Association of Endocrine Factors (Insulin-Like Growth Factor-II and Binding Protein-3) with Litter Size in Pigs

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ABSTRACT : Litter size has been one of the important economic traits in porcine reproduction. The insulin-like growth factor (IGF) system has been shown to mediate actions of the steroid hormone or to synergize with other endocrine factors so that it consequently plays roles in reproductive processes, including ovulation, implantation, maintenance of pregnancy, and fetal development. However, the effect of the serum IGF system on porcine litter size has not been deeply studied. Therefore, this study was conducted to relate serum IFG-II concentration and IGF binding protein-3 (IGFBP-3) expression with porcine litter size. Moreover, the possible association of those with estrogen receptor (ER) as a candidate gene for litter size was investigated. Swine were separated into two groups showing high and low litter sizes, and sera were collected from sows in the estrous cycle to postnatal growth of their female progeny. Serum IFG-II concentration was measured by radioimmunoassay and IGFBP-3 expression was detected by Western ligand blotting. During the estrous cycle, IGFBP-3 expression in both groups decreased moderately from metestrus to estrus, but IFG-II concentration showed a reverse pattern. Also, IFG-II concentration and IGFBP-3 expression decreased gradually as pregnancy proceeded. Unlike IGFBP-3, IFG-II decreased moderately as newborn pigs grew. Significant differences in serum IFG-II amount between the two groups were detected at 60 ($p<0.01$), 75, 90, and 105 d ($p<0.05$) of pregnancy and at 60 ($p<0.01$), 45, and 105 d ($p<0.05$) of postnatal growth. Furthermore, based on ER genotypes, a high litter size group with genotypes AB and BB showed lower IFG-II concentration than a low litter size group with a genotype AA during pregnancy. Taken together, the results indicate that the serum IFG-II and IGFBP-3 are correlated with the litter size in pigs. (*Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 3 : 307-315*)

Key Words : Pig, IGF-II, IGFBP-3, ER, Litter Size

INTRODUCTION

Growth factors are multifunctional proteins that affect the synthesis of regulatory proteins for cell proliferation and/or differentiation. With this capacity, growth factors, especially insulin-like growth factors (IGFs), may constitute cellular signals to facilitate the coordinate progression of development in the embryo and uterus (Jones and Clemmons, 1995).

IFG-II is a single-chain polypeptide of 67 amino acids found in high concentrations in fetal and neonatal sera of rodents, and the transcription of its gene is strongly down-regulated postnatally, whereas in humans IFG-II levels increase after birth (Moses et al., 1980; Zapf et al., 1981). IFG-II is found in plasma as well as a variety of biological fluids and is known to reach the target tissue in endocrine as well as paracirne/autocrine manner (Daughaday et al., 1989).

Like IFG-I, IFG-II expression has been shown to be stimulated or inhibited by IGF binding proteins (IGFBPs). In particular, IGFBP-3, a 43 - 39 kDa peptide, plays several important endocrine roles. First, IGFBP-3 functions as a transporter that carries IGFs and regulates the efflux from the vascular system (Zapf et al., 1984). Second, IGFBP-3 prolongs the half-life of IGFs, regulating the metabolic clearance. Third, it provides the tissue- and cell type-specific localization of IGFs. In addition, IGFBP-3 directly regulates receptor binding so that it ultimately regulates biological actions of IGFs. Finally, binding proteins themselves affect cellular functions independent of IGFs. Also, IGFs and IGFBP-3 constitute a 150 kDa complex with an acid labile subunit that can stabilize the IGF binding complex (Furlanetto, 1980).

On the respect of animal breeding and feeding management, several studies about litter size have been reported. However, because the litter size has a low heritability and depends on other factors such as environment, these studies posses some limitation. Recently, it has progressed to the study of fertilization, ovulation-rate, embryo viability, and uterus capacity. In particular, Young et al. (1993) studied ovulation-rate as how it offers the first chance for increasing the litter size. Also, Johnson et al. (1999) reported that ovulation-rate has a positive correlation with embryo survival. On the other hand, there was a report that the litter size was related to uterus capacity including secretion, size and shape (Ribeiro et al., 1997). But, though the above three factors (ovulation-rate, embryo survival, and uterus capacity) have partial relationships

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with litter size, further studies are required to clearly define any correlations. Therefore, this study was performed to relate serum IFG-II concentration and IGFBP-3 expression with porcine litter size and to investigate the possible association of those endocrine factors with estrogen receptor (ER) as a candidate gene for the litter size.

MATERIALS AND METHODS

Animals

The high litter size group was comprised of Yorkshire producing a total number born per litter (TNB) higher than 12; sows with TNB less than 11 were included in the low litter size group.

In each group, blood was collected from the jugular veins of pigs in the estrous cycle (proestrus, estrus, and metestrus), in pregnancy (45, 60, 75, 90, and 105 d), and from their female progeny at ages of 30, 45, 60, 75, 90, and 105 d. Sera were made by allowing blood samples to stand at room temperature for 1 to 2 h, to be centrifuged at 1,000×g for 15 min, and stored at -20℃.

IFG-II iodination

One microgram of recombinant human IFG-II (GroPep Pty Ltd.) was iodinated to a specific activity of 270~300 μCi/μg protein using 1 mCi Na ^{125}I (Amersham) by the chloramine T method (Lee and Henricks, 1990). Iodinated IFG-II was purified on a Sephadex G-50 column and aliquots were stored at -20℃ until used.

