—Original Article—

Expression of Insulin-like Growth Factor (IGF) Family Members in Porcine Pregnancy

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Abstract. Prenatal mortality is a prime concern for commercial swine industry in North America. Fetal losses occur throughout gestation but cluster in early (~day20) and mid (~day50) pregnancy. Adequate vascularization of the attachment site has emerged as a key factor contributing to fetal success. Since Insulin-Like Growth Factor (IGF) family members regulate angiogenesis in addition to promoting fetal development and growth, we hypothesized that conceptus success is governed by members of the IGF family. Using quantitative real time PCR, we analyzed expression of IGF family members (*IGF-I*, *IGF-II*, *IGF-I Receptor (IGF-IR)*, *IGF-IIR* and their binding proteins, *IGFBPs*) in matched maternal and fetal tissues of healthy and arresting conceptuses at gestation days (gd) 20 and 50. *IGF-II* transcripts were 100 fold increased in both maternal and fetal tissues compared to *IGF-I*, but receptor transcripts were found in similar abundance irrespective of health status and gestation point. *IGFBP3* was the most abundantly transcribed of the binding proteins. Using immunohistochemistry we confirmed the expression of IGF family members in maternal luminal and glandular epithelial cells, the endothelium of blood vessels and some scattered stromal cells. Our results suggest that IGF-I and II and their receptors are differentially expressed at the maternal and fetal components of the attachment site.

Key words: Angiogenesis, Epitheliochorial placenta, Insulin like growth factor, Pregnancy, Spontaneous fetal loss (J. Reprod. Dev. 58: 51–60, 2012)

Normal ovulation rates in North American pigs (*Sus scrofa*) lie in the range of 15–16 ova per cycle, and 95% of these fertilize [1,2]. However, after embryos attach to the uterine epithelium, up to 40% of conceptuses are lost over the 114 day course of gestation, resulting in an average live birth rate of 10.5 piglets per gestational period [3]. These fetal losses cluster during the period of endometrial attachment from gestation day (gd) 10–30, in which ~30% of the conceptuses are lost and again between gd50–70 when approximately 10–15% are lost [4]. The loss of these conceptuses has not yet been attributed to any specific factors [3–6].

The development of each placenta occurs in concert with attachment site specific angiogenesis in preparation for the increased demand for nutrition and gas exchange by the growing fetus [7]. Deficits in angiogenesis have been implicated in cases of fetal loss [5–9], and among the many molecules contributing to angiogenesis in the placenta, vascular endothelial growth factor (VEGF) and its receptors VEGFR1 and VEGFR2 are most significant [8, 9]. Other molecules involved in promoting vascular development include placenta growth factor (PlGF), which also binds VEGFR1, and Angiopoietin (Ang)1 and 2 and their receptor Tie2 [10]. Previous studies have determined that the attachment sites of smaller, less developed porcine conceptuses have reduced vasculature as compared to their more robust littermates and that this reduction is associated with reduced transcripts of VEGF and its receptors [5, 6,

8, 9]. Although there was no direct evidence that the arresting conceptuses identified at early or mid-gestation will be lost later during gestation, our studies provided concrete evidence that aberrant angiogenesis at the maternal-fetal interface is a major cause leading to growth arrest of developing conceptuses.

Members of the Insulin-Like Growth Factor (IGF) family are known to influence VEGF expression and thus, angiogenesis [11–13]. The IGFs, similar in structure to insulin, consist of IGF-I and IGF-II which effect their functions upon binding primarily to receptor IGF-IR but also to IGF-IIR [14]. The availability of IGF to their receptors is regulated through binding proteins IGFBP1 through 6 and these are also found in placenta of human and mice [15–17]. IGFs circulate at high concentrations, and stimulate DNA, RNA, and protein synthesis. However, their actions are tissue and cell dependent, thus are considered paracrine or autocrine hormones rather than endocrine [18]. Neither IGF nor their binding proteins cross the placenta [15]. Similar to IGF functions in mice [19, 20], IGF-I and II participate in the regulation of embryonic and fetal growth in porcine pregnancy [21]. IGF-I is most critical in the peri-implantation period; transcripts rises in porcine endometrium at gd12, in response to peak estrogen production by the fetus [22] and coinciding with conceptus elongation. IGF-II transcripts however increase as pregnancy progresses, suggesting that it is more relevant to later growth events [23, 24]. Treatment with somatotropin during pregnancy increase fetal size but does not affect transcription of either IGF-I or IGF-II [25]. In contrast, feed deprivation during pregnancy induces a reduction in IGF-I particularly, but also in IGF-II and results in small for gestation age fetuses [26]. The preferred, but not exclusive receptor for IGF-I,

