mM proline										
Net P5C	0.46[f]	0.67[e]	0.89[c]	1.20[b]	0.88[c]	0.77[d]	0.76[d]	0.44[f]	0.42[f]	0.03
Ornithine	0.57[f]	0.78[e]	1.05[c]	1.32[b]	1.08[c]	0.89[d]	0.90[d]	0.54[f]	0.53[f]	0.02
Total P5C	1.03[f]	1.45[e]	1.94[c]	2.51[b]	1.97[c]	1.66[d]	1.67[d]	0.98[f]	0.95[f]	0.04
2 mM proline										
Net P5C	1.02[f]	1.39[e]	1.91[c]	2.54[b]	2.04[c]	1.71[d]	1.69[d]	1.06[f]	0.98[f]	0.07
Ornithine	1.28[f]	1.50[e]	2.21[c]	2.91[b]	2.24[c]	1.86[d]	1.84[d]	1.20[f]	1.16[f]	0.08
Total P5C	2.31[f]	2.89[e]	4.12[c]	5.44[b]	4.28[c]	3.57[d]	3.54[d]	2.26[f]	2.14[f]	0.12

[a] Data are means for six gilts on each day of gestation. On each day of gestation, the transport rate of proline at 2 mM was higher ($P < 0.01$) than that at 0.5 mM.
[b–f] Means with different superscript letters within the same row are different ($P < 0.01$).

These results indicate that proline, but not arginine, is the major amino acid for the synthesis of putrescine, spermidine, and spermine in the porcine placenta.

Despite a previous report that dealt with polyamine synthesis from arginine in ovine placentae [13], little is known about changes in placental polyamine synthesis associated with conceptus development in pigs. Arginine is considered to be a major substrate for ornithine production via arginase in animal tissues [11, 26]. However, the present results clearly indicate that neither arginase activity nor conversion of arginine into ornithine was detected in porcine placentae, allantoic fluid, or amniotic fluid. These results are in sharp contrast to those that we recently reported for ovine placentae and fetal fluids, in which a relatively high arginase activity plays an important role in the production of ornithine and, thus, polyamines from arginine [13]. The lack of arginine degradation via arginase in the porcine placenta and fetal fluids maximizes placental transfer of arginine from maternal and fetal blood. This finding aids in explaining the unusual abundance (4–5 mM) of arginine in porcine allantoic fluid during early gestation [15].

Proline oxidase is the only known enzyme for initiating proline catabolism in animal cells [27]. Results of the present study demonstrate that proline was extensively catabolized via proline oxidase and OAT to yield P5C and, subsequently, ornithine in the porcine placenta. In this tissue, concentrations of glutamate were three- to fivefold greater than those of ornithine [18], and concentrations of P5C were two- to threefold greater than those of α-ketoglutarate (3.5–6 nmol/g tissue; unpublished data). Thus, the equilibrium of the placental OAT reaction favors the formation of ornithine. The proline-derived ornithine was readily used for the synthesis of putrescine, spermidine, and spermine via ODC, spermidine synthase, and spermine synthase, respectively (Table 6). These results suggest that proline oxidase is coupled efficiently with OAT in placental mitochondria and that the proline-derived ornithine readily enters the cytoplasm to serve as the substrate for ODC. A similar finding has been reported for the porcine small intestine [19]. Thus, either extracellular or mitochondrially generated ornithine is used for the synthesis of polyamines in the porcine tissues. Interestingly, P5C dehydrogenase was not detectable in porcine placentae between Days 20 and 110 of gestation in the present study. The lack of conversion of P5C to glutamate limits the irreversible loss of P5C while maximizing the formation of ornithine from P5C via OAT. This mechanism helps to conserve proline for polyamine production, because P5C can be recycled into proline by the cytosolic P5C reductase, a widespread enzyme in animals [27], including fetal pigs [28]. Notably, the rate of placental polyamine synthesis from proline increased with increasing extracellular concentrations of proline from 0.5 to 2 mM (Table 6) for the provision of P5C at all days of gestation, indicating a high capacity for this biosynthetic pathway. Additionally, our results suggest that proline oxidase, which catalyzes the first and irreversible reaction in proline catabolism, is a rate-controlling enzyme in placental synthesis of polyamines.