IFG-II radioimmunassay (RIA)

Serum IGFBPs were removed using the acid-ethanol method (Daughaday et al., 1980). Briefly, 0.2 ml of each sample was acidified with 0.8 ml of acid-ethanol (87.5% ethanol, 12.5% HCl) and stabilized for 30 min at room temperature. After samples were centrifuged at 1,000×g for 30 min, they were neutralized with 0.2 ml of 0.855 M Tris-base. IGFBPs-removed supernatant was added to 0.1 ml of RIA buffer (30 mM sodium phosphate, 0.02% protamine sulfate, 10 mM EDTA, 0.05% Tween-20, 0.02% sodium azide; pH 7.5), and incubated with rabbit anti-human IFG-II polyclonal antiserum (GroPep Pty Ltd.; final dilution of 1:10,000) and 15,000~20,000 cpm [^{125}I] IFG-II in RIA buffer for 16 h at 4℃. Then, 0.1 ml of goat anti-rabbit IgG antibody (GroPep Pty Ltd.) was added and the mixture was incubated for 1 h followed by additional incubation with 0.1 ml of normal rabbit serum at 4℃. After the addition of 1 ml RIA buffer, the tubes were centrifuged for 30 min at 1,000×g at 4℃. The supernatant was aspirated and the pellet was measured in a gamma-counter. IFG-II amount was determined by logit - log plots, and the intra assay and inter assay coefficients of variation were 3% and 3.8%, respectively.

[^{125}I] IFG-II Western ligand blotting

[^{125}I] IFG-II Western ligand blotting was performed as described previously (Hossenlopp et al., 1986). Briefly, samples were heated at 95℃ with a 2x nonreducing sample buffer for 3 min and subjected SDS-PAGE on gels consisting of a 5% stacking gel and a 12.5% separating gel in electrophoresis buffer (25 mM Tris, 192 mM glycine, 0.1% SDS ; pH 8.3). Separated protein bands were electrophoretically transferred onto a nitrocellulose membrane in the presence of transfer buffer (25 mM Tris, 192 mM glycine, 20% methanol ; pH 8.3). After transfer, the membrane was dried for 30 min at room temperature and rinsed with TBS (0.01 M Tris, 0.15 M NaCl, 0.05% NaN_3 ; pH 7.4). The rinsed membrane was then incubated with 1% blotto and washed with TBS - 0.1% Tween-20. The membrane was incubated for 5 h at room temperature with TBS - 0.1% Tween-20, 1% BSA, and 0.5 to 1.0×10^6 cpm [^{125}I] IGF-II. The membrane was then rinsed two times (15 min each) in TBS - 0.1% Tween-20 and rinsed twice (15 min each) in 0.1% TBS. The membrane was dried and exposed onto Kodak X - Omat AR film at -80℃ for 7 d using intensifying screens (Kasei Optonix, Ltd. Tokyo, Japan). A scanning densitometry (MultiImage™ Light Cabinet, Alpha Imager 2200, Alpha Innotech Co.) of the autoradiograph was used to obtain relative IGFBP-3 levels.

DNA extraction and PCR-RFLP

Porcine genomic DNA was extracted from the clotted blood (Seo et al., 1999). In brief, 250 μl of lysis solution (360 μg/ml proteinase K, 150 mM sodium chloride, 50 mM EDTA, 2% SDS) was mixed to lyse the clotted blood, and the mixture was incubated at 55℃ for 3 h. After 5.5 M NaCl and 600 μl of phenol : chloroform (25:24) were added, it was centrifuged for 10 min at 5,000×g. The supernatant was mixed with absolute ethanol and the mixture was centrifuged again at the same condition. The pellet was dried, resuspended with TE solution (10 mM Tris-Cl, 1 mM EDTA) and stored at -20℃.

ER genotypes were analyzed by PCR-RFLP using primers as reported (Short et al., 1997). Primers were as follows : Forward 5'- CCT GTT TTT ACA GTG ACT TTT ACA GAG - 3', and Reverse 5'- CAC TTC GAG GGT CAG TCC AAT TAG - 3'. The reaction was performed with AccuPower™Premix-Top (BIONEER Co., Korea) including 50 ng of extracted DNA under the following conditions : 1 cycle at 94℃ for 4 min, 55℃ for 1 min, and 70℃ for 1 min; 31 cycles of 94℃, 55℃ for 1 min, and 70℃ for 1 min;

1 cycle at 72℃ for 8 min ; hold at 4℃ (Short et al., 1997). The PCR reaction was done with a GeneAmp PCR System 2400 (Perkin Elmer Co.). RFLP was performed by adding 0.5 μl of *Pvu*II to each sample followed by incubation at 37℃ overnight. DNA samples were separated using 12.5% polyacrylamide gel electrophoresis.

Statistical analysis

Using the Duncan method of the one way ANOVA procedure in SAS package (1995), a comparison of porcine serum IFG-II concentration between high and low litter size groups from estrous cycle to growth of newborn was analyzed. Also, data were analyzed according to ER genotypes.

RESULTS

IFG-II radioimmunoassay

Serum concentration of IFG-II in the high litter size group decreased from metestrus to estrus, whereas IFG-II expression in the low litter size group increased (table 1). In general, the high litter size group showed higher levels of IFG-II than the low litter size group. Although significant differences in IFG-II amount between the two groups during the extrous cycle were not detected, the IFG-II of the high litter size group at proestrus showed the highest concentration at 489.8 ng/ml, and at metestrus. The IFG-II of cycling gilts in the low litter size showed the lowest concentration at 269.7 ng/ml.

