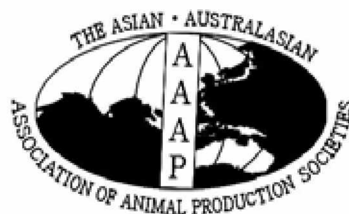

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Peroxisome Proliferator-activated Receptor γ Is Involved in Weaning to Estrus of Primiparous Sows by Regulating the Expression of Hormone Genes in Hypothalamus-pituitary-ovary Axis*

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ABSTRACT : The objective of this study was to determine whether peroxisome proliferator-activated receptor γ (*PPARγ*) is involved in the regulation of weaning to estrus of primiparous sows. Twelve sows composed of 6 groups of 2 full-sibs in a similar age (325.2 d), body weight (BW; 152.4 kg) and backfat thickness (BFT; 27.0 mm) at start of lactation, were allocated to accept 31 MJ (restricted group, R-group) or 53 MJ (control group, C-group) DE/d treatment, respectively. The experimental results indicated that the low energy intake resulted in excessive losses of BW and BFT during lactation in R-group sows, which may be related to decrease of serum 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2), a ligand of *PPARγ*. The obvious peak and the frequency of LH, FSH and estradiol (E_2) were only observed in C-group sows. Except for E_2 at d 1 and 2, serum FSH, LH and E_2 concentrations in R-group were lower than those in C-group sows after weaning. However, the serum progesterone (P_4) level in R-group sows was always more than that in C-group. The expression abundances of *PPARγ* and *GnRH* receptor (*GnRH-R*) in pituitary, *FSH* receptor (*FSH-R*), *LH* receptor (*LH-R*), estrogen receptor (*ES-R*) and aromatase in ovary of anestrous sows were lower than those of estrous sows. Neither the BFT nor the BW was associated with the mRNA abundance of *PPARγ* in hypothalamus during lactation. Expressions of *PPARγ* in pituitary and ovary were affected evidently by the BFT changes and only by the loss of BW of sows during and after lactation. Furthermore, *PPARγ* mRNA level in ovary was significantly related to the expression abundances of *GnRH-R*, *FSH-R*, *ES-R* and aromatase, and *GnRH-R* was obviously associated with *PPARγ* expression in pituitary. However, *PPARγ* expression in hypothalamus likely has no effects on these genes expression and no obvious difference for all sows. Not serum E_2 or P_4 alone but the ratios of E_2 to P_4 and 15d-PGJ_2 to P_4, and serum FSH and LH were evidently related to *PPARγ* expression in pituitary and ovary. It is concluded that *PPARγ* is associated with body conditions, reproduction hormones and their receptor expression, which affected the functions of pituitary and ovary and ultimately the estrus after weaning of primiparous sows. (**Key Words :** Primiparous Sows, Body Condition, Hypothalamus-pituitary-ovary Axis, Estrus, Peroxisome Proliferator-activated Receptor γ, Hormone Receptor)

INTRODUCTION

The MLC Pig Year Book 1995 (MLC, 1995) analyses the reasons for culling sows in UK and shows that the major reason for 18% of all sow disposals is mainly reproductive failure. With a confined intensive raising system and more modernizing managing strategy, reproductive failures commonly occur and maybe get more and more serious on pig farms. One of the major reproductive problems that a producer encounters is anestrus in gilts and postweaning sows, which is more severe in primiparous than that in multiparous sows (Quesnel et al., 1998). In some moderate managing level farms, the percentage of postweaning primiparous anestrous sows are up to 10% or more, which result in an increase in the proportion of sows annually removed from the breeding herd and affect enormously the economic interest.

Anestrus is the state of ovarian acyclicity, reflected by complete sexual inactivity without exhibition of estrus (Wright and Malmo, 1992). Several factors may affect the prolonged return to estrus following weaning (Meredith, 1984; Gourdine et al., 2006), but the factor of most concern to many nutritionists and producers is the nutritional and metabolic status of the sow at weaning, such as excessive losses of weight and body tissue stores during lactation

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(Prunier and Quesnel, 2000; Boyd et al., 2000; Cheng et al., 2001). The deficiency of energy intaken by lactating sows was considered as one of the important factors related to the anestrus of primiparous or multiparous sows, especially for primiparous sows (Rozeboom et al., 1993; Caroll et al., 1996; van den Brand et al., 2000). Furthermore, there is a very obvious correlation between the levels of body weight and backfat thickness of sows at weaning and probability of anestrus occurred postweaning (Mullan and Williams, 1989).