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Table 1. PCR primer sequences

Gene	Forward primer	Reverse primer
<i>IGF-I</i>	TGCTCTCCTTCACCAGCTCT	GCCTCCTCAGATCACAGCTC
<i>IGF-IR</i>	TGGAGATCATCAGCAGCATC	TCAGCCTTGTGTCCTGAGTG
<i>IGF-II</i>	ACACCCTCCAGTTTGTCTGC	GGGGTATCTGGGGAAGTTGT
<i>IGF-IIR</i>	CAGGAAGTGTCTTCTGAGCA	TGGAATCTGCCTTTTTCACC
<i>IGFBP1</i>	ACGGCATCTTAACCGATGTC	GAAACTTGCTCCACGTGTCA
<i>IGFBP3</i>	GCCTGACTCCAACTCCACT	GGAAGTTGAGGTGGTTCAGC
<i>IGFBP4</i>	CTCCTGCCAGAGTGAGCTG	GGAAGCTTCACTCCCGTCTT
<i>IGFBP5</i>	GGTTTGCCTCAACGAAAAGA	ACGAAGTTGGACTGGGTCAG
<i>IGFBP6</i>	GGGGGAGAATCCTAAGGAGA	GGAAGACCTCGGTCTGGAGT

IGF-IR is present weakly only in endometrial cells at gd20, but has been found to be higher in smaller fetuses than average sized littermates from day 65 through to 100 [26]. IGF-IIR, which preferentially binds IGF-II, is expressed in greater abundance than IGF-IR in both fetal and maternal cells but is highly variable between embryos irrespective of their size [27]. Differential expression of binding proteins 3 and 5 were also noted during late gestation, suggesting that these proteins play a significant role in fetal development [26]. These studies focus on the role of IGFs in later gestation but reports of their presence in early porcine pregnancy have not yet been addressed. Here, we evaluate expression of IGF family members in attachment sites of robust and poorly developed porcine littermate conceptuses during early (gd20) and mid gestation (gd50).

Materials and Methods

Porcine tissue sample collection

Porcine endometrial and trophoblast samples were obtained from specific pathogen-free Yorkshire gilts housed at the Arkell Swine Research Station of the University of Guelph. These studies had been approved by the University of Guelph Animal Care Committee and all work was done in accordance with approved protocols. First time cycling gilts were slaughtered and uteri collected from non-pregnant (NP) (n=8, diestrus), gd20 (n=8) and gd50 (n=8) stages. Immediately following slaughter, uteri were transported on ice, to an RNase decontaminated laboratory. From non-pregnant uteri, random samples of mesometrial endometrium were collected. At gd20 and gd50, healthy and arresting littermates were identified and endometrial and trophoblast samples were taken from the attachment sites. Arresting or dying conceptuses were identified by decreased size and lower placental vascularity at gd20, and decreased conceptus length and weight measurements at gd50 [8, 9]. Samples were collected from three healthy attachment sites, and all arresting conceptus attachment sites per uterus. Each sample was snap-frozen at -80 C for RNA extraction, another was frozen at -80 C in optimal cutting temperature cryomatrix for cryo-sectioning, and the last was placed in 4% paraformaldehyde for paraffin embedded immunohistochemistry.