Table 2 shows serum IFG-II concentrations in both groups during pregnancy (45, 60, 75, 90 and 105 d). Generally, the serum IFG-II of pregnant pigs increased in both groups from 45 until 105 d. Contrary to the IFG-II profile during the estrous cycle, serum IFG-II concentration of the low litter size group was larger than that of the high litter size group. Significant differences ($p<0.05$) in IFG-II amount between high and low litter size groups were detected at 75 (344.6 vs. 406.9 ng/ml), 90 (384.5 vs. 494.2 ng/ml), and 105 d (407.3 vs. 550.4 ng/ml). Especially, the mean serum IFG-II concentration showed the most significant difference between high and low litter size groups (317.6±89.4 ng/ml and 436.6±79.5 ng/ml; $p<0.01$) at 60 d.

Table 1. Comparison of porcine serum IFG-II concentrations between high and low litter size groups during estrous cycle

	Serum IFG-II concentration (ng/ml)		
	Metestrus	Proestrus	Estrus
High litter size (n=5)	471.0 ±210.2	489.8 ±243.3	423.0 ±103.7
Low litter size (n=3)	269.7 ±79.8	357.0 ±48.1	356.0 ±30.4

Note: No differences were detected between high and low litter size groups during the estrous cycle.

The concentrations of serum IFG-II in both groups decreased as newborn pigs grew (table 3). Like the IFG-II pattern of pregnant piglets, the IFG-II level in the low litter size group was 1.5 fold higher than that in the high litter size group. Significant differences ($p<0.05$) in IFG-II amount between high and low litter size groups were detected at ages of 45 d (638.6 vs. 948.8 ng/ml) and 105 d (523.3 vs. 707.7 ng/ml). The mean serum IFG-II concentration showed the most significant difference between high and low litter size groups (623.8±120.7 ng/ml and 926.3±137.2 ng/ml; $p<0.01$) at 60 d.

Western ligand blotting of IGBP-3

The expression of IGFBP-3 between the high and low litter size groups from metestrus to estrus by Western ligand blotting is shown in figure 1 and table 4 A. Serum IGFBP-3 of cycling gilts in both groups was expressed by 43 kDa and 39 kDa doublet bands during the estrous cycle (figure 1). With an exception at estrus, the expression of IGFBP-3 in the low litter size group was greater than that in the high litter size group through the entire period. Also, the IGFBP-3 levels of the high litter size group did not change, whereas the low litter size group showed a declining tendency from metestrus to estrus (table 4 A).

Gilts from 45 to 105 d of pregnancy in both groups also expressed IGFBP-3 (figure 2, table 4 B). Especially, the IGFBP-3 expression of the low litter size group at 45 d is more intense than at any time during pregnancy (figure 2). Differences in IGFBP-3 expression between the two groups during pregnancy

Table 2. Comparison of porcine serum IFG-II concentrations between high and low litter size groups during pregnancy

	Serum IFG-II concentration (ng/ml)				
	Day 45	Day 60	Day 75	Day 90	Day 105
High litter size (n=12)	335.3 ± 66.7	317.6 ± 89.4[a]	344.6 ± 61.7[c]	384.5 ± 67.8[c]	407.3 ± 93.3[c]
Low litter size (n=10)	390.2 ± 89.9	436.6 ± 79.5[b]	406.9 ± 55.8[d]	494.2 ± 117.1[d]	550.4 ± 162.8[d]

[a,b] Means±SD within a column with different superscripts differ ($p<0.01$).
[c,d] Means±SD within a column with different superscripts differ ($p<0.05$).

Table 3. Comparison of porcine serum IFG-II concentrations between newborn female piglets from high and low litter size groups at ages from 45 to 105 days

	Serum IFG-II concentration (ng/ml)				
	Day 45	Day 60	Day 75	Day 90	Day 105
High litter size (n=5)	1079.0 ±202.0	638.6 ± 85.8[c]	623.8 ± 120.7[a]	452.3 ±89.5	523.3 ± 25.3[c]
Low litter size (n=5)	1227.3 ±374.0	948.8 ±287.2[d]	926.3 ± 137.2[b]	617.0 ±78.9	707.7 ± 106.5[d]

[a,b] Means ± SD within a column with different superscripts differ ($p<0.01$).
[c,d] Means ± SD within a column with different superscripts differ ($p<0.05$).

were smaller than during the estrous cycle. The expression of IGFBP-3 in the high litter size group decreased at 60 d, and was then sustained. However, its expression in the low litter size group changed little from 45 to 105 d (table 4 B).

As shown in figure 3, the expression of IGFBP-3 in newborn pigs was detected as 43 kDa and 39 kDa doublet bands, which increased as newborn pigs grew. The IGFBP-3 expression at 90 d from the low litter size group was the most intense (table 4 C). Overall, the IGFBP-3 levels in the lower litter size group were more intense than in the high litter size group during newborn pig growth stages, showing 1.5 fold higher expression.

Comparison of IFG-II concentration by ER genotype

Chromosomal DNA fragments digested by *PvuII* were separated into three subtypes for ER (AA genotype : 120 bp, AB genotype : 120 bp and 65 bp and BB genotype : 65 bp and 55bp ; Short et al., 1997). Based on the analyses of estrogen receptor (ER) genotypes as a candidate gene for the litter size, pigs were separated into two groups showing high and low litter sizes. As pregnancy proceeded, the concentration of IFG-II in each group was moderately increased (table 5). Although significant differences among genotypes were not detected, the concentration of IFG-II in the AA genotype known for low litter size (Park et al., 2000) was higher than that of the BB genotype known for high litter size from 60 to 105 d.

The serum IFG-II concentrations during growth of the newborn piglets were compared on the basis of ER genotypes (table 6) and piglets were separated into three groups. Generally, IFG-II concentrations in newborn piglets declined with age, but significant differences between the two groups based on genotypes were not detected.

DISCUSSION

In the present study, circulating levels of IFG-II and IGFBP-3 in pigs from the estrous cycle stage to the growth of newborns were measured to investigate differences between high and low litter size groups.