Another novel finding of the present study is that porcine allantoic and amniotic fluids contain both proline oxidase and OAT (Table 8). These two proteins may be secreted from allantoic and amniotic membranes of the placenta as well as the uterus. In the fetal fluids, concentrations of glutamate were approximately 50% lower than those of ornithine [15], but concentrations of P5C were 3- to 10-fold greater than those of α-ketoglutarate (2.3–3.5 nmol/ml; un-

TABLE 6. Polyamine synthesis (pmol g tissue^{-1} h^{-1}) from proline in porcine placentae.[a]

Polyamine	Day of gestation									Pooled SEM
	20	30	35	40	45	50	60	90	110	
0.5 mM proline										
Putrescine	145[f]	188[e]	269[c]	294[b]	261[c]	227[d]	215[d]	160[f]	153[f]	12
Spermidine	249[f]	304[e]	446[c]	639[b]	437[c]	360[d]	352[d]	258[f]	250[f]	19
Spermine	220[f]	286[e]	401[c]	598[b]	388[c]	344[d]	318[d]	224[f]	217[f]	16
Total	616[f]	778[e]	1115[c]	1532[b]	1087[c]	930[d]	887[d]	640[f]	620[f]	43
2 mM proline										
Putrescine	322[f]	471[e]	655[c]	751[b]	662[c]	572[d]	559[d]	366[f]	351[f]	31
Spermidine	580[f]	733[e]	1104[c]	1561[b]	1215[c]	953[d]	921[d]	632[f]	624[f]	56
Spermine	517[f]	675[e]	987[c]	1395[b]	951[c]	802[d]	783[d]	561[f]	535[f]	53
Total	1421[f]	1879[e]	2746[c]	3706[b]	2830[c]	2326[d]	2265[d]	1561[f]	1510[f]	97

[a] Data are means for six gilts on each day of gestation. On each day of gestation, rate of polyamine synthesis at 2 mM ornithine was higher ($P < 0.01$) than that at 0.5 mM.
[b–f] Means with different superscript letters within the same row are different ($P < 0.01$).

TABLE 7. Polyamine synthesis (pmol g tissue^{-1} 3 h^{-1}) from ornithine in porcine placentae.[a]

Polyamine	Day of gestation									Pooled SEM
	20	30	35	40	45	50	60	90	110	
0.5 mM ornithine										
Putrescine	396[f]	529[e]	790[c]	941[b]	832[c]	699[d]	672[d]	426[f]	413[f]	35
Spermidine	690[f]	878[e]	1298[c]	2026[b]	1377[c]	1112[d]	1064[d]	725[f]	718[f]	62
Spermine	627[f]	841[e]	1187[c]	1967[b]	1254[c]	1094[d]	973[d]	679[f]	651[f]	66
Total	1714[f]	2250[e]	3274[c]	4936[b]	3401[c]	2906[d]	2710[d]	1828[f]	1781[f]	147
2 mM ornithine										
Putrescine	914[f]	1361[e]	1966[c]	2573[b]	2108[c]	1807[d]	1724[d]	1057[f]	984[f]	92
Spermidine	1678[f]	2148[e]	3450[c]	5217[b]	3706[c]	2954[d]	2877[d]	1793[f]	1816[f]	174
Spermine	1494[f]	1964[e]	3040[c]	4862[b]	3153[c]	2531[d]	2471[d]	1648[f]	1571[f]	166
Total	4088[f]	5472[e]	8454[c]	12 653[b]	8969[c]	8293[d]	7071[d]	4450[f]	4372[f]	503

[a] Data are means for six gilts on each day of gestation. On each day of gestation, the transport rate of polyamine synthesis at 2 mM was higher ($P < 0.01$) than that at 0.5 mM ornithine.
[b–f] Means with different superscript letters within the same row are different ($P < 0.01$).

published data). Thus, as in the placenta, the equilibrium of the OAT reaction favors the generation of ornithine from proline. Importantly, the production of P5C and ornithine in the fetal fluids represents a hitherto-unrecognized pathway for extracellular metabolism of proline in mammals. The high activity of proline oxidase would actively catabolize proline to generate P5C in porcine allantoic and amniotic fluids, thereby explaining the relatively low concentrations of proline [15, 29]. Indeed, we have reported previously that proline is the second least abundant nonessential amino acid in porcine allantoic and amniotic fluids throughout pregnancy [29]. Interestingly, concentrations of P5C in porcine fetal fluids (Table 2) are approximately 10- to 20-fold higher than those in the plasma of fetal pigs (~1.0–1.4 μM), pregnant gilts (~0.6 μM; unpublished data), and adult humans (~0.4 μM) [30], suggesting an important role for P5C in conceptus development. In this regard, it is noteworthy that P5C regulates cell redox state, proliferation, and differentiation in cultured cell lines [30, 31]. Whether P5C is a signaling molecule that regulates placental metabolism and function remains to be determined. Nonetheless, our discovery of proline oxidase in the conceptus opens a new avenue for future studies regarding the role of proline in placental and fetal development.

