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Select Nutrients in the Ovine Uterine Lumen. III. Cationic Amino Acid Transporters in the Ovine Uterus and Peri-Implantation Conceptuses[1]

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ABSTRACT

Arginine is an essential amino acid for conceptus (embryo/fetus and trophoblast/placenta) growth and development; however, the mechanisms for arginine transport into the uterine lumen and uptake by conceptuses are largely unknown. In this study, expression of System y^+ (*SLC7A1*, *SLC7A2*, and *SLC7A3*) cationic amino acid transporters in uteri of cyclic and pregnant ewes and conceptuses was studied, and effects of pregnancy, progesterone (P4), and interferon tau (IFNT) on their expression were investigated. *SLC7A1* mRNA was most abundant in endometrial luminal (LE) and superficial glandular (sGE) epithelia on Day 16 of the estrous cycle and on Days 16–20 of pregnancy, whereas *SLC7A2* mRNA was most abundant in LE and mid to deep glandular (GE) epithelia on Days 14–20 of gestation. Expression of *SLC7A1* and *SLC7A2* was enhanced in pregnant ewes in a cell-specific manner, but abundance of *SLC7A3* was not affected by day of the estrous cycle or by pregnancy status. *SLC7A1*, *SLC7A2*, and *SLC7A3* mRNAs were expressed in trophectoderm and endoderm of conceptuses. In ovariectomized ewes, short-term treatment of ewes with P4 and IFNT did not affect endometrial *SLC7A1* mRNA, while long-term treatment with P4 stimulated *SLC7A1* in LE and GE, and IFNT tended to increase *SLC7A1* abundance in LE. *SLC7A2* mRNA abundance increased 4.1-fold in response to short-term P4 treatment and an additional 1.7-fold by IFNT primarily in endometrial LE/sGE, and these effects were ablated by a P4 receptor antagonist. These results indicate that coordinate changes in *SLC7A1*, *SLC7A2*, and *SLC7A3* expression in uterine endometria and conceptuses are likely important in transport of arginine that is critical to conceptus growth, development, and survival.

cationic amino acid transporters, conceptus, interferon tau, pregnancy, progesterone, sheep, uterus

INTRODUCTION

Arginine, an essential amino acid for fetal-placental growth and development [1], is required for synthesis of substances, including nitric oxide (NO) and polyamines, that have versatile functions [2]. As a major regulator of angiogenesis [3] and utero-placental-fetal blood flows, NO determines the rate of transfer of nutrients and oxygen from mother to fetus [4]. Polyamines are essential for DNA and protein synthesis and for proliferation and differentiation of cells [5]. In addition, arginine regulates key metabolic pathways critical for nutrient utilization and protein deposition through FKBP12-rapamycin complex-associated protein 1 and NO signaling pathways [6–8].

In support of a crucial role for arginine in embryogenesis and conceptus growth and development [9], we reported that arginine increased 10-fold in the uterine lumen of ewes between Days 10 and 15 of pregnancy (i.e., the peri-implantation period) [10]. A sufficient supply of arginine to the conceptus may be particularly important for ruminants and pigs, which have synepitheliochorial and epitheliochorial placentae, respectively, and for conceptuses that undergo rapid elongation during a protracted peri-implantation period [11]. The mechanisms for arginine transport into the uterine lumen and its uptake by conceptuses are largely unknown.

L-arginine transport is mediated primarily by the Na^+-independent System y^+ for cationic amino acids, which has low affinity but high capacity in cells. The cationic amino acid transporter (CAT) system consists of three members, *CAT1*, *CAT2*, and *CAT3*, which are encoded by *SLC7A1*, *SLC7A2*, and *SLC7A3* genes, respectively. These CATs are distributed differentially among cells, and their affinities for basic amino acids vary greatly for arginine, lysine, and ornithine [12, 13]. Notably, *Slc7a1* and *Slc7a2* were identified in mouse blastocysts [14], but little information is available on developmental changes in expression of CATs in uteri or conceptuses of any animal. Therefore, the objectives of this study were to determine 1) temporal and spatial (cell-specific) changes in expression of *SLC7A1*, *SLC7A2*, and *SLC7A3* in ovine conceptuses and uteri during the peri-implantation of pregnancy and the estrous cycle and 2) effects of progesterone (P4), interferon tau (IFNT), and a P4 receptor (PGR) antagonist on their expression in the ovine uterus.

MATERIALS AND METHODS

Animals

Crossbred Suffolk ewes (*Ovis aries*) were observed daily for estrus in the presence of vasectomized rams and used in experiments after they had exhibited at least two estrous cycles of normal duration (16–18 days). All experimental and surgical procedures were in compliance with the Guide for the Care and Use of Agricultural Animals in Teaching and Research and were approved by the Institutional Animal Care and Use Committee of Texas A&M University.

Experimental Design

Experiment 1. This experiment determined temporal and spatial (cell-specific) changes in *SLC7A1*, *SLC7A2*, and *SLC7A3* mRNA abundance in ovine uteri during the estrous cycle and early pregnancy. At estrus (Day 0), ewes were mated to an intact fertile or a vasectomized ram and then

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TABLE 1. Primer designs for PCR amplification.

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)	GenBank accession no.	Product size (bp)
SLC7A1	tggcactctcctggcttact	cgttcaacgaagatgctcag	AF212146	483
SLC7A2	aaggaaatgtggcaaactgg	ttgaaaagcaacccatcctc	XM_865568.2	490
SLC7A3	taccagcctcttgggctcta	aaagcagtggaatggaccac	BC126655	473

hysterectomized (n = 5 ewes/day) on either Day 10, 12, 14, or 16 of the estrous cycle or Day 10, 12, 14, 16, 18, or 20 of pregnancy as we described previously [15]. On Days 10–16 after mating, uteri were flushed with 20 ml of sterile saline, and pregnancy was confirmed by the presence of a morphologically normal conceptus. Sections (~0.5 cm) from the midportion of each uterine horn ipsilateral to the corpus luteum were fixed in fresh 4% paraformaldehyde (prepared in PBS, pH 7.2) for 24 h and then in 70% ethanol for 24 h. The fixed tissues were dehydrated through a graded series of alcohol to xylene and then embedded in Paraplast-Plus (Oxford Labware, St. Louis, MO). The remaining endometrium was physically dissected from myometrium, frozen in liquid nitrogen, and stored at −80°C for subsequent RNA extraction.