Since Issemann and Green (1990) found peroxisome proliferator-activated receptors (PPARs), tremendous progress has been made toward understanding the role of PPARs in whole body physiology and in many human diseases including diabetes, obesity, atherosclerosis, hypertension, and cancer (Desvergne and Wahli, 1999). *PPARγ*, belongs to a subclass of the *PPARs*, is mainly expressed in white and brown adipose tissues and has a function in adipogenesis (Tontonoz et al., 1994). And 15d-PGJ_2, derived from PGD_2, was shown to be a high-affinity nature ligand for *PPARγ* (Kliewer et al., 1995). Recent reports have indicated that *PPARγ* is also expressed in urinary tract (Guan et al., 1997), rat and human placenta (Capparuccia et al., 2002; Asami-Miyagishi et al., 2004), suggesting roles for *PPARγ* in regulation of expression of myriad genes that regulate energy metabolism, cell differentiation and proliferation, apoptosis and inflammation, embryo development and implantation (Houseknecht et al., 2002).

However, there is limited information about the distribution of *PPARγ* expression in porcine. Whether *PPARγ* is related to reproduction performance of sows, such as estrous cycle, is still a secret. Therefore, we established the estrous and anestrous models by supplying the paired sows with sufficient or insufficient energy intake daily to compare the *PPARγ* profiles in primiparous sows returned to cycles postweaning with those in their paired anestrous sows, and to determine the relationships between expression level of *PPARγ* mRNA, and the levels of relative hormones and expression levels of their receptors, body weight and backfat thickness of primiparous sows after weaning.

MATERIALS AND METHODS

Animals, treatments, and management

The protein and energy intake of Landrace×Rongchang pig primiparous sows (Breed Farm of Chongqing Swine Science Academy, Chongqing Rongchang, China), comprising 7 groups of two full-sibs in a similar age (325.2±10.3 d), was manipulated during gestation such that all gilts were of very similar body weight (BW) (151.34±3.25 kg) and backfat thickness (26.93±0.80 mm) at the start of lactation. Both total piglets (10 piglets) and the litter weight (19.26±1.02 kg) were similar in all paired sows until weaning by means of fosteraging, to standardized the nursed and weaned number of piglets. The sows were ablactated at the same day, and the lactation period was approximate 4 wks (SD = 1.2 day). One sow of each pair was allocated randomly to one of the paired treatments, comprising two levels of energy intake daily (high: 53 MJ DE/d or low: 31 MJ DE/d) and 7 animals per treatment. Initially 8 groups of 2 to 3 full-sibs were selected to ensure that there would be 2 full-sib sows per family to allocate to the experiment. Sows were housed in crates individually with free access to water throughout the study and ambient temperature was maintained a minimum temperature of 18 to 24°C in environment-controlled, light-tight rooms. The photoperiod was maintained at 14 h light: 10 h dark.

Similar to the methods of Rozeboom et al. (1993) and van den Brand et al. (2000), the desired DE intakes (53 MJ/d or 31 MJ/d) were achieved by feeding two corn-soybean diets (12.9 MJ DE/kg and 13.9% CP in C-group; 11.9 MJ DE/kg and 21.9% CP in R-group) formulated using similar ingredients, at a different feeding level (4.1 kg/d vs. 2.6 kg/d). Each diet provided equal daily amounts of crude protein (570 g), minerals and vitamins, which meet the estimated maintenance requirement (NRC, 1998). Two levels of dietary digestible energy were imposed to cause different rates of body tissue catabolism and different effects on the intervals of weaning to estrus. Fresh feed was offered at 08:00 and 17:00 h. After weaning, sows remained on their individual diets and received the same amount of feed as fed during lactation until they were determined to be estrous or anestrous and slaughtered.

The BW and BFT were recorded on the farrowing day, once a week during lactation until weaning, on the day when sows in high energy intake group (control group, C-group) exhibited standing heat, and on the day 12 after weaning for anestrous sows in low energy intake group (restricted group, R-group). Backfat thickness was measured ultrasonically (1600AGROSCAN, Japan) at 65 mm off of the midline at the 10th rib. The average backfat thickness for the left and right sides was used.