Although early anatomical studies suggested that the allantoic sac served as a reservoir for fetal wastes, it is now clear that allantoic fluid nutrients can be absorbed by the allantoic epithelium into the fetoplacental circulation and used by fetal tissues [16]. In addition, amniotic fluid is actively swallowed by the fetus and is a significant source of nutrients for the gut and other fetal tissues [32]. Allantoic and amniotic fluids derive, in part, from secretions and transport of water and solutes across the placenta and endometrium [16, 32]. Consistent with this notion, the pattern of changes in concentrations of total polyamines in allantoic and amniotic fluids between Days 20 and 110 of gestation (Table 10) closely matched the rates of placental polyamine synthesis from proline (Table 6) and ornithine (Table 7). With an increase in the transport capacity of fetal enterocytes [33], amniotic fluid taken up by the fetus provides a significant source of polyamines for supporting the proliferation and differentiation of intestinal epithelial cells. The nutritional significance of amniotic fluid is 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 pigs [34].

Transport of proline across the plasma membrane represents the first step for its utilization by the placenta. Our results show that the rates of placental transport of proline (Table 4) were the highest at Day 40 of gestation. The active transport of proline ensures an adequate supply of intracellular proline for its metabolism by placentae. Several results are notable: First, the activities of proline oxidase, OAT, and ODC were maximal at Day 40 of gestation, which would maximize the production of ornithine and, subsequently, of all polyamines from proline in porcine placentae. Second, concentrations of ornithine in allantoic fluid were highest at Day 40 of gestation (Table 2), and the dynamic exchange of nutrients between this fluid and the placenta provides an additional source of ornithine for enhancing polyamine synthesis in the placenta. Third, placental production of glutamate (a major substrate for OAT) and glutamine (a stimulator of ODC activity) from branched-chain amino acid catabolism [18] as well as their concentrations in porcine placentae [18] and allantoic fluid [15, 29] were highest at Day 40 of gestation. Fourth, placental concentrations of methionine and its metabolite, S-adenosylmethionine, which provides a methyl group for the syn-

TABLE 8. Proline oxidase, OAT, and ODC activities in pig placentae.[a]

Enzyme	Day of gestation									Pooled SEM
	20	30	35	40	45	50	60	90	110	
Proline oxidase (μmol g tissue^{-1} min^{-1}	0.44[f]	0.63[e]	0.83[c]	1.02[b]	0.85[c]	0.74[d]	0.72[d]	0.50[f]	0.47[f]	0.02
OAT (μmol g tissue^{-1} min^{-1})	0.93[f]	1.17[e]	1.88[c]	2.31[b]	1.97[c]	1.65[d]	1.53[d]	0.91[f]	0.89[f]	0.03
ODC (nmol g tissue^{-1} min^{-1})	0.10[f]	0.15[e]	0.26[c]	0.34[b]	0.27[c]	0.21[d]	0.20[d]	0.11[f]	0.11[f]	0.03

[a] Data are means for six gilts per day of gestation.
[b–f] Means with different superscript letters within the same row are different ($P < 0.01$).

TABLE 9. Proline oxidase and OAT activities (nmol ml^{-1} min^{-1}) in porcine allantoic and amniotic fluids.[a]

Enzyme	Day of gestation									Pooled SEM
	20	30	35	40	45	50	60	90	110	
Allantoic fluid										
Proline oxidase	21[f]	32[e]	81[d]	187[b]	135[c]	90[d]	83[d]	35[e]	38[e]	5.2
OAT	15[f]	24[e]	105[d]	242[b]	188[c]	96[d]	92[d]	27[e]	29[e]	6.8
Amniotic fluid										
Proline oxidase	ND	45[e]	76[d]	136[b]	103[c]	82[d]	75[d]	51[e]	48[e]	5.7
OAT	ND	15[e]	23[d]	42[b]	31[c]	20[d]	21[d]	14[e]	15[e]	1.0