Experiment 2. This experiment determined changes in *SLC7A1*, *SLC7A2*, and *SLC7A3* mRNA abundance in ovine conceptuses during the peri-implantation period of early pregnancy. At estrus (Day 0), ewes were mated to a fertile ram and were assigned randomly to surgery on Day 13, 14, 15, or 16 of pregnancy (n ≥ 5 ewes/day), when uteri were flushed with sterile Tris buffer (10 mM, pH 7.0). Conceptuses were collected from each uterine flushing. On Day 18 of pregnancy, ewes were hysterectomized (n = 5 ewes/day), and conceptuses were separated from uterine tissues, fixed in fresh 4% paraformaldehyde, and then embedded in Paraplast-Plus as described for experiment 1.

Experiment 3. This experiment determined effects of short-term treatment with P4 and IFNT on abundance of *SLC7A1* and *SLC7A2* mRNAs in ovine uteri. Cyclic ewes (n = 20) were ovariectomized and fitted with intrauterine catheters on Day 5 after estrus as described previously [16]. Ewes were assigned randomly (n = 5 ewes/treatment) to receive daily i.m. injections of P4 (Sigma Chemical Co., St. Louis, MO) or P4 plus a PGR antagonist (ZK 136317; Schering AG, Berlin, Germany) and intrauterine infusions of either control (CX) serum proteins or recombinant ovine IFNT as follows: 1) 50 mg of P4 (Days 5–15) and 200 μg of CX serum proteins (Days 11–15) (P4+CX), 2) P4 and 75 mg of ZK 136317 (Days 11–15) and CX serum proteins (P4+ZK+CX), 3) P4 and IFNT (2×10^7 antiviral U, Days 11–15) (P4+IFN), or 4) P4, ZK, and IFNT (P4+ZK+IFN). The IFNT was prepared in a yeast bacterial system and was assayed for biological activity using an antiviral assay as described previously [17]. Control serum proteins and IFNT were prepared for intrauterine injections as described previously [18]. All ewes were hysterectomized on Day 16 after estrus, and the uterus was processed as described for experiment 1.

Experiment 4. This experiment determined the effects of treatment of ewes with P4 for 20 days and intrauterine infusions of IFNT on uterine expression of *SLC7A1*. As described previously [19], cyclic ewes (n = 20) were ovariectomized and fitted with intrauterine catheters on Day 5 after estrus. Ewes were then assigned randomly (n = 5 ewes/treatment) to receive daily i.m. injections of P4 or P4 and PGR antagonist (ZK 136317) and intrauterine infusions of either CX serum proteins or recombinant ovine IFNT as follows: 1) 50 mg of P4 (Days 5–24) and 200 μg of CX serum proteins (Days 11–24) [P4+CX], 2) P4 and 75 mg of ZK 136317 (Days 11–24) and CX serum proteins (200 μg) [P4+ZK+CX], 3) P4 and IFNT (2×10^7 antiviral U, Days 11–24) [P4+IFN], or 4) P4, ZK, and IFNT [P4+ZK+IFN]. All ewes were hysterectomized on Day 25 after estrus, and their uteri were processed as described in experiment 1.

RNA Isolation

Total cellular RNA was isolated from endometrium from the uterine horn ipsilateral to the corpus luteum (experiment 1) using Trizol reagent (Gibco-BRL) according to the manufacturer's recommendations and stored at −80°C. The quantity and quality of tRNA were determined by spectrometry and denaturing agarose gel electrophoresis, respectively.

Cloning of Partial cDNA for Ovine Endometrial SLC7A1, SLC7A2, and SLC7A3 mRNA

Partial cDNAs for ovine endometrial *SLC7A1*, *SLC7A2*, and *SLC7A3* were amplified by RT-PCR using tRNA from endometria collected from uteri of ewes hysterectomized on Day 20 of pregnancy (Table 1). For *SLC7A1*, the sense primer (5′-tggcactctcctggcttact-3′) and antisense primer (5′-cgttcaacgaagatgctcag-3′) were derived from the ovine *SLC7A1* mRNA coding sequence (GenBank accession No. AF212146). For *SLC7A2*, the sense primer (5′-aaggaaatgtggcaaactgg-3′) and antisense primer (5′-ttgaaaagcaacccatcctc-3′) were derived from the bovine *SLC7A2* mRNA coding sequence (GenBank accession No. XM_865568.2). For *SLC7A3*, the sense primer (5′-taccagcctcttgggctcta-3′) and antisense primer (5′-aaagcagtggaatggaccac-3′) were derived from the bovine *SLC7A3* mRNA coding sequence (GenBank accession No. BC126655). The PCR amplification was as follows: 1) 95°C for 2 min; 2) 95°C for 30 sec, 50°C for 45 sec, and 72°C for 1 min for 35 cycles; and 3) 72°C for 7 min. Partial ovine *SLC7A1*, *SLC7A2*, and *SLC7A3* cDNAs (483, 490, and 473 bp, respectively) were cloned into pCRII using a T/A Cloning Kit (Invitrogen, San Diego, CA), and their sequences were verified using an ABI PRISM Dye Terminator Cycle Sequencing Kit and an ABI PRISM automated DNA sequencer (Perkin-Elmer Applied Biosystems, Norwalk, CT).