Table 4. Comparison of IGFBP-3 density between high and low litter size groups from estrous cycle to growth of newborn

A. IGFBP-3 density during estrous cycle

	IGFBP-3 (mean density ± SD)		
	Metestrus	Proestrus	Estrus
High litter size (n=5)	64.4 ± 36.2	61.2 ± 28.4	65.8 ± 43.1
Low litter size (n=3)	67.0 ± 25.6	63.0	53.0 ± 12.5

B. IGFBP-3 density during pregnancy

	IGFBP-3 (mean density ± SD)				
	Day 45	Day 60	Day 75	Day 90	Day 105
High litter size (n=12)	20.1 ± 7.1	17.0 ± 6.1	17.3 ± 5.5	16.5 ± 5.2	15.9 ± 6.8
Low litter size (n=10)	15.0 ± 3.4	13.8 ± 3.3	13.4 ± 4.2	14.2 ± 3.0	13.2 ± 2.8

C. IGFBP-3 density during growth of newborn

	IGFBP-3 (mean density ± SD)					
	Day 30	Day 45	Day 60	Day 75	Day 90	Day 105
High litter size (n=5)	23.4 ± 17.4	23.6 ± 15.3	36.4 ± 28.5	37.1 ± 27.6	44.3 ± 33.3	69.7 ± 30.3
Low litter size (n=5)	25.6 ± 10.1	42.4 ± 24.0	55.4 ± 28.3	56.2 ± 30.4	72.6 ± 26.4	62.7 ± 33.0

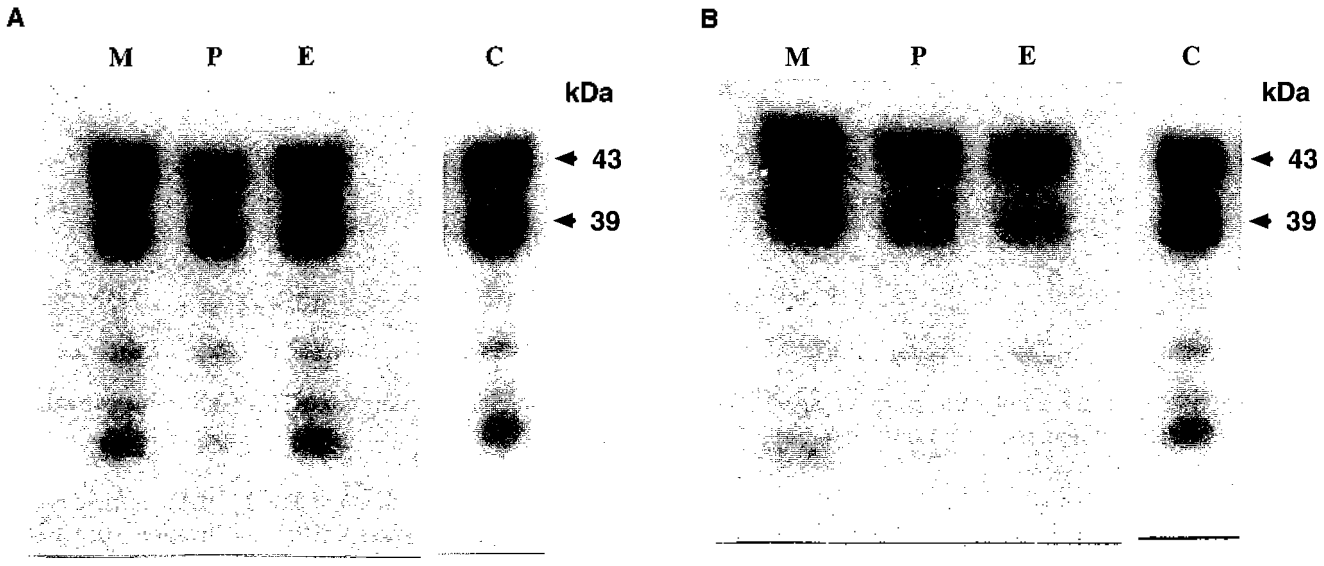

Figure 1. Representative autoradiogram of an [^{125}I] IGF-II Western ligand blotting for IGFBP-3 during estrous cycle. A : High litter size, B : Low litter size. * Abbreviation: M (metestrus), P (proestrus), E (estrus), C (control).

Although concentrations of IFG-II in cycling gilts did not show either increasing or declining tendencies during the cycle (table 1) in both groups, the expression of IFG-II increased throughout pregnancy period in dams (table 2) and rapidly decreased as the growth of newborns proceeded (table 3). Similar studies have reported that the placental expression of IFG-II mRNA in pregnant rats increases, beginning at 13 d, and reaches maximal levels at 17 to 20 d (Pescovitz et al., 1991), and that IFG-II levels are high during fetal life and decrease immediately after birth in rats and sheep (Moses et al., 1980; Mesiano et al., 1989). Moreover, Carr et al. (1995) reported that circulating IFG-II and IGFBP-2 are high in the fetus in mid to late gestation and decrease towards adult levels near term, while circulating IFG-I and IGFBP-3 are lower in early to mid gestation and increase throughout gestation to reach adult values. Contrary to the IFG-I profile during pregnancy (Yang et al., 1999; figure 4), serum concentration of IFG-II in the low litter size group is higher than that in the high litter size group (table 2). These reverse patterns between IFG-I and -□ profile could be explained by the observation that increased maternal IFG-I concentration may exhibit negative feedback on maternal IFG-II concentration, as reported by Sterle et al. (1995). IFG-I, acting as a placental growth factor, is a known stimulator of pig conceptus P450