RNA extraction

Thirty milligrams of minced, frozen tissue was sonicated in a

DNase, RNase-free tube in RNeasy buffer, following manufacturer's instructions (RNeasy, Qiagen, Mississauga, Canada). The cleared tissue lysate was mixed with 700 μ l of 70% ethanol and loaded into an RNeasy mini column. Columns were centrifuged at 8000 g for 30 sec. After washing the column with wash buffer 1 and 2 provided with the kit, RNA was eluted using 50 μ l nuclease-free water by centrifugation at 8000 g for 1 min. RNA concentration and purity was assessed by RNA/DNA calculator and RNA was frozen at -80 C until required.

cDNA synthesis

Following RNA quantification, cDNA was prepared using first strand cDNA synthesis kit (GE Healthcare, Mississauga, ON). Total RNA (1 μ g) extracted from endometrial and trophoblast tissue samples was brought to 20 μ l using nuclease-free water and incubated in a thermocycler (10 min, 65 C). To this, 1 μ l dithiothreitol, 1 μ l Not-1(dT) primer, and 11 μ l bulk first-strand cDNA mix were added. This reaction mixture was incubated (37 C, 60 min, then 90 C, 5 min) and then placed on ice. The resulting cDNA was quantified and stored at -20 C.

Quantitative real time polymerase chain reaction

Primers for genes of interest, listed in Table 1, were designed (Primer3 software: <http://biotools.umassmed.edu/cgi-bin/primer3plus/primer3plus.cgi>) from published porcine templates and purchased from Laboratory Services (University of Guelph, Guelph, Canada). Primers were reconstituted in nuclease free water to a concentration of 0.5 μ M and stored at -20 C.

The PCR was optimized to quantify the target genes relative to the reference gene β actin. A standard curve was prepared from 8 serial dilutions of the PCR product or plasmid inserts of the gene of interest which had been demonstrated to be specific by including a negative and positive controls in the same run, an appropriate size on an agarose gel and sequence confirmed (Laboratory Services, University of Guelph). A mastermix of SybrGreen (LightCycler® 480 SYBR Green I Master, Roche, Mississauga, Canada), 5 nM each of forward and reverse primers was prepared such that each sample could be run in duplicate and three replicates of each dilution of the standards along with a negative (water in place of cDNA) could be run. Eight microlitres of master mix for the gene of interest were pipetted into the first 180 wells of a 384 well plate. Eight microlitres of mastermix for the reference gene were added to

Table 2. Antibodies dilutions

Antibody	Source	Dilution -IHC
Goat polyclonal to IGF-I (ab50179)	AbCam Inc, Cambridge MA, USA	1:200
Rabbit polyclonal to IGF-IR (ab5497)	AbCam Inc, Cambridge MA, USA	1:250
Goat polyclonal to IGF-II (ab10730)	AbCam Inc, Cambridge MA, USA	1:400
Rabbit polyclonal to mannan 6 phosphate R (ab32815)	AbCam Inc, Cambridge MA, USA	1:300
IGFBP1 (H-120) sc-13097	Santa Cruz Biotechnology, CA, USA	1:200
IGFBP3 (H-98) sc-9028	Santa Cruz Biotechnology, CA, USA	1:200
Peroxidase conjugated goat anti-rabbit IgG	Thermo Scientific, Fisher, Mississauga, ON, Canada	1:5000
Peroxidase conjugated rabbit anti-goat IgG	Pierce, Fisher, Mississauga, ON, Canada	1:10000

the next 180 wells. Then 2 μ l of cDNA of each sample were pipetted into 2 wells containing target gene master mix and 2 wells containing reference gene mastermix. Finally, triplicate samples of the standard dilutions for the target gene and for the reference gene were added to the wells containing the matching mastermix. The plate was sealed with a film, briefly centrifuged to and placed in a LightCycler 480 (Roche). A program consisting of denaturation for 5 min 95 C, annealing at 53–57 C, amplification by 45 cycles of 10 sec melt at 95 C, 20 sec and elongation for 15 sec. The final stage was a melting program to determine melting points. The data was analyzed using Roche LightCycler Software 1.5.0.