Slot-Blot Hybridization Analyses

Steady-state levels of *SLC7A1*, *SLC7A2*, and *SLC7A3* mRNAs in ovine endometria were assessed by slot-blot hybridization as described previously [20]. Radiolabeled antisense cRNA probes were generated by in vitro transcription with [α-^{32}P]-uridine triphosphate (UTP). Denatured total endometrial RNA (20 μg) from each ewe was hybridized with radiolabeled antisense cRNA probes. To correct for variation in tRNA loading, a duplicate RNA slot membrane was hybridized with radiolabeled antisense 18S cRNA (pT718S; Ambion, Austin, TX). Following washing, the blots were digested with ribonuclease A, and radioactivity associated with slots was quantified using a Typhoon 8600 MultiImager (Molecular Dynamics, Sunnyvale, CA).

In Situ Hybridization Analyses

Cell-specific localization of *SLC7A1*, *SLC7A2*, and *SLC7A3* mRNAs in sections (5 μm) of ovine uteri was determined by radioactive in situ hybridization analyses as described previously [20]. Briefly, deparaffinized, rehydrated, and deproteinated uterine tissue sections were hybridized with an [α-^{35}S]-UTP radiolabeled antisense or sense cRNA probe. After hybridization, washing, and ribonuclease A digestion, slides were dipped in NTB-2 liquid photographic emulsion (Eastman Kodak, Rochester, NY) and exposed at 4°C for 4 days. Slides were developed in Kodak D-19 developer, counterstained with Gill hematoxylin (Fisher Scientific, Pittsburgh, PA), and then dehydrated through a graded series of alcohol to xylene. Coverslips were then affixed with Permount (Fisher Scientific). Images of representative fields were recorded under brightfield and darkfield illumination using a Nikon Eclipse 1000 photomicroscope (Nikon Instruments Inc., Natick, MA) fitted with a Nikon DXM1200 digital camera.

In experiment 4, the relative abundance of *SLC7A1* mRNA in the endometrial glandular (GE) and luminal (LE) epithelia was measured as optical density using the public domain imaging program ImageJ (National Institutes of Health, Bethesda, MD [http://rsb.info.nih.gov/nih-image/]). Briefly, more than six representative areas of the uterus from each ewe were photographed in darkfield illumination, and the photographs were saved as TIFF files. The noise in the original photograph was filtered by median filter, followed by conversion to mask using binary function. With Image Calculator (Image Metrology A/S; Hørsholm, Denmark), a new photograph was created by combining the original and converted photographs. After setting a threshold, the optical density of hybridization signals in endometrial GE and LE was measured separately, and data were analyzed statistically.

Statistical Analysis

All quantitative data were subjected to least-squares regression ANOVA using the general linear models procedures of the Statistical Analysis System (SAS Institute, Cary, NC). Slot-blot hybridization data were corrected for differences in sample loading using 18S rRNA values as the covariate. Data from experiment 1 were analyzed for effects of day, pregnancy status (cyclic or pregnant), and their interactions. Within pregnancy status, least-squares

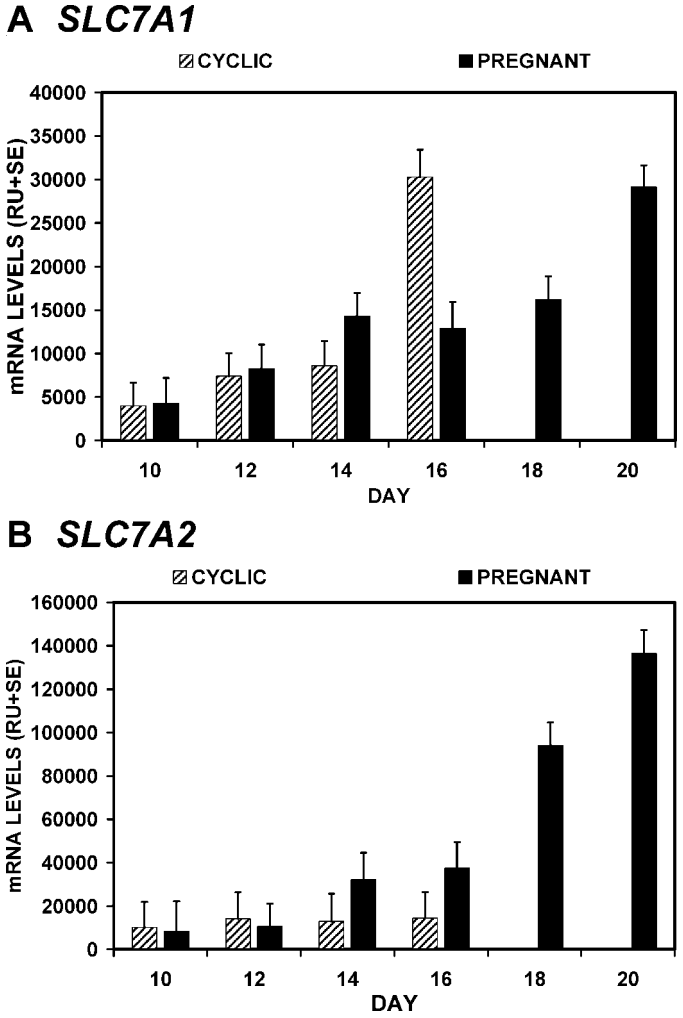

FIG. 1. Slot-blot hybridization analysis of steady-state mRNAs for *SLC7A1* (**A**) and *SLC7A2* (**B**) in endometria of cyclic and pregnant ewes (Days 10–20). Data (relative U [RU]) are expressed as least-squares means ± SEM, n = 5. **A**) *SLC7A1* mRNA levels were affected by a day-×-pregnancy status interaction ($P < 0.05$). In cyclic ewes, *SLC7A1* mRNA abundance was affected by day ($P < 0.01$) between Days 10 and 16. An effect of day in pregnant ewes for *SLC7A1* mRNA was due to a 4-fold increase between Days 10 and 14 and a further increase between Days 16 and 20 ($P < 0.01$). **B**) Steady-state mRNA levels for endometrial *SLC7A2* were not affected by a day-×-pregnancy status interaction ($P > 0.1$) or by day ($P = 0.2$) between Days 10 and 16 but were affected by pregnancy status ($P < 0.05$) due to greater abundance of *SLC7A2* mRNA in pregnant ewes. In cyclic ewes, *SLC7A2* mRNA levels did not change between Days 10 and 16; however, in pregnant ewes, *SLC7A2* mRNA levels increased 3.9-fold from Days 10 to 14 and then another 2.6-fold and 4.2-fold to Days 18 and 20, respectively (quadratic effect of day, $P < 0.01$).

