

Select nutrients and glucose transporters in pig uteri and conceptuses

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Glucose present in the intrauterine environment can be metabolized, activate cell signaling pathways or be converted to a "storage" form. Total recoverable glucose in uterine fluid of pregnant, but not cyclic pigs increases from Day 12 after onset of estrus in concert with conceptus elongation (Bazer *et al.* 1991). Transport of glucose into the ovine uterus and its uptake by conceptuses involves sodium-dependent and facilitative glucose transporters (Gao *et al.* 2009). Glucose can activate FRAP1/mTOR "nutrient sensing" pathway in which protein kinases activate p70S6 through phosphorylation to increase translation of 5'TOP mRNAs (terminal oligopyrimidine tract) (Wen *et al.* 2005). Activated FRAP1 also regulates differentiation of trophoctoderm (Tr) via Ras transformation by phosphorylating eukaryotic initiation factor 4E binding protein 1 (eIF4EBP1), a translational repressor of CAP-dependent translation (De Benedetti & Rhoads 1990). Select nutrients that stimulate FRAP1 activity in Tr include glucose, arginine (Arg), leucine (Leu) and glutamine (Gln) which may increase expression of *IGF2*, *ODC* and *NOS* mRNAs (Nielsen *et al.* 1995; Kimball *et al.* 1999; Martin & Sutherland 2001) which are required for conceptus development, differentiation and implantation through effects on production of NO (Kaliman *et al.* 1999) and polyamines (Van Winkle & Campione 1983). FRAP1 null mice die shortly after implantation due to impaired cell proliferation and hypertrophy in both the embryonic disc and Tr (Murakami *et al.* 2004). There are 14 isoforms of facilitative glucose transporters and 6 sodium-dependent glucose transporters. Of these, *SLC2A1*, *SLC5A1* and *SLC5A11* mRNAs are most abundant in endometria and *SLC2A3* is uniquely expressed by ovine conceptus Tr and endoderm (Gao *et al.* 2009). The objective of this study with sexually mature gilts was to identify effects of pregnancy, long-term treatment of ovariectomized gilts with progesterone (P4) and estradiol-induced pseudopregnancy (PP) on changes in amounts of select nutrients (glucose, Arg, Leu and Gln) in uterine fluid and expression of glucose transporters in endometria and conceptuses.

Experiment 1 determined effects of day of the estrous cycle and pregnancy on total recoverable glucose, Arg, Leu and Gln in uterine flushings from gilts on Days 5, 9, 12 and 15 of the estrous cycle (Cy) and Days 9, 10, 12, 13, 14 and 15 of pregnancy (Px). Total recoverable glucose, Arg, Leu and Gln increased ($P < 0.05$) with day in Cy and Px gilts, but only Arg increased more in Px than Cy ewes (day x pregnancy status; $P < 0.05$) between Days 12 and 15.

Experiment 2 determined recoverable amounts of selected nutrients in uterine flushings of gilts ovariectomized on Day 12 and treated daily with either corn oil (OVX-CO; $n = 4$) or 200 mg progesterone (OVX-P4; $n = 5$) to Day 39 and hysterectomized on Day 40. Values (mean \pm SEM; nmol) were greater for OVX-P4 than OVX-CO gilts for glucose (4,955 \pm 2,534 vs 726 \pm 133), Arg (207,112 \pm 160,979 vs 7,409 \pm 2,877) and Leu (248,255 \pm 178,599 vs 13,983 \pm 5,225), but differences were not significant due to high variability and small sample size.

Experiment 3 determined amounts of selected nutrients in uterine flushings of gilts on Day 90 of pseudopregnancy (PP) induced by treatment with 5 mg/day estradiol benzoate on Days

11 to 15 after onset of estrus. The flushings (109 ± 24 ml) contained significant amounts (nmol) of glucose ($14,007 \pm 3,946$), Arg ($9,051 \pm 1,959$), Gln ($4,949 \pm 1,449$) and Leu ($2,455 \pm 771$).

Results from examination of uterine and conceptus tissues by in situ hybridization for expression of Facilitative Glucose Transporters *SLC2A1*, *SLC2A2*, *SLC2A3* and *SLC2A4* mRNAs indicated that: 1) *SLC2A3* mRNA was weak or not detectable in conceptus and uterine tissues; 2) *SLC2A1* mRNA was expressed by all cell types in conceptuses and abundant in uterine luminal (LE) epithelium of Cy, Px, PP and OVX-P4 gilts; 3) *SLC2A4* mRNA was moderately abundant in LE of Px, but not Cy gilts between Days 13 and 25 of Px and by LE and GE of OVX-P4 gilts, but not detectable in LE or GE of PP gilts; 4) *SLC2A2* mRNA was most abundant in conceptuses from Days 12 to 40 of Px, decreased to Day 50 and then increased and was maintained specifically in placental areolae and apical regions of interdigitating endometrial folds to Day 80 of Px. *SLC2A2* was expressed in uterine LE of Px and PP gilts and LE and GE of OVX-P4 gilts.

Results indicated that: 1) glucose and Arg in particular, but also Leu and Gln, increase in uterine fluids of Cy and Px gilts; 2) these select nutrients are abundant in uterine flushings of PP and OVX-P4 gilts; and 3) temporal and cell specific changes occur in expression of specific glucose transporters in the uterus and conceptus. These select nutrients likely stimulate FRAP1 cell signaling in trophectoderm cells of conceptuses to influence proliferation, migration, attachment and gene expression necessary for conceptus development and survival in pigs.

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