OMEGA-3 FATTY ACID SUPPLEMENTATION AND THE INSULIN-LIKE GROWTH FACTOR (IGF) SYSTEM IN EARLY PREGNANCY IN PIGS

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Summary

The IGF system of growth factors, receptors and binding proteins functions from early in pregnancy. Recent evidence indicates improved embryo survival in gilts fed supplemental omega-3 fatty acids beginning before conception. Here we report effects of supplementing a corn-soybean meal diet (control) with a marine source of protected omega-3 fatty acids (PFA, 1.5% of diet) on mRNA expression for IGF-I, IGF-II, IGF Binding Protein-3 (IGFBP-3) and IGFBP-5 in the porcine gravid uterus. The PFA (Gromage™) contained equal amounts of eicosapentanoic (EPA) and docosahexanoic (DHA) acids and replaced corn in the diet beginning when gilts were approximately 170 d old (n = 13/treatment).

Gilts were artificially inseminated at approximately 205 d of age. Conceptus and endometrial samples were collected on d 11, 15, and 19 of gestation. All gilts were pregnant.

In the conceptus, message for IGF-II and IGFBP-3 increased (P<0.001) from d 15 to d 19, while there was an increase (P < 0.001) in IGF-I and IGFBP-5 from d 11 to 15 and a decrease (P<0.001) to d 19. In the endometrium, message for IGF-I was stable over the interval, but message for IGF-II and IGFBP-5 were increased by d 15 and IGFBP-3 by d 19 (P<0.01). There were trends for omega-3 fatty acid supplementation to increase endometrial IGF-II (P = 0.09) and IGFBP-5 (P = 0.12) on d 15. In the d-19 conceptus, embryonic but not extraembryonic IGF-I mRNA tended to be greater (P = 0.13) for PFA compared to control gilts. During d 11 to 19 the conceptus is elongating, attaching to the uterus, and the embryonic disc is differentiating from a homogenous tissue to form the tissues and organs of the adult. One mechanism for omega-3 fatty acid effects in early pregnancy could involve epigenetic effects on mRNA expression for the IGF and IGFBP proteins.

(Key words: reproduction, IGF, omega-3 fatty acids.)

Introduction

Published research indicates that the insulin-like growth factor system includes three hormones, IGF-I, IGF-II, and insulin, and six binding proteins, IGFBP-1 through IGFBP-6. Insulin-like growth factor (IGF)–I and –II are proteins that are important regulators of fetal and postnatal growth.

IGF-I is present in most fetal tissues as early as the embryonic stage. IGF-II is the prominent growth factor during fetal growth and development. IGFBP-3 is the most abundant binding protein, and it binds approximately 90% of the IGFs in circulation. IGFBP-5 is the most conserved binding protein across species and is an essential regulator of physiological processes.

Materials and Methods

At 150 d of age, 26 gilts (PIC 327MQ × 1050; BW = 131 kg) were exposed to boars to induce puberty. At 170 d of age, gilts were moved to gestation stalls and dietary treatments were initiated.

Gilts were randomly assigned to one of two dietary treatments. The control treatment consisted of a typical corn-soybean meal diet. The treatment called PFA was the control diet with an added protected fish source of omega-3 fatty acids. This product, Gromega™, was added at 1.5 percent of the diet in place of corn.

At approximately d 190, a 14-d Matrix® treatment was applied for estrus synchronization. After the Matrix® treatment and upon heat detection, gilts were artificially inseminated with semen from the PIC line 1050 boars. Dietary treatments continued until days eleven, fifteen, and nineteen of gestation (d 0 = onset of estrus) when gilts underwent surgery to remove embryos and tissue samples. Prior to the trial, gilts were pen fed, *ad libitum*. Once moved to gestation stalls, gilts were fed 5 lb per day for the remainder of the trial.

Thirteen gilts were allotted per treatment. All were pregnant at surgery and provided samples for mRNA measurement. Total RNA was isolated from endometrium, extraembryonic membrane, and embryos by using the RNeasy Mini Kit (Qiagen; Valencia, CA). The concentration of RNA was determined by absorbance at 260 nm. Electrophoresis of total RNA through a 1% agarose-formaldehyde gel followed by ethidium bromide staining allowed visualization of 28S and 18S ribosomal RNA (rRNA) and was used to assess the integrity of RNA. One microgram of total RNA was reverse-transcribed to produce the first-strand complementary DNA (cDNA) using TaqMan reverse transcriptase (Applied Biosystems, Foster City, CA) following the protocol recommended by the manufacturer.