regression analyses were used to determine effects of day on endometrial mRNA levels. Data from experiments 3 and 4 were analyzed for effect of treatment. Preplanned orthogonal contrasts were used to determine main effects of treatment. All tests of significance were performed using the appropriate error terms according to the expectation of the mean squares for error. $P \leq 0.05$ was considered significant, whereas $P = 0.06$ to $P = 0.10$ was considered to indicate a trend toward significance. Data are presented as least-square means ± SEM.

RESULTS

Steady-State Levels of SLC7A1 *and* SLC7A2 *mRNAs in Ovine Endometrium (Experiment 1)*

Steady-state levels of *SLC7A1* and *SLC7A2* mRNAs in endometria of cyclic and pregnant ewes were determined by slot-blot hybridization analysis (Fig. 1). For *SLC7A1* mRNA levels, there was a day-×-pregnancy status interaction ($P < 0.05$). For cyclic ewes, there was a linear effect of day ($P < 0.01$) on *SLC7A1* mRNA abundance, as values were similar between Days 10 and 14 and then increased on Day 16. In pregnant ewes, *SLC7A1* mRNA levels increased 4-fold between Days 10 and 14 and an additional 2-fold between Days 16 and 20 (linear effect of day, $P < 0.01$) (Fig. 1A). Steady-state levels of mRNA for endometrial *SLC7A2* were not affected by a day-×-pregnancy status interaction ($P > 0.1$) or by day ($P = 0.2$) but were affected by pregnancy status ($P < 0.05$) because of higher levels in pregnant ewes. In cyclic ewes, *SLC7A2* mRNA levels did not change between Days 10 and 16; however, in pregnant ewes, *SLC7A2* mRNA abundance increased 3.9-fold from Days 10 to 14 and another 2.6-fold and 4.2-fold on Days 18 and 20, respectively (quadratic effect of day, $P < 0.01$) (Fig. 1B).

Localization of SLC7A1, SLC7A2, *and* SLC7A3 *mRNAs in Ovine Uteri and Conceptuses (Experiments 1 and 2)*

In situ hybridization analysis was used to detect *SLC7A1*, *SLC7A2*, and *SLC7A3* mRNAs in a cell-specific manner in uteri and conceptuses of cyclic and pregnant ewes. *SLC7A1* mRNA was most abundant in uterine LE/superficial GE (sGE) and GE on Day 16 of the estrous cycle and on Days 16–20 of pregnancy and was detectable in conceptuses at low abundance between Days 13 and 18 of pregnancy (Fig. 2). *SLC7A2* mRNA in LE/sGE was increasingly abundant in LE/sGE between days Days 14 and 20 of pregnancy but did not change in the estrous cycle (Fig. 3). *SLC7A2* mRNA was detectable in uterine endometria from two of five cyclic ewes on Day 14 of the estrous cycle. In pregnant ewes, *SLC7A2* mRNA abundance was weak in two of five ewes but was expressed in greater abundance in the other ewe on Day 14 of pregnancy as shown in Figure 3. Day 14 of pregnancy may represent a transition from weak to strong expression in uterine endometria of ewes during early pregnancy. *SLC7A2* mRNA was weakly expressed in trophectoderm and endoderm of conceptuses from Days 13 to 18 of pregnancy (Fig. 3). *SLC7A3* mRNA was weakly detectable in LE, GE, and stroma from cyclic and pregnant endometria, as well as trophectoderm and endoderm of conceptuses (Supplemental Fig. 1 available at www.biolreprod.org).

Effects of P4 and IFNT on SLC7A1 *mRNA in Ovine Uterine Endometria (Experiments 3 and 4)*

Short-term treatment of ewes with P4 and/or intrauterine IFNT to mimic Day 16 of pregnancy (experiment 3) affected expression of *SLC7A1* mRNA in ovine uteri. There was only a weak signal for *SLC7A1* mRNA in uterine LE, GE, and stromal cells of ewes in all treatment groups (data not shown). However, long-term treatment of ewes with P4 (experiment 4) induced expression of *SLC7A1* in uterine LE, GE, and stromal cells, which was inhibited by the PGR antagonist ZK 136317 (Fig. 4). Levels of *SLC7A1* mRNA in LE and GE uteri of ewes treated with P4+CX were 1.8-fold and 1.5-fold higher ($P < 0.01$), respectively, compared with values of ewes treated with P4+ZK+CX. Intrauterine treatment with IFNT tended to stimulate *SLC7A1* mRNA abundance in LE in the presence of P4 ($P = 0.057$, P4+IFNT vs. P4+CX) but not in ewes treated with ZK 136317 ($P > 0.8$, P4+ZK+IFNT vs. P4+ZK+CX) (Fig. 4B). *SLC7A1* expression in GE was not affected by IFNT ($P > 0.1$, P4+IFNT vs. P4+CX; $P > 0.7$, P4+ZK+IFNT vs. P4+ZK+CX) (Fig. 4C). These results indicate that long-term