Immunohistochemistry

Matched endometrial tissues from healthy and arresting conceptus attachment sites fixed in 4% paraformaldehyde were embedded in paraffin. The sections were cut at 7 micron thickness and mounted on positively charged slides (SuperfrostPlus, Fisher Scientific, Mississauga, ON). After deparaffinizing in graded alcohol baths, antigen retrieval was performed using a citrate buffer at 80 C for 20 min. After rinsing in Phosphate buffer saline (PBS), tissues were blocked with 3% H₂O₂ and rinsed in PBS. Sections were then blocked with universal block (Dako Canada, Mississauga, ON, Canada) prior to an overnight incubation in a humidified chamber at 4 C with either an isotype control (rabbit IgG or goat IgG, Dako Canada) or primary antibody (Table 2). The following morning, slides were rinsed and a biotinylated secondary antibody was applied (Dako Canada) for 15 min. After rinsing with PBS, the provided streptavidin peroxidase solution was applied for 10 min. Slides were then rinsed again before applying the substrate-chromagen solution. After colour development, the solution was rinsed off with distilled water. Specimens were then counterstained with hematoxylin (Fisher Scientific) for 2 min, rinsed, then dipped 10 times into a bath of 1% HCl and in 70% isopropanol prior to dehydration through a series of graded alcohol and xylene baths before mounting with Cytoseal XYL (ThermoScientific). Slides were examined under microscopy (Leica DMLB) and photographed using QCapture Pro software (QImaging Corporation, Surrey, BC, Canada).

Statistics

Data generated by Quantitative PCR were log transformed to achieve normal distribution, and analyzed by two-way ANOVA with a Holm-Sidak post-test. $P < 0.05$ was considered significant.

Results

Analysis of IGF family members by quantitative real time PCR

PCR analysis was completed for all genes of interest, with the exception of *IGFBP2*, which repeatedly failed to amplify product in pooled cDNA from porcine samples. Negative controls, positive samples to create a standard series, and sequencing were completed for all other genes. Nucleotide sequences were submitted to BLAST (Basic Local Alignment Search Tool) to confirm identity of the genes of interest. All returned a 98% or higher homology to the target genes, with the exception of *IGFBP4*, which returned a 92% homology.

IGF-I was detected in all tissues examined (Fig. 1A), and was transcribed at consistent levels relative to β *actin* in both maternal and fetal tissues at both studied timepoints in gestation. However, there was a significant decline in *IGF-I* transcription at gd20 endometrium from healthy attachment sites relative to virgin endometrium ($P = 0.004$) and to gd20 endometrium from arresting sites ($P = 0.03$). No significant differences were found in *IGF-I* transcripts between healthy and arresting conceptuses in gd20 trophoblast and gd50 endometrial and trophoblast samples. On the other hand, *IGF-II* transcripts showed great variability between tissues and between timepoints (Fig. 1B), but was consistently much higher than for *IGF-I*. *IGF-II* transcripts decreased in trophoblast samples between gd20 and gd50 ($P = 0.027$) and endometrium between the same timepoints ($P = 0.030$).

As shown in Fig. 1C, *IGF-IR*, which preferentially binds *IGF-I*, was down-regulated between gd20 and gd50 in trophoblast, but not in endometrial samples ($P = 0.014$). The preferred receptor for *IGF-II*, *IGF-IIR*, did not differ between these timepoints in maternal tissues, or between healthy and arresting littermates (Fig. 1D).