Real-time quantitative-PCR was used to measure the quantity of mRNA for IGF-I, IGF-II, IGFBP-3, and IGFBP-5 and 18S rRNA in total RNA isolated from endometrium, extraembryonic membranes, and embryos. Measurement of the relative quantity of cDNA was carried out using TaqMan Universal PCR Master Mix (Applied Biosystems), 900 n*M* of the appropriate forward and reverse primers, 200 n*M* of the appropriate TaqMan detection probe, and 1 µL (0.5 µg cDNA) of the cDNA mixture. Commercially available eukaryotic 18S rRNA primers and probes were used as an endogenous control (Applied Biosystems; Genbank Accession no. X03205). Assays were preformed in an ABI Prism 7000 sequence detection system (Applied Biosystems) using thermal cycling parameters recommended by the manufacturer (50 cycles of 15 sec at 95ºC and 1 min at 60ºC). Relative expressions of mRNA of IGF-I, IGF-II, IGFBP-3, and IGFBP-5 were normalized to the 18S rRNA endogenous control and expressed in arbitrary units. Primers and probes were used for the real-time quantitative-PCR.

Data were analyzed using the MIXED procedure of SAS (SAS, 2000; SAS Inst. Inc., the Satterthwaite degrees of freedom).

Results

In the endometrium, as day of gestation increased, the mRNA for all of the IGF proteins except IGF-I increased. Within the conceptus, the mRNAs for all the IGF proteins increased ($P < 0.001$) with increasing day of gestation. Within the conceptus on d 19 of gestation, the embryo contained more ($P = 0.056$) IGF-I mRNA than the extraembryonic tissue, and there was a trend for a tissue by treatment interaction ($P = 0.13$) because PFA tended to increase IGF-I message in the embryo but not in the extraembryonic tissue. There was also a tendency ($P<0.09$) for the extraembryonic membranes to contain more IGFBP-3 message (30.2 ± 5.6) than the embryo (15.6 ± 5.6).

In the endometrium, IGF-II message was greater ($P<0.05$) on d 15 and d 19 than on d

11, and there was a tendency ($P = 0.09$) for a d × treatment interaction because PFA tended to increase IGF-II message on d 15 but not on other days. In the endometrium IGFBP-5 mRNA was greater ($P<0.05$) on d 15 and d 19 than on d 11, and a trend for a treatment × day effect ($P<0.12$) because PFA tended to increase IGFBP-5 mRNA on d 15 but decrease its message on d 19.

Discussion

There were no strong treatment effects of omega-3 fatty acid supplementation on IGF-I, IGF-II, IGFBP-3, or IGFBP-5 in the endometrium; however, tendencies for d × treatment interactions for IGF-II and IGFBP-5 occurred because PFA tended to increase their mRNAs on d 15 but not other days. Because the mRNA for these two proteins also increased on d 15 it may be that this period of transition is responsive to omega-3 fatty acid supplementation. There was also a tendency for PFA to increase IGF-I mRNA in the embryo on d 19. The dietary fatty acid fed to the sow could have impacted the fetuses' rate of transcription of mRNA stability of the IGF mRNA by increasing its half-life.

There were day effects detected in the endometrium and conceptus for all the IGF genes studied. In particular the interval from d 11 to d 15 seems to be a period when the IGF system is upregulated in these tissues. In the conceptus IGF-II and IGFBP-3 continued to increase to d 19, while IGFBP-3 showed a similar but less dramatic increase from d 15 to d 19 in the endometrium. These changes occur during a period of blastocyst elongation and attachment, embryogenesis and growth of tissues.

It is important to determine whether maternal fatty acid supplementation can modulate IGF signaling during this critical period of pregnancy. More data on protein expression and potentially including samples for the individual days from d 11 to 19 would expand our ability to evaluate this possibility. It will ultimately be important to evaluate downstream physiologies such as steroid synthesis.