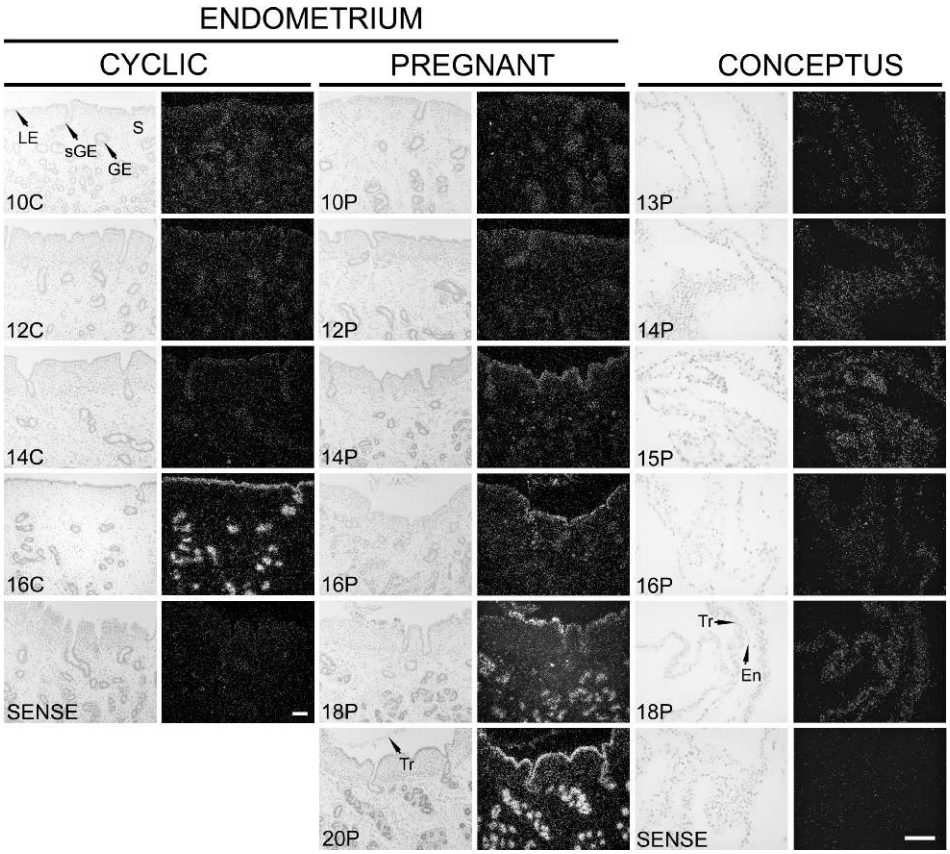

FIG. 2. In situ hybridization analysis of *SLC7A1* mRNA in endometria from cyclic (C) and pregnant (P) ewes. *SLC7A1* mRNA was present in uterine LE, GE, and stromal cells of cyclic (Days 10–16) and pregnant (Days 10–20) ewes, as well as trophectoderm and endoderm of conceptuses (Days 13–18). *SLC7A1* mRNA was most abundant in LE/sGE and GE on Day 16 of the estrous cycle and on Days 16–20 of pregnancy. In the conceptus, the *SLC7A1* mRNA was detectable at low levels in both trophectoderm and endoderm. S, stroma; Tr, trophectoderm; En, endoderm; bar = 10 μm.

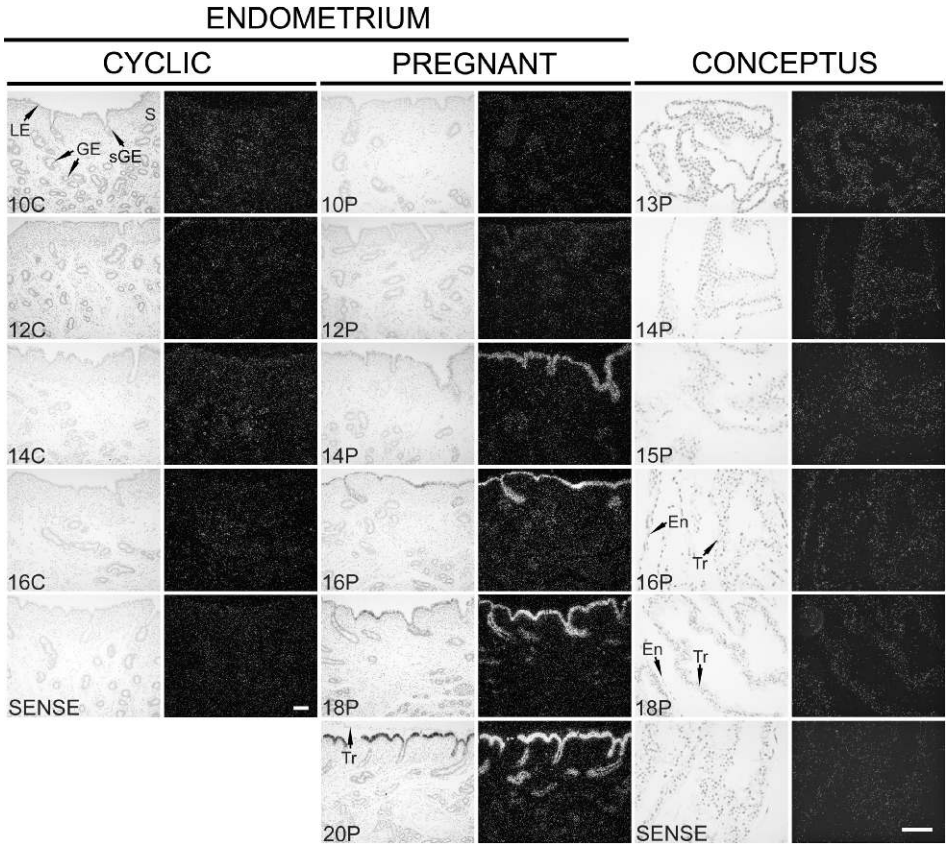

FIG. 3. In situ hybridization analysis of *SLC7A2* mRNA in endometria from cyclic (C) and pregnant (P) ewes. *SLC7A2* mRNA was present in very low amounts in LE/sGE and stroma of cyclic (Days 10–16) ewes, was abundant in LE/sGE of pregnant uteri between Days 16 and 20, and was just detectable in trophectoderm and endoderm of conceptuses (Days 13–18). S, stroma; Tr, trophectoderm; En, endoderm; bar = 10 μm.