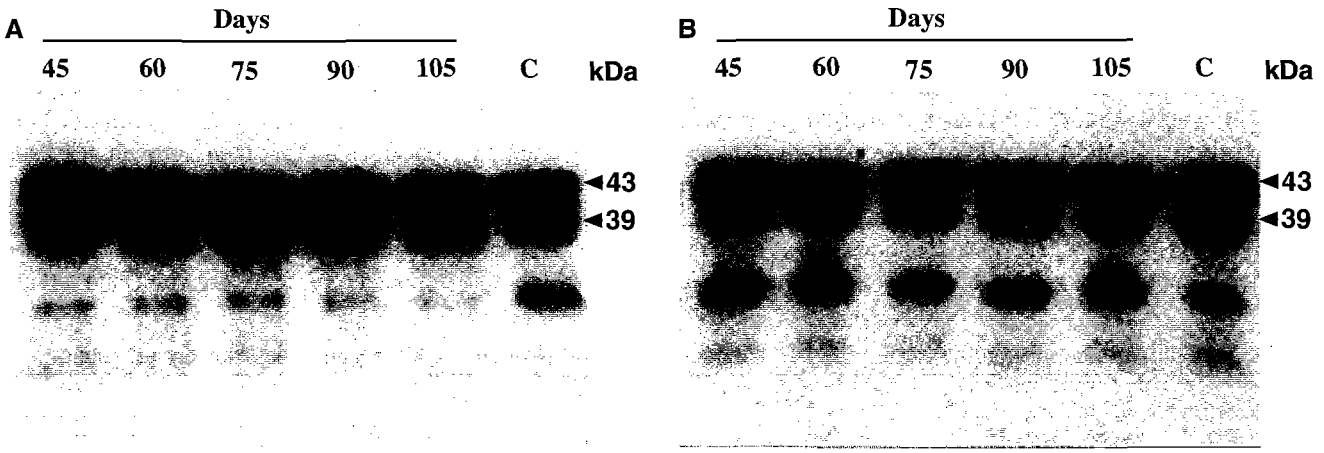

Figure 2. Representative autoradiogram of an [^{125}I] IFG-II Western ligand blotting for IGFBP-3 during pregnancy. A: High litter size; B: Low litter size; C: control.

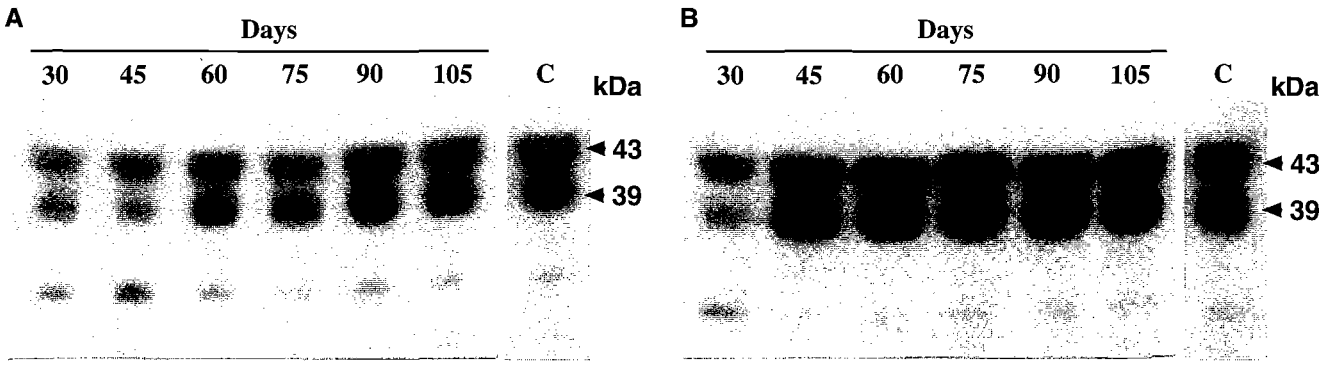

Figure 3. Representative autoradiogram of an [^{125}I] IFG-II Western ligand blotting for IGFBP-3 during growth of newborn. A: High litter size; B: Low litter size; C: control.

aromatase activity (Hofig et al., 1991; Simmen et al., 1992). IFG-II is also postulated to function as a placental growth factor; it has been shown to inhibit human placental P 450 aromatase activity *in vitro* (Fant et al., 1986; Nestler, 1990; Hofig et al., 1991). So, IGFs regulate the expression of E_2 that plays important roles for fetus growth and environments, which results in providing the most suitable environment to fetus and mother.

Significant differences in IFG-II concentration between high and low litter size groups in pregnancy were detected from 60 d until parturition (table 2), especially at 60 d (317.6±89.4 ng/ml and 436.6±79.5 ng/ml; $p<0.01$). Compared with IFG-I level, it is of interest that the IFG-II levels of pregnant pigs are not significantly different until mid-pregnancy. This may be related to the differential expression of endometrial IFG-I and IFG-II mRNAs during pregnancy, suggesting preferential roles for IFG-I at preimplantation and for IFG-II at post-implantation stages, respectively (Simmen et al., 1992).

During growth of newborns, expressions of IFG-II in both groups were higher than those of IFG-I (table 3). Similar results were also reported that circulating concentrations of IFG-II are higher than those of IFG-I during postnatal life in humans, sheep, and cattle (Zapf et al., 1981; Mesiano et al., 1989). However, concentrations of IFG-II decreased as growth proceeded, while those of IFG-I increased moderately for the increased endocrine mode of IFG-I action (Moses et al., 1980; Mesiano et al., 1989).