[a] Data are means for six gilts per day of gestation. ND, Not determined.
[b–f] Means with different superscript letters within the same row are different ($P < 0.01$).

thesis of spermidine and spermine from putrescine (Fig. 1), were highest during early pregnancy (Table 3). Collectively, our results indicate metabolic coordination among several integrated pathways that support maximal polyamine synthesis in the porcine placenta at Day 40 of gestation (Tables 6 and 7), when placental growth and morphological changes are most rapid [3]. Similarly, polyamine synthesis is highest in the ovine placenta during early gestation (Day 60) [13]. This common phenomenon from two divergent mammalian species supports the view that polyamines play a crucial role in promoting conceptus development [7]. Thus, modulation of the polyamine-synthetic pathways may provide a novel and useful method to regulate fetal growth and survival.

Based on the water content (~82%) of porcine placentae between Days 20 and 110 of gestation [35], concentrations of total polyamines in this tissue were estimated to be 220–550 μM. These values are substantially higher than concentrations of polyamines in maternal or fetal plasma (4–6 μM; unpublished data), indicating an abundance of polyamines in the porcine placenta, as we have reported previously for the ovine placenta [13]. Thus, polyamines are substantially concentrated in mammalian placentae, which is consistent with their crucial role in placental growth [7]. Indeed, in porcine placentae, polyamines are more abundant than most essential amino acids [14], and polyamine concentrations were highly correlated with placental growth in the first half of pregnancy (Tables 1 and 10).

The present findings raise important questions regarding the physiologic significance of polyamine synthesis in fetoplacental nutrition and development. In this regard, it is noteworthy that maternal dietary protein deficiency (0.5% protein) decreased the activities of proline oxidase and OAT in porcine placentae, allantoic fluid, and amniotic fluid by 70–80% at Day 40 of gestation (unpublished data); concentrations of proline and ornithine in porcine fetal plasma, placentae, and allantoic fluid [36]; as well as placental and fetal growth [37]. In addition, marked changes were found in placental tissue growth and vascularity during pregnancy [38]. In both pigs [8] and humans [39], low birth weight is a major factor contributing to high neonatal morbidity and mortality and is associated with the development of chronic disease (e.g., diabetes, hypertension, and coronary heart disease) later in life [40–42]. New knowledge about placental synthesis of polyamines may have important implications for preventing both intrauterine growth retardation and fetal origins of adult-onset diseases.

In conclusion, the present results indicate that polyamine synthesis and concentrations were highest in porcine placenta at Day 40 of gestation. Importantly, metabolic coordination occurs among the several integrated pathways that support high rates of polyamine synthesis in the placenta during early pregnancy. Our discovery of the novel pathway for the synthesis of polyamines from proline via proline oxidase in the porcine conceptus provides a new framework for future studies to define the roles of amino acids in fetoplacental growth and development.

ACKNOWLEDGMENTS

We thank research personnel in our laboratories for technical assistance, Kenton Lillie for assistance with animal husbandry, Dr. Darrell A. Knabe for helpful discussion, and Frances Mutscher for office support.

TABLE 10. Concentrations of polyamines in porcine placentae and fetal fluids.[a]

Polyamine	Day of gestation									Pooled SEM
	20	30	35	40	45	50	60	90	110	
Placentae (nmol/g tissue)										
Putrescine	41[e]	56[d]	72[c]	89[b]	74[c]	62[d]	57[d]	43[e]	45[e]	2.8
Spermidine	66[e]	109[d]	134[c]	174[b]	138[c]	115[d]	110[d]	53[f]	50[f]	3.5
Spermine	68[f]	117[d]	157[c]	198[b]	162[c]	131[d]	127[d]	87[e]	92[e]	4.0
Total	179[e]	282[d]	361[c]	464[b]	375[c]	308[d]	295[d]	184[e]	189[e]	9.2
Allantoic fluid (nmol/ml)										
Putrescine	0.64[f]	1.58[e]	3.02[c]	3.85[b]	3.07[c]	2.53[d]	1.72[e]	0.61[f]	0.57[f]	0.19
Spermidine	1.03[f]	2.27[e]	3.70[c]	4.61[b]	3.85[c]	3.19[d]	2.11[e]	0.93[f]	0.88[f]	0.24
Spermine	1.14[f]	2.40[e]	3.98[c]	4.83[b]	4.12[c]	3.06[d]	2.65[e]	1.27[f]	1.21[f]	0.27
Total	2.81[f]	6.25[e]	10.6[c]	13.4[b]	11.0[c]	8.72[d]	6.53[e]	2.89[f]	2.73[f]	0.55
Amniotic fluid (nmol/ml)										
Putrescine	ND	0.42[f]	0.76[d]	1.33[b]	1.05[c]	0.70[de]	0.62[e]	0.36[f]	0.38[f]	0.12
Spermidine	ND	0.68[f]	0.95[de]	1.99[b]	1.41[c]	1.13[d]	0.89[e]	0.65[f]	0.62[f]	0.18
Spermine	ND	0.60[f]	0.81[e]	1.73[b]	1.33[c]	1.02[d]	0.84[e]	1.29[c]	1.68[b]	0.22
Total	ND	1.73[g]	2.54[de]	5.05[b]	3.80[c]	2.92[d]	2.37[ef]	2.25[f]	2.74[d]	0.41