Transcripts of *IGFBP1* remained consistent in all tissues regardless whether of maternal or fetal origin, or of the health status of the conceptus, or of the timepoint of gestation (Fig. 2A). Relative to β *actin*, *IGFBP3* was transcribed at higher rates than *IGFBP1*. Transcripts were found to be increased in trophoblast from arresting relative to healthy conceptuses at gd50 ($P = 0.05$), but this was not seen at gd20 (Fig. 2B). Significant differences were also found in the relative amounts of *IGFBP4* expression (Fig. 2C) in the endometrial samples between gd20 and gd50 ($P = 0.004$). Analyses of *IGFBP5* showed only one significant difference; transcription of *IGFBP5* (Fig. 2D) declined in endometrium after pregnancy was established ($P = 0.020$). Likewise *IGFBP6* transcripts (Fig. 2E) in

Fig. 1. Quantitative PCR analyses of IGF family members in porcine reproductive tissues. Relative quantification was done by comparing the crossing points of the genes of interest IGF-I (A), IGF-II (B), IGF-IR (C), IGF-IIR (D) with reference gene, β actin in the same sample and are presented as a ratio. Each tissue type was assessed in samples from eight animals. The first box represents data from virgin endometrium, followed by endometrium from healthy and arresting conceptus attachment sites at gd20, endometrium from healthy and arresting conceptus attachment sites at gd50, trophoblast from healthy and arresting conceptus attachment sites at gd50. All data was log transformed and analysed by two way ANOVA. A difference of $P < 0.05$ was considered significant.

gd20 endometrium declined compared with endometrium from non-pregnant animals ($P = 0.004$).

Localization of IGF family members using immunohistochemistry

Figures 3, 4 and 5 show immunolocalization of IGF family members in porcine endometrium obtained from healthy and arresting porcine conceptus attachment sites at gd20 and gd50. Overall,

no difference in staining intensity for IGF-I was found between gd20 and gd50 endometrium, or between healthy and arresting attachment sites. At gd20, IGF-I was detected in the endothelial cells of endometrial blood vessels (red arrows, Fig. 3A). Positive staining for IGF-I was also found in the endometrial glands (green arrows) and in several scattered stromal cells (Fig. 3A and 3B). At gd50, all endothelium (red arrow) and epithelium (blue arrow) appeared positive for IGF-I (Fig. 3D), but at arresting conceptus

Fig. 2. Quantitative PCR analyses of IGF binding protein family members in porcine reproductive tissues. Crossing points of the gene of interest IGFBP1 (A), IGFBP3 (B), IGFBP4 (C), IGFBP5 (D), IGFBP6 (E) were compared with the crossing point of a reference gene (β actin) generated by the same sample and are presented as a ratio. Each tissue type was assessed in samples from eight animals. Amongst all the IGFBPs studied, transcripts for IGFBP3 were found to be increased in trophoblast from arresting conceptus attachment sites relative to healthy conceptuses at gd50 ($P < 0.05$). No significant differences were observed for any of the IGFBPs in endometrium or trophoblast samples between healthy and arresting conceptus attachment sites at gd20 and gd50.

Fig. 3. Immunohistochemical localization of IGF-I (A–E), and IGF-II (G–L) in porcine endometrium from healthy and arresting conceptus attachment sites at gd20 and gd50. Overall, no difference in staining intensity for IGF-I was found between gd20 and gd50 endometrium, or between healthy and arresting attachment sites. IGF-I was mostly detected in the endothelial cells blood vessels, while IGF-II expression was restricted mainly to luminal and glandular epithelium. Red arrows indicate positive blood vessel staining, green arrows endometrial glands and blue arrows luminal epithelium. Size bars represent 100 μ m.

attachment sites stromal cells no longer stained positive for IGF-I (Fig. 3E). At gd20, IGF-II expression was restricted mainly to luminal and glandular epithelium (blue and green arrows respectively, Fig. 3G, H, J and K), but a few blood vessels also appear to

express it (red arrow). Similar staining was also found at gd50 in blood vessels glands and luminal epithelium (Fig. 3J).