IGFBP-3, a 43 - 39 kDa peptide, plays several important endocrine roles. IGFBP-3 functions as a transporter, prolongs the half-life of IGFs, provides the tissue- and cell type-specific localization of IGFs, directly regulates receptor binding, and itself affects cellular functions independent of IGFs (Furlanetto et al., 1980; Zapf et al., 1984). The result showed the difference in relative intensity of the 43 kDa, 39 kDa, and 34 kDa bands among cycling (figure 1), pregnant (figure 2), and newborn pigs (figure 3). Moreover, these IGFBPs were generally similar to previous data from humans, pigs and rats (Hardouin et al., 1987; Yang et al., 1989; Lee et al., 1991). Wang et al. (1995) reported that maternal serum levels of IGFBP-3 increased gradually as pregnancy progressed, so the concentration of IGFBP-3 was positively associated with the gestational age. However, our Western ligand blotting showed that levels of IGFBP-3 in pregnant pigs were declining as pregnancy proceeded (figure 2). As compared with our IFG-I data previously reported (Yang et al., 1999; figure 4), this pattern of circulating IGFBP-3 correlates positively with circulating IFG-I, as previously reported (Carr et al., 1995). Also, IGFBP-3 appears to be the principal binding protein for circulating IFG-I (Wang et al., 1995), which is important for endocrine roles of IFG-I in the regulation of fetal growth during preimplantation (Simmen et al., 1995; Wilson and Ford, 1997). Especially, IGFBP-3 modulates functions of IFG-I that is positively associated with E_2 secreted by preimplantation conceptuses for their growth (Wilson and Ford, 2000). So, IFG-I and IGFBP-3 also appear to be regulated in a coordinated manner, increasing with gestational age with a significant positive relationship to parameters of fetal growth (Fant et al.,

Table 5. Comparison of porcine serum IFG-II concentration by estrogen receptor genotypes during pregnancy

ER Genotype	Serum IFG-II concentration (ng/ml)				
	Day 45	Day 60	Day 75	Day 90	Day 105
AA (n=3)	310.7 ± 30.2	404.0 ±116.8	394.7 ± 35.0	471.7 ±119.8	507.3 ±320.8
AB (n=12)	360.6 ± 93.3	344.1 ± 96.1	360.6 ± 56.6	437.9 ±124.3	436.6 ±123.2
BB (n=7)	377.7 ± 70.0	388.6 ±113.2	381.0 ± 90.8	409.0 ± 80.5	470.9 ± 43.0

Table 6. Comparison of porcine serum IFG-II concentration by estrogen receptor genotypes during growth of newborn female piglets

ER Genotype	Serum IGF-II concentration (ng/ml)					
	Day 30	Day 45	Day 60	Day 75	Day 90	Day 105
AA (n=3)	1131.5±244.0	730.0±126.8	703.3±161.6	675.7±167.8	568.7±119.5	650.5±157.7
AB (n=6)	1077.0±417.1	828.0±332.9	773.2±253.2	521.3±137.5	500.7±129.7	606.8±115.8
BB (n=1)	1277.0	880.3	777.3	641.0	-	480.0

1993).

Similar to the IFG-I patterns, the level of IGFBP-3 in both groups increased as postnatal growth progressed. It is thought that this postnatal increase in serum 43kD and 39kD IGFBP-3 is driven by GH action, and the levels of circulating IGFBP-3 during growth of newborns also positively correlate with circulating IFG-I, while circulating IGFBP-2 is positively correlated with circulating IFG-II (Carr et al., 1995). In addition, expressions of IGFBP-3 in the high litter size group were smaller than those of IGFBP-3 in the low litter size, similar to IFG-I levels.

The study of the ER locus demonstrated that the ER B allele is positively associated with litter size (Short et al., 1997), and PCR products digested with restriction enzyme *PvuII* were separated into three subtypes (AA, AB, BB) for ER (Yang et al., 1999). As pregnancy proceeded, the concentration of IFG-II in each group showed an increasing tendency (table 5). The concentration of IFG-II in the AA genotype known, for low litter size, was higher than that in the BB genotype, known for high litter size, during pregnancy except at 45 d (table 5). These results are identical to IFG-II profiles (table 2) that show low litter size groups express higher IFG-II concentrations than high litter size groups. IFG-II concentrations rapidly declined with age in newborn piglets, but any differences between the two genotypes were not detected (table 6).

In summary, we have examined possible associations of IFG-II and IGFBP-3 with litter size in pigs during estrous cycle, pregnancy, and postnatal growth. Although a significant difference in IGFBP-3 between the high and low litter size groups was not detected, results of this study support that the expression of IGFBP-3 in the preimplantation stage is associated with litter size. Moreover, because level of IFG-II during mid-gestation was then the most significantly different between high and low litter size groups, it is suggested that IFG-II also shows the possible