[a] Data are means for six gilts on each day of gestation. ND, Not determined.
[b–g] Means with different superscript letters within the same row are different ($P < 0.01$).

REFERENCES

1. Reynolds LP, Redmer DA. Angiogenesis in the placenta. Biol Reprod 2001; 64:1033–1040.
2. Ford SP. Control of blood flow to the gravid uterus of domestic livestock species. J Anim Sci 1995; 73:1852–1860.
3. Knight JW, Bazer FW, Thatcher WW, Frank DE, Wallace HD. Conceptus development in intact and unilaterally hysterectomized-ovariectomized gilts: interrelationships among hormonal status, placental development, fetal fluids and fetal growth. J Anim Sci 1977; 44:620–637.
4. Reynolds LP, Redmer DA. Uteroplacental vascular development and placental function. J Anim Sci 1995; 73:1839–1851.
5. Wilson ME. Role of placental function in mediating conceptus growth and survival. J Anim Sci 2002; 80(suppl 2):E195–E201.
6. Geisert RD, Schmitt RAM. Early embryonic survival in the pig: can it be improved? J Anim Sci 2002; 80(suppl 1):E54–E65.
7. Wu G, Bazer FW, Cudd TA, Meininger CJ, Spencer TE. Maternal nutrition and fetal development. J Nutr 2004; 134:2169–2172.
8. Pond WG, Maner JH. Swine Production and Nutrition. Westport, CT: AVI Publishing Company; 1984.
9. Igarashi K, Kashiwagi K. Polyamines: mysterious modulators of cellular functions. Biochem Biophys Res Commun 2000; 271:559–564.
10. Chattopadhyay MK, Tabor CW, Tabor H. Polyamines protect *Escherichia coli* cells from the toxic effect of oxygen. Proc Natl Acad Sci U S A 2003; 100:2261–2265.
11. Wu G, Morris SM Jr. Arginine metabolism: nitric oxide and beyond. Biochem J 1998; 336:1–17.
12. Tabor CW, Tabor H. Polyamines. Annu Rev Biochem 1984; 53:749–790.
13. Kwon H, Wu G, Bazer FW, Spencer TE. Developmental changes in polyamine levels and synthesis in the ovine conceptus. Biol Reprod 2003; 69:1626–1634.
14. Wu G, Pond WG, Flynn SP, Ott TL, Bazer FW. Maternal dietary protein deficiency decreases nitric oxide synthase and ornithine decarboxylase activities in placenta and endometrium of pigs. J Nutr 1998; 128:2395–2402.
15. Wu G, Bazer FW, Tuo W, Flynn NE. Unusual abundance of arginine and ornithine in porcine allantoic fluid. Biol Reprod 1996; 54:1261–1265.
16. Bazer FW. Allantoic fluid: regulation of volume and composition. In: Brace RA, Ross MG, Robillard JE (eds.), Reproductive and Perinatal Medicine, Vol. 11: Fetal and Neonatal Body Fluids. Ithaca, NY: Perinatology Press; 1989:135–155.
17. National Research Council. Nutrient Requirements of Swine. Washington, DC: National Academy Press; 1998.
18. Self JT, Spencer TE, Johnson GA, Hu J, Bazer FW, Wu G. Glutamine synthesis in the developing porcine placenta. Biol Reprod 2004; 70: 1444–1451.
19. Wu G, Flynn NE, Knabe DA. Enhanced intestinal synthesis of polyamines from proline in cortisol-treated piglets. Am J Physiol Endocrinol Metab 2000; 279:E395–E402.
20. Mezl VA, Knox WE. Properties and analysis of a stable derivative of pyrroline-5-carboxylic acid for use in metabolic studies. Anal Biochem 1976; 74:430–440.
21. Wu G. Synthesis of citrulline and arginine from proline in enterocytes of postnatal pigs. Am J Physiol Gastrointest Liver Physiol 1997; 272: G1382–G1390.