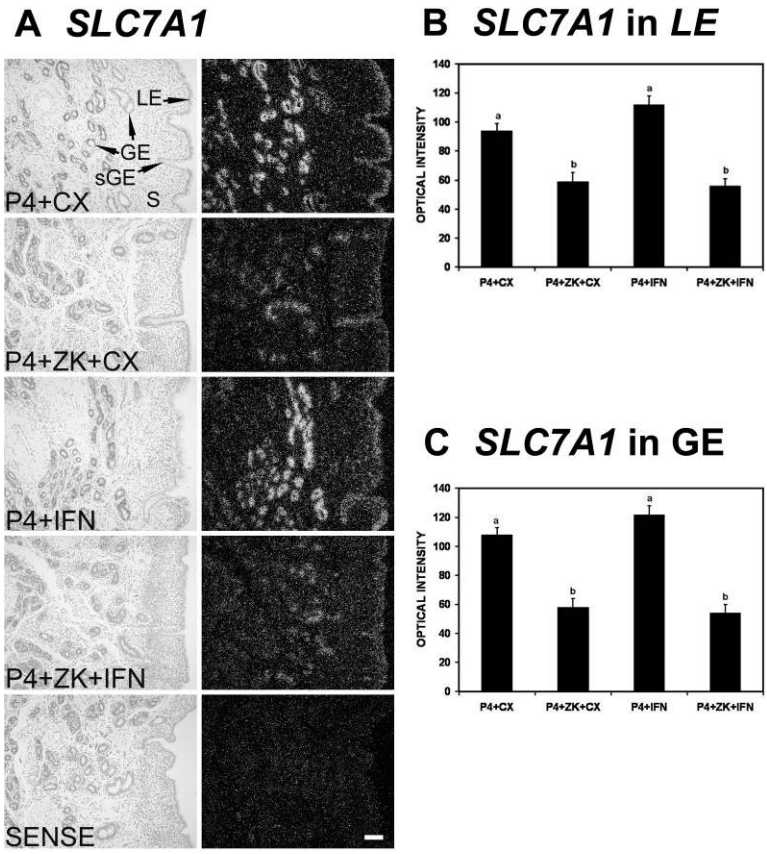

FIG. 4. Effects of long-term treatment of ewes with P4 and IFNT on endometrial *SLC7A1* mRNA. **A**) In situ hybridization analysis detected abundant *SLC7A1* mRNA in endometrial LE/sGE, GE, and stromal cells of ewes treated with P4+CX or P4+IFNT, and it was detectable at low levels in uterine LE/sGE and GE of ewes treated with P4+ZK+IFN and P4+ZK+CX. S, stroma; bar = 10 μm. **B**) The optical intensity of in situ hybridization signals on *SLC7A1* mRNA in uterine LE. Levels of *SLC7A1* mRNA in LE in P4+CX-treated ewes were 1.5-fold higher ($P < 0.01$) compared with P4+ZK+CX-treated ewes, and IFNT tended to stimulate *SLC7A1* expression in LE ($P = 0.057$, P4+IFNT vs. P4+CX) but not in ewes treated with the PGR antagonist ($P > 0.8$, P4+ZK+IFNT vs. P4+ZK+CX). The different letters (a and b) indicate significant differences in steady-state mRNA levels among the four treatment groups. **C**) The optical intensity of in situ hybridization signals for *SLC7A1* mRNA in GE. Levels of *SLC7A1* mRNA in GE of P4+CX-treated ewes were 1.8-fold higher ($P < 0.01$) than for P4+ZK+CX-treated ewes, but there was no effect of IFNT ($P > 0.1$, P4+IFNT vs. P4+CX; $P > 0.7$, P4+ZK+IFNT vs. P4+ZK+CX).

treatment with P4 induces *SLC7A1* expression in endometrial GE and LE.

P4 Induces and IFNT Stimulates SLC7A2 *mRNA in Ovine Uterine Endometria (Experiment 3)*

The abundance of *SLC7A2* mRNA increased 4.1-fold in response to P4 ($P < 0.01$, P4+CX vs. P4+ZK+CX) and another 1.7-fold in response to IFNT ($P < 0.01$, P4+CX vs. P4+IFN), and these effects were blocked by the PGR antagonist ($P > 0.10$, P4+ZK+CX vs. P4+ZK+IFN) (Fig. 5A). In situ hybridization analyses revealed that effects of P4 and IFNT on *SLC7A2* mRNA abundance were specific to endometrial LE/sGE (Fig. 5B). The results indicate that *SLC7A2* is induced by P4 and stimulated by IFNT in ovine uterine LE/sGE during early pregnancy.