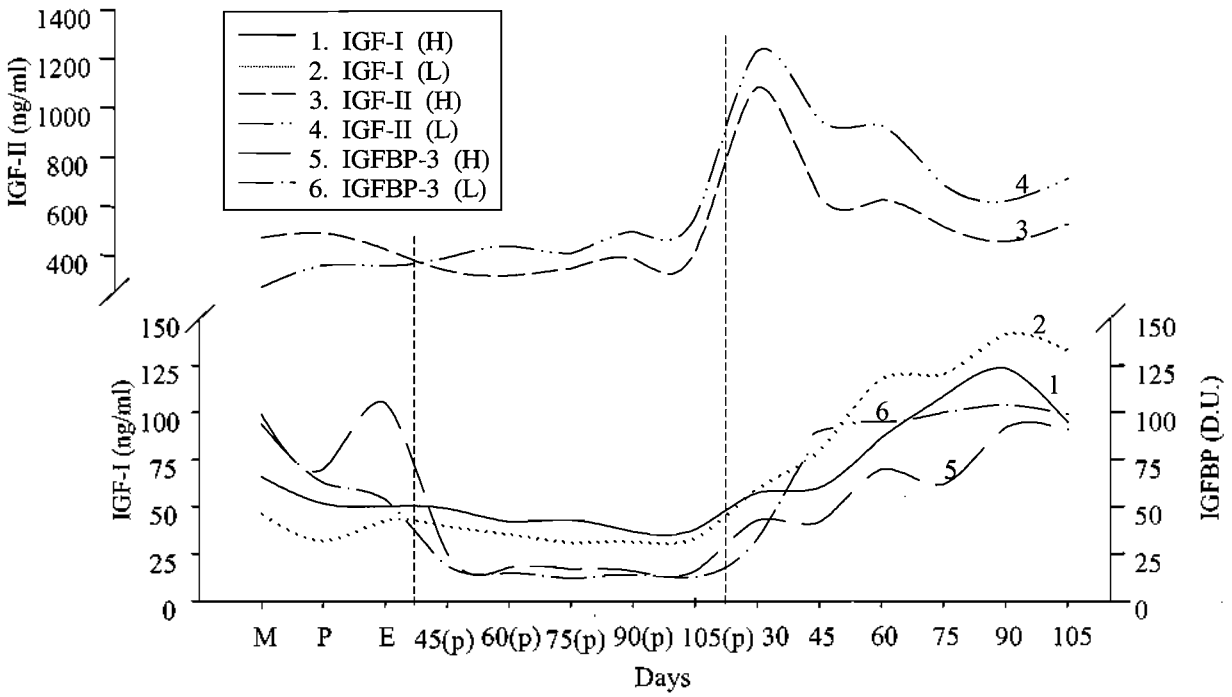

Figure 4. Comparison of porcine serum IFG-I, IFG-II, and IGFBP-3 expression between high and low litter size groups from estrous cycle to postnatal growth. [The results for IFG-I concentration have been described in Yang et al. (1999)]

relationship with litter size during definite pregnancy. Although significant differences in ER genotype from pregnancy to postnatal growth were not detected, IFG-II levels by ER genotype during pregnancy show a similar tendency to those between the high and low litter size groups.

IMPLICATION

Recent studies have investigated porcine litter size by monitoring litter size-related markers, such as estrogen receptor gene. But because reproductive processes, including ovulation and pregnancy, are organized with a series of alterations of hormones, cytokines, and growth factors modulated by environmental and nutritional factors, the examination at gene level only may not be an accurate estimation. Therefore, if endocrine factors including growth factors are also monitored during postnatal growth and pregnancy, it is considered that the estimation of litter size at the gene level could be more assured.

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REFERENCES

Carr, J. M., J. A. Owens, P. A. Grant, P. E. Walton, P. C. Owens and J. C. Wallace. 1995. Circulating insulin-like growth factors (IGFs), IGF-binding proteins (IGFBPs) and tissue mRNA levels of IGFBP-2 and IGFBP-4 in the ovine fetus. J. Endocrinol. 145:545-557.

Daughaday, W. H., I. K. Mariz and S. L. Blethen. 1980. Inhibition of access of bound somatomedin to membrane receptor and immunobinding sites: a comparison of radioreceptor and radioimmunoassays of somatomedin in native and acid-ethanol-extracted serum. J. Clin. Endocrinol Metab. 51:781-788.

Daughaday, W. H. and P. Rotwein. 1989. Insulin-like growth factor I and II. Peptide, nessenger ribonucleic acid gene structures, serum, and tissue concentrations. Endocr. Rev. 10:68-91.

Fant, M., C. Salafia, R. C. Baxter, J. Schwander, C. Vogel, J. Pezzullo and F. Moya. 1993. Circulating levels of IGFs and IGF binding proteins in human cord serum: relationship to intrauterine growth. Regulatory Peptides. 48:29-39.

Fant, M., H. Munro and A.C. Moses. 1986. An autocrine/ paracrine role for insulin-like growth factors in the regulation of human placental growth. Endocrinology. 63:499-505.

Furlanetto, R. W. 1980. The somatomedin C binding protein: evidence for a heterologus subunit structure. J. Clin. Endocrinol Metab. 51:12-19.

Hardouin, S., P. Hossenlopp, B. Segovia, D. Seurin, G. Protolan, C. Lassarre and M. Binoux. 1987. Heterogeneity of insulin-like growth factor binding proteins and relationships between structure and affinity. I. Circulating form in man. Eur. J. Biochem. 170:121- 132.

Hofig, A., F. A. Simmen, F. W. Bazer and R. C. M. Simmen. 1991. Effects of insulin-like growth factor-I on aromatase cytochrome P450 activity and oestradiol biosynthesis in preimplantation porcine conceptuses *in vitro*. J. Endocrinol. 130:245-250.

Hossenlop, P., D. Seurin, B. Segovia-Quinson, S. Hardouin and M. Binoux. 1986. Analysis of serum insulin-like growth factor binding proteins using Western ligand blotting : use of the method for titration of the binding proteins and competitive binding studies. Anal. Biochem. 154:138-143.

Johnson, R. K., M. K. Nielsen and D. S. Casey. 1999. Responses in ovulation rate, embryonal survival and litter traits in swine to 14 generations of selection to increase litter size. J. Anim. Sci. 77:541-557.

Jones, J. I. and D. R. Clemmons. 1995. Insulin-like growth factors and their binding proteins: biological actions. Endcr. Rev. 16:3-34.

Lee, C. Y., F. W. Bazer, T. D. Etherton and F. A. Simmen. 1991. Ontogeny of insulin-like growth factors (IGF-I and IGF-II) and IGF-binding proteins in porcine serum during fetal and postnatal development. Endocrinology. 128: 2336-2344.