22. O'Quinn PR, Knabe DA, Wu G. Arginine catabolism in lactating porcine mammary gland. J Anim Sci 2002; 80:467–474.
23. Wu G, Knabe DA, Flynn NE. Synthesis of citrulline from glutamine in pig enterocytes. Biochem J 1994; 299:115–121.
24. Wu G, Knabe DA, Flynn NE, Yan W, Flynn SP. Arginine degradation in developing porcine enterocytes. Am J Physiol Gastrointest Liver Physiol 1996; 271:G913–G919.
25. Steel RGD, Torrie JH, Dickey DA. Principles and Procedures of Statistics. New York: McGraw-Hill; 1997.
26. Morris SM Jr. Recent advances in arginine metabolism. Curr Opin Clin Nutr Metab Care 2004; 7:45–51.
27. Adams E, Frank L. Metabolism of proline and the hydroxyprolines. Annu Rev Biochem 1980; 49:1005–1061.
28. Dekaney CM, Wu G, Jaeger LA. Gene expression and activity of enzymes in the arginine biosynthetic pathway in porcine fetal small intestine. Pediatr Res 2003; 53:274–280.
29. Wu G, Bazer FW, Tuo W. Developmental changes of free amino acid concentrations in fetal fluids of pigs. J Nutr 1995; 125:2859–2868.
30. Phang JM. The regulatory functions of proline and pyrroline-5-carboxylic acid. Curr Top Cell Regul 1985; 25:91–132.
31. Yeh GC, Phang JM. Pyrroline-5-carboxylate stimulates the conversion of purine antimetabolites to their nucleotide forms by a redox-dependent mechanism. J Biol Chem 1983; 258:9774–9779.
32. Ross MG, Nijland MJM. Development of ingestive behavior. Am J Physiol Regul Integr Comp Physiol 1998; 274:R879–R893.
33. Wu G, Jaeger LA, Bazer FW, Rhoads JM. Arginine deficiency in preterm infants: biochemical mechanisms and nutritional implications. J Nutr Biochem 2004; 15:442–451.
34. Sangild PT, Schmidt M, Elnif J, Bjornvad CR, Westrom BR, Buddington RK. Prenatal development of gastrointestinal function in the pig and the effects of fetal esophageal obstruction. Pediatr Res 2002; 52:416–424.
35. McPherson RL, Ji F, Wu G, Blanton JR, Kim SW. Growth and compositional changes of fetal tissues in pigs. J Anim Sci 2004; 82:2534–2540.
36. Wu G, Pond WG, Ott T, Bazer FW. Maternal dietary protein deficiency decreases amino acid concentrations in fetal plasma and allantoic fluid of pigs. J Nutr 1998; 128:894–902.
37. Schoknecht PA, Newton GR, Weise DE, Pond WG. Protein restriction during pregnancy alters fetal and placental growth and allantoic fluid proteins in swine. Theriogenology 1994; 42:217–226.
38. Vonnahme KA, Wilson ME, Ford SP. Relationship between placental vascular endothelial growth factor expression and placental/endometrial vascularity in the pigs. Biol Reprod 2001; 64:1821–1825.
39. McIntire DD, Bloom SL, Casey BM, Leveno KJ. Birth weight in relation to morbidity and mortality among newborn infants. N Engl J Med 1999; 340:1234–1238.
40. Osgerby JC, Wathes DC, Howard D, Gadd TS. The effect of maternal undernutrition on ovine fetal growth. J Endocrinol 2002; 173:131–141.
41. Symonds ME, Budge H, Stephenson T, McMillen IC. Fetal endocrinology and development—manipulation and adaptation to long-term nutritional and environmental challenges. Reproduction 2001; 121: 853–862.
42. Barker DJP, Clark PM. Fetal undernutrition and disease in later life. Rev Reprod 1997; 2:105–112.