At gd20, in endometrium sections, IGF-IR staining was strong in both healthy and arresting sections and localized to luminal epithe-

Fig. 4. Immunohistochemical localization of IGF-IR (A–E) and IGF-IIR (G–L) in porcine endometrium from healthy and arresting conceptus attachment sites at gd20 and gd50. IGF-IR and IIR staining was localized in endometrial sections from both healthy and arresting conceptus attachment sites. Predominantly, luminal epithelium (blue arrows), glandular epithelium (green arrows) and vascular endothelium (red arrows) were found positive for both IGF-IR and IIR. Size bars represent 100 μ m.

lium (blue arrows), glandular epithelium (green arrows) and vascular endothelium (red arrows, Fig. 4A and B). In addition, stromal cells also showed positive staining (Fig. 4B). IGF-IR was still present in gd50 endometrial sections and localized to glandular

and luminal epithelium of healthy tissues (Fig. 4D). Gestation day 20 endometrium demonstrated strongly positive luminal (blue arrow) and glandular (green arrow) epithelial cell staining for IGF-IIR (Fig. 4G and H). At gd50, positive staining was restricted to

Fig. 5. Immunohistochemical localization of IGFBP1 (A–E) and IGFBP3 (G–L) in porcine endometrium from healthy and arresting conceptus attachment sites at gd20 and gd50. IGFBP1 was ubiquitously expressed in stroma, endothelium and luminal epithelium. IGFBP3 on the other hand was strongly expressed in all the endometrial compartments at gd20 but staining appeared weak at gd50. Red arrows indicate positive blood vessel staining, green arrows endometrial glands and blue arrows luminal epithelium. Size bars represent 100 μ m.

luminal and glandular epithelium (blue and green arrows respectively in Fig. 4K).

At gd20 in both healthy and arresting conceptus attachment site tissues, the endometrial glands stained strongly for IGFBP1 (green

arrow) while vascular endothelium is only faintly positive at both (red arrow in Fig. 5A and B). Luminal epithelium is also positive at gd20. In gd50 endometrium staining is less intense in the glands and endothelial cells (Fig. 5D and E). At gd20 IGFBP3-positive

endometrial glands are seen along with strongly positive blood vessels, and luminal epithelium at both healthy and arresting sites (Fig. 5G and H). At gd50 there is only weak positive staining in luminal and glandular epithelium (blue and green arrows respectively) in arresting endometrium, with almost no positive staining in healthy endometrium (Fig. 5J and K).

Discussion

Deficits in angiogenesis at the maternal-fetal interface are one of the prime causes associated with gestational losses in pigs around gd15–25, while mid-gestational losses around gd50 appear to have etiologies particularized to their timepoint [28]. The IGF family is highly implicated in both fetal development and regulation of placental development, including angiogenesis, but previous pig specific studies have focussed on the concentration of IGFs in normal porcine endometrium in the peri-attachment period [23] and on its regulation by endocrine factors produced by the fetus [24, 29]. Here, we took advantage of the clear separation of maternal and fetal tissues found in the epitheliochorial placenta of pigs to design a study of maternal cells and facing fetal cells of two littermates, one well-developed and one poorly developed for gestational age, from each of eight pigs to investigate the potential contributions of the IGF family in establishing optimal attachment sites in porcine pregnancy.

Endometrial transcripts of *IGF-I* and *II* relative to a housekeeping gene are similar in endometrium sampled during diestrus. However, once pregnancy is established, endometrial transcripts of *IGF-II* greatly exceed those of *IGF-I*, and remained much higher despite a decline in transcription at gd50. In contrast, *IGF-II* transcripts in trophoblast, while still exceeding *IGF-I*, are much lower than their neighbouring endometrial cells, with the exception of samples of trophoblast from arresting fetuses at gd20. In any case, transcripts in either endometrium or trophoblast did not differ between healthy versus arresting attachment sites. Serum concentrations of *IGF-I* have been reported to peak at gd12, the time of conceptus elongation, then decline as pregnancy progresses [23]. We did not find a significant decline in *IGF-I* between gd20 and 50. *IGF-II* is 70% similar in structure to that of *IGF-I*. *IGF-II* is considered to be the key regulator of trophoblast development in the human, guinea pig and mouse, as well as the main factor leading to increased nutrient transfer and fetal weight in guinea pigs [11, 30, 31]. Both *IGF-I* and *II* are reported to induce *VEGF* expression [13], to promote cellular differentiation and migration. Decline in these molecules could be expected to be associated with increased cell death, but this was not seen here.