Lee, C. Y. and D. M. Henricks. 1990. Comparisons of various acidic treatments of bovine serum on insulin-like growth factor-I immunoreactivity and binding activity. J Endocrinol. 127:139-148.

Mesiano, S., I. R. Young, A. W. Hey, C. A. Browne and G. D. Thorburn. 1989. Hypophysectomy of the fetal lamb leads to a fall in the plasma concentration of insulin-like growth factor I (IGF-I), but not IGF-II. Endocrinology. 124:1485-1491.

Moses, A. C., S. P. Nissley, P. A. Short, M. M. Rechler, R. M. White, A. B. Knight and O. Z. Higa. 1980. Increased levels of multiplication-stimulating activity, an insulin-like growth factor, in fetal rat serum. Proc. Natl. Acad. Sci. USA. 77:3649-3653.

Nestler, J. E., 1990. Insulin-like growth factor II is a potent inhibitor of the aromatase activity of human placental cytotrophoblasts. Endocrinology. 127:2064-2070.

Park, H. B., S. H. Yang, D. S. Seo, K. D. Kim, Y. Ko, S. S. Park and K. C. Hong. 2000. Association of porcine estrogen receptor locus variation with litter size in yorkshire swine. J. Anim. Sci Technol. (Kor.). 42:125-134.

Pescovitz, O. H., N. B. Johnson and S. A. Berry. 1991. Ontogeny of growth hormone releasing hormone and insulin-like growth factors-I and -II messenger RNA in rat placenta. Pediatr. Res. 29:510-516.

Riberio, E. L., M. K. Nielsen, K. A. Leymaster and G. L. Bennett. 1997. A stimulation model including ovulation rate, potential embryonic viability and uterine capacity to explain litter size in mice: II. Responses to alternative criteria of selection. J. Anim. Sci. 75:652-656.

Roberts, R. M., S. Xie and W. E. Trout. 1993. Embryo-uterine interactions in pigs during week 2 of pregnancy. J. Reprod. Fertil. 48:171-186.

SAS. 1995. SAS/STAT Software for PC. Release 6.11, SAS Institute, Cary, NC, USA.

Seo, D. S., S. H. Yang, H. B. Park, S. S. Park, K, C, Hong and Y. Ko. 1999. Utilization of porcine clotted and dried blood for estrogen receptor gene PCR-RFLP. Korean J. Anim. Reprod. 23:159-163.

Short, T. H., M. F. Rothschild, O. I. Southwood, D. G. McLaren, A. de Vries, H. van der Steen, G. R. Eckardt, C. K. Tuggle, J. Helm, D. A. Vaske, A. J. Mileham and G. S. Plastow. 1997. Effect of the estrogen receptor locus on reproduction and production traits in four commercial pig lines. J. Anim. Sci. 75:3138-3142.

Simmen, F. A., R. C. Simmen, R. D. Geisert, F. Martinat-Botte, F. W. Bazer and M. Terqui. 1992. Differential expression, during the estrous cycle and pre- and post-implantation conceptus development, of messenger ribonucleic acids encoding components of the pig uterine insulin-like growth factor system. Endocrinology. 130: 1547-1556.

Simmen, R. C. M., M. L. Green and F. A. Simmen. 1995. IGF system in periimplantation uterus and embryonic development. Molecular and Cellular Aspects of Periimplantation Process. pp. 185-204.

Sterle, J. A., T. C. Cantley, W. R. Lamberson, M. C. Lucy, D. E. Gerrard, R. L. Matteri and B. N. Day. 1995. Effects of recombinant porcine somatotropin on placental size, fetal growth and IFG-I and IFG-II concentration in pigs. J. Anim. Sci. 73:2980-2985.

Wang, H. S., B. J. Cheng and Y. K. Soong. 1995. Serum levels of insulin-like growth factor-binding protein-3 in normal pregnancy. Eur. J. Obstet. Gynecol. Reprod. Biol. 61:157-160.

Wilson, M. E. and S. P. Ford. 1997. Differences in trophectoderm mitotic rate and P450 172 α-hydroxylase expression between late preimplantation Meishan and Yorkshire conceptuses. Biol. Reprod. 56:380-385.

Wilson, M. E. and S. P. Ford. 2000. Effects of estradiol-17 β administration during the time of conceptus elongation on placental size at term in Meishan pigs. J. Anim. Sci. 78:1047-1052.

Yang, S. H., D. S. Seo, H. B. Park, K. D. Kim, C. W. Kang, K. S. Choi, S. S. Park, K. C. Hong, Y. Ko. 1999. Studies on the possible relationship of porcine serum insulin-like growth factor-I with litter size. Korean J. Anim. Reprod. 23:213-220.

Yang, Y. W., J. F. Wang, C. C. Orlowski, S. P. Nissely and M. M. Rechler. 1989. Structure, specificity and regulation of the insulin-like growth factor-binding proteins in adult rat serum. Endocrinology. 125:1540-1555.

Young, C. R., S. P. Ford, L. K. McGinnis and L. H. Anderson. 1993. Investigation into the control of litter size in swine: I. Comparative studies on in vitro development of Meishan and Yorkshire preimplantation embryos. J. Anim. Sci. 71:1561-1565.

Zapf, J., C. H. Schmid and E. R. Froesch. 1984. Biological and immunological properties of insulin-like growth factors (IGF) I and II. Clin. Endocrinol. Metab. 13:3-30.

Zapf, J., H. Walter and E. R. Froesch. 1981. Radio-immunologcal determination of insulin-like growth factors I and II in normal subjects and in patients with growth disorders and extrapancreatic tumor hypoglycemia. J. Clin. Invest. 68:1321-1330.