DISCUSSION

Arginine is hypothesized to have an important role in conceptus growth and development [1]. In most animals, including sheep, citrulline (the precursor of arginine) is synthesized from glutamine and proline in enterocytes of the small intestine [2]. However, this synthetic pathway is absent from uteri, placenta, and endometria of sheep [21]. We found that total recoverable arginine in the uterine lumen increased 10-fold in pregnant but not cyclic ewes during the peri-implantation period between Days 10 and 15 of pregnancy [10]. This was also the case for other cationic amino acids (lysine, histidine, and ornithine) in ovine uterine flushings [10]. Thus, arginine in ovine uterine fluid must be derived from maternal blood and/or tissue fluid via transport through the vascular endothelium and uterine epithelia. During the preimplantation period, there is down-regulation of tight and adherent junctions in endometrial LE by P4, which facilitates selective transudation of molecules from serum and tissue fluids into the uterine lumen [22]. To our knowledge, this is the first report of temporal and spatial (cell type) changes in expression of System y^+ amino acid transporters in ovine uteri and conceptuses. Results of the present study indicate marked increases in expression of mRNAs for *SLC7A1* and *SLC7A2* in LE/sGE and GE of ovine uterine endometria during early pregnancy, while *SLC7A3* mRNA was constitutively and weakly expressed in uterine GE and stromal cells. The abundance of *SLC7A1*, *SLC7A2*, and *SLC7A3* mRNAs was low in ovine conceptuses during the peri-implantation period. Thus, combined effects of SLC7A1, SLC7A2, and SLC7A3 likely contribute to increased arginine transport into the uterine lumen to exert effects on conceptuses.

The System y^+/CAT members are differentially distributed in organs, cells, and intracellular compartments. *SLC7A2A* was not detected in ovine uterine endometria or conceptuses (data not shown) but is expressed in liver, while *SLC7A2B* is expressed in many cell types in humans. In contrast, SLC7A1 is absent from the liver in humans and is expressed ubiquitously in extrahepatic tissues and cells [12]. SLC7A3 is mainly distributed in central neurons and peripheral tissues of human embryos and adults [23]. The differential expression of these CATs suggests that they have different roles in the provision of basic amino acids. For example, SLC7A1 is associated with caveolin, which contains endothelial NO synthase in endothelial cells [24]. Similarly, in macrophages, SLC7A1 and SLC7A2B may be responsible for transporting arginine into exchangeable cationic amino acid pools for use by inducible NO synthase [25]. Whether colocalization of SLC7A1 and caveolin-like protein or the association of CAT family members with specific isoforms of NO synthase exists in uterine endometria is unknown. In addition, the unique

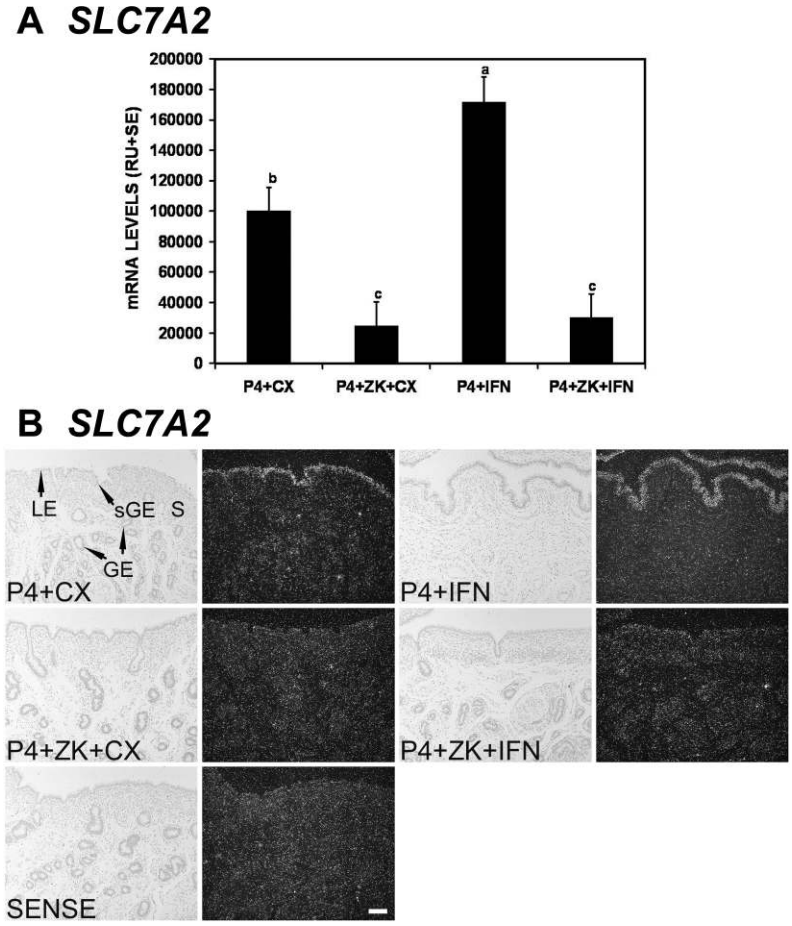

FIG. 5. Effects of treatment of ewes with P4 and IFNT on endometrial *SLC7A2* mRNA. **A**) Slot-blot hybridization analysis of steady-state *SLC7A2* mRNA in endometria. Endometrial *SLC7A2* mRNA abundance increased 4.1-fold in response to P4 ($P < 0.01$, P4+CX vs. P4+ZK+CX) and another 1.7-fold in response to IFNT ($P < 0.01$, P4+CX vs. P4+IFN). The effects of P4 and IFNT were blocked by the PGR antagonist ($P > 0.1$, P4+ZK+CX vs. P4+ZK+IFN). Data (relative U [RU]) are expressed as least-squares means ± SEM, n = 5. The different letters (a, b, and c) indicate significant differences in steady-state mRNA levels among the four treatment groups. **B**) In situ hybridization analysis of *SLC7A2* mRNA indicated that P4 induces and IFNT stimulates *SLC7A2* abundance only in LE/sGE. S, stroma; bar = 10 μm.

function and subcellular localization of other CAT members are unclear, although subcellular localization of CATs may be essential for transport of cationic amino acids by the placenta [26].