Studies in primates, rodents and ruminants suggest that neither *IGF-I* nor *IGF-II* cross the placenta [15] so IGF effects are considered highly localized. These effects are realized through binding to their receptors *IGF-IR* and *IGF-IIR*, which are present on target cell membranes. *IGF-I* preferentially binds *IGF-IR*, while *IGF-II* prefers *IGF-IIR*, but each is capable of binding with the other [30]. A decline in *IGF-IR* transcripts between gd20 and gd50 was detected in trophoblast samples but no difference was found in *IGF-IIR* expression. Both were transcribed at similar levels rela-

tive to β -actin. *IGF-IR* is expressed on endometrium and *IGF-II* on both endometrial and trophoderm cells in very early porcine pregnancy (gd8–10) but their relationship to embryonic health is not clearly established [27]. In mice, expression of *IGF-IIR* is highest during early pregnancy, when the most trophoblast proliferation and migration is occurring [11]. In contrast, it has also been reported that the expression of murine *IGF-IR* does not change significantly during fetal development [16]. The role of IGF receptors in endometrium are not defined, but the clear dominance of *IGF-IIR* over *IGF-IR* in endometrial samples described here suggest that *IGF-IIR* may be quite significant, particularly in early pregnancy, when a decrease in endometrial expression is associated with arresting attachment sites.

The IGFs circulate at high concentrations, either free with a 15–20 min half-life, or bound to an IGFBP acting as a carrier. Six IGFBPs have been characterized and a seventh has recently been identified, but its function is unclear [32]. IGFBPs act as carriers for IGF-I and II, generally reducing their bioavailability, but sometimes enhancing their activity. While IGFBP1 through 4 all have approximately equal affinity to IGF-I and II, IGFBP5 and 6 bind IGF-II with 10 and 100 times higher affinity respectively [30]. Two mechanisms determine interactions between IGFs and IGFBPs: proteolytic cleavage which decreases IGF binding affinity, and binding of the IGFBP to the extracellular matrix which potentiates IGF actions. We found very little variation in transcription of the IGFBP in the porcine samples. IGFBP1 is reportedly not found in porcine endometrium [23, 33] but we detected IGFBP1 transcripts at the lowest level of any of the binding proteins and located IGFBP1 expression by immunohistochemistry in gd20 and 50 in endometrial epithelium, stroma and arteries. IGFBP3 was transcribed at higher levels relative to β actin than other binding proteins. Almost 80% of circulating IGFs are bound to IGFBP3 [30], the major binding protein involved with IGF functions. Significantly more IGFBP3 transcripts are present in arresting conceptus trophoblast tissue at gd50 compared to healthy, suggesting potential sequestration of IGFs. IGFBP4 was the only binding protein which altered transcripts between timepoints of pregnancy, increasing at gd50. Both IGFBP5 and IGFBP6 showed a decline in transcript numbers after pregnancy was established, but did not differ between healthy and arresting conceptus attachment sites and remained low at gd50. It has been reported that IGFBP2 and 3 disappear from uterine flushings after gd12, in response to increasing progesterone [34]. Our findings suggest that transcription of IGFBP3 is consistent between gd20 and 50, but recovers in IGFBP4 at gd50 and remains depressed in IGFBP5 and 6. An increase in binding proteins decreases the immediate bioavailability of IGF-I and IGF-II, but preserves the molecules from degradation. From this data, IGFBP3 appears to have the significant role in preserving IGF molecules in porcine pregnancy.

In summary, this study was designed to examine the expression of the IGF family in adjacent healthy and arresting conceptus attachment sites in pig pregnancy. Our results suggest that IGF-I and II and their receptors are differentially regulated at the maternal and fetal components of the attachment site.

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