Results of in vivo and in vitro studies indicate complex compartmentalization of arginine metabolism in mammals due to cell-specific and organ-specific expression of proteins involved in arginine transport, synthesis, and catabolism [2]. In human placentae, System y^+ and System y^+L transporters are distributed on the maternal-facing basal membrane, and System y^+L is also present on the fetal-facing basal membrane [27]. This differential distribution of CATs may be responsible for low rates of arginine transport from placenta to fetus compared with transport from uterus to placenta in sheep [28]. *SLC7A1* and *SLC7A2* expression were more abundant in uterine endometria than in peri-implantation conceptuses in the present study (Figs. 2 and 3), whereas *SLC7A3* was expressed very weakly in uterine endometria and conceptuses (Supplemental Fig. 1). We were unable to evaluate abundance of CAT proteins in ovine uteri and conceptuses because of the lack of available antibodies. It is possible that other CATs contribute to the uptake of arginine by ovine conceptuses. Future studies are warranted to test this hypothesis.

We have reported that total recoverable arginine in the uterine lumen increased 10-fold in pregnant but not cyclic ewes between Days 10 and 15 of pregnancy [10]. Similarly, concentrations of other cationic amino acids (lysine, histidine, and ornithine) in ovine uterine flushings increased markedly during the peri-implantation period of pregnancy [10]. In the present study, ovine endometrial *SLC7A1* and *SLC7A2* mRNA levels increased 3.0-fold and 4.2-fold from Days 10 to 14 and Days 14 to 16 of pregnancy, respectively (Fig. 1). This indicates that increases in endometrial *SLC7A1* and *SLC7A2* expression likely contribute largely to the increased total recoverable arginine in uterine flushings during early pregnancy; however, other CATs may also have a role in the arginine transport into uterine lumen. Because concentrations of arginine in ovine maternal plasma do not change substantially during pregnancy [29], an increase in the rate of arginine transport across uterus is necessary to provide sufficient arginine to meet the need for rapid growth and elongation of conceptuses during the peri-implantation period of pregnancy in sheep. Thus, increases in CAT expression and arginine transport are likely crucial to successful pregnancy outcomes in mammals. In support of this view, increasing dietary arginine in gilts [30] and rats [31] increased availability to conceptuses and significantly increased litter size and survival of offspring in pigs [30]. Conversely, in humans, intrauterine growth retardation is associated with reduced activity and expression of the System y^+/hCAT-1 and y^+/hCAT-2B transporters in umbilical vein endothelial cells [32]. In addition, autophagic degradation of proteins to amino acids within early embryos is essential for preimplantation development in mammals [33], and autophagy is induced under starvation conditions [34]. The increase in expression of endometrial *SLC7A1* and *SLC7A2* that is coordinate with increased availability of arginine in the ovine uterine lumen and weak expression of *SLC7A1*, *SLC7A2*, and *SLC7A3* in ovine conceptus may preclude the need for conceptus autophagy in sheep during the peri-implantation period.

There are marked changes in circulating levels of steroid hormones and cytokines during pregnancy. However, little is

known about endocrine regulation of expression of amino acid transporters in the uterus or conceptus. Of note, there are reports that IFN gamma (a type II interferon), interleukin 6 [35], and lipopolysaccharide [36] stimulate *SLC7A1* and *SLC7A2* expression and L-arginine uptake in cells through the p38 MAPK pathway. A novel and important finding from the present study is that IFNT increases *SLC7A1* and *SLC7A2* expression in the uterus. The pregnancy recognition signal in ruminants, IFNT, is produced by mononuclear cells of ovine conceptus trophectoderm between Days 10 and 21 of pregnancy [37], and *SLC7A2* expression in LE/sGE was found to increase from Days 14 to 20 of pregnancy (Fig. 1), which is consistent with evidence that P4 can induce and IFNT can further stimulate *SLC7A2* expression in ovine endometrial LE/sGE in closest proximity to conceptus trophectoderm (Fig. 5). Furthermore, *SLC7A1* mRNA was most abundant on Day 16 of the estrous cycle, when circulating concentrations of estradiol peak before ovulation and expression of estrogen receptors is increasing in uterine epithelia [38]. Estrogen can increase NOS3 (eNOS) activity, while SLC7A1 and NOS3 are colocalized in endothelial cells [24] and ovine uterine endometria (Gao, Wu, and Bazer, unpublished results). Thus, estradiol may be involved in increasing *SLC7A1* expression in ovine uteri. On the other hand, effects of P4 on *SLC7A1* expression in ovine uteri required continuous treatment of ewes with P4 for 20 days (experiment 4). Similarly, expression of *SPP1* [39, 40], *STC1* [41], *UTMP* [42], and *GRP* [43] in the ovine uterus requires long-term treatment with P4; however, the underlying mechanism is unknown. In addition, the increase in endometrial SLC7A1 mRNA between Days 16 and 20 of pregnancy is coordinate with the onset of production of placental lactogen by binucleate cells of ovine conceptus trophectoderm [44]. This may indicate that *SLC7A1*, like *SPP1* [45], *UTMP* [43], and other genes, is under the control of a servomechanism involving the timely interplay of estrogen, P4, IFNT, placental lactogen, and placental growth hormone. It is worth noting that the long-term effects of P4, with or without intrauterine IFNT, were not blocked by the PGR antagonist (Fig. 4A). Future studies are required to elucidate mechanisms that control SLC7A1 and SLC7A2 expression in ovine uteri.

In conclusion, results of the present study revealed temporal and spatial expression of *SLC7A1*, *SLC7A2*, and *SLC7A3* in ovine uteri and conceptuses. Expression of *SLC7A1* in LE and GE, as well as *SLC7A2* in LE and sGE, was stimulated by P4 and/or IFNT and enhanced during early gestation in association with increased amounts of arginine and other basic amino acids in uterine fluid. These coordinate changes in expression of these CATs are likely important for conceptus survival, growth, and development. These novel findings may contribute to prevention of early pregnancy loss and to improved reproductive performance in humans and animals.

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