All sows were checked for estrus three times a day (07:00, 15:00 and 23:00 h) from d 3 to 11 after weaning using physical and behavioral signs elicited in response to exposure to a mature vasectomized Yorkshire boar and with the back pressure test. Once not exhibited estrus by d 11 postweaning, the sows were considered being anestrous. Seven sows in C-group were all exhibited estrus and slaughtered after the last blood sampling (one exhibited estrus at 15:00 h on d 5 postweaning, four at 07:00 h and two at 15:00 h on d 6 postweaning). Six sows in R-group were not observed being estrous and slaughtered on d 12 postweaning. The last one in R-group was abrogated from

the experiment because it appeared to be estrous on d 9 postweaning. When sows were slaughtered, hypothalamus, pituitary and ovary were removed quickly and shock-frozen in liquid nitrogen and then stored -80°C until total RNA extraction. The development status (size and number) of follicle cells and corpora lutea were examined before the ovary was viscerated.

Blood sampling, and hormone and 15d-PGJ_2 assay

All sows were nonsurgically fitted with indwelling jugular vein cannulas upon arrival at 4 d prior to weaning. Blood samples were collected at 8-h intervals (07:30, 15:30 and 23:30 h) from the first day of postweaning to the day when the estrous full-sib sow was slaughtered for all sows. Additional blood samples were collected at 15-min intervals for 6 h from both full-sib sows when the standing heat of one full-sib in C-group emerged. Then, the estrous full-sib sows were electrically stunned and killed by exsanguination. On d 12 postweaning, before the anestrous full-sib sows in R-group were slaughtered, approximately 10 ml blood samples were collected and allowed to clot at room temperature prior to centrifugation to harvest serum. Serum samples were frozen at -20°C until RIA for luteinizing hormone (LH), follicle-stimulating hormone (FSH), 17-β estradiol (E_2) and progesterone (P_4) concentrations. At the same time, additional blood samples were collected in a 5 ml heparinized vacutainer tube (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA) and centrifugated (3,500×g for 5 min) to collect plasma. Plasmas were stored at -20°C until analysis for 15d-PGJ_2.

Except for 15d-PGJ_2, all hormone assays were measured in duplicate with the double-antibody RIA kit production for porcine (^{125}I-labelled) (SINO-UK Institute of Biological Technology, Beijing, China) using the GC-911-Gamma Radioimmunoassay Counter (USTC S&T Enterprises Group, Hefei, China). The analysis technique parameters of all RIA kits were described in brief as following (intra-assay and inter-assay coefficients of variation in parentheses): FSH (2.4 and 6.2%), the assay sensitivity was 84 pg/ml and the cross-reactivity was 3.0, 1.0 and 0.01% for TSH, LH and HCG respectively; LH (2.2 and 5.9%), the assay sensitivity was 0.25 ng/ml, and the cross-reactivity was 4.0, 2.0 and 12.0% for TSH, FSH and HCG respectively; E_2 (6.2 and 9.3%), the assay sensitivity was 3.5 pg/ml and the cross-reactivity was 0.2% for estriol (E_3); P_4 (5.3 and 7.9%), the assay sensitivity was 82 pg/ml, and the cross-reactivity was 0.03, 0.01 and 0.01% for pregnenolone, androstenedione and estradiol, respectively.

15d-PGJ_2 was tested using Correlate-EIA kit (Assay Designs, Inc., Ann Arbor, MI, USA). The sensitivity of this kit was 36.8 pg/ml with 5.7 and 13.0% intra- and inter-assay coefficient of variance, respectively. The recovery of 15d-PGJ_2 in porcine plasma was 105%. Assays were performed in duplicate and analyzed according to the manufacture's instructions.

RNA analyses

RNA extraction and complementary DNA preparation : Adopting the procedure described by Ziecik et al. (1989), each ovary follicles were excised with scissors to isolate total RNA after one ovary of a sow was thawed. The left one ovary of the same sow was isolated total RNA as the whole. The RNA was isolated from hypothalamus, pituitary, whole ovary and follicular cell using TRIzol Reagent (Life Technologies, Inc., Gaithersburg, MD, USA) according to the manufactures instructions, and then stored at -80°C. The purity and integrity of RNA was electrophoretically verified by ethidium bromide staining and by optical density (OD) absorption ratio OD_{260}/OD_{280} and rRNA (28s/18s) ratios, respectively.

Reverse transcription-polymerase chain reaction (RT-PCR) was performed using 2.0 μg of total RNA isolated from each tissue in 40 μl of reaction volume. The positive and negative control templates supplied by the reverse transcriptase kit were used for detecting the response system. To remove genomic DNA contamination, RNA samples were treated with RNase free DNase (Life Technologies, Inc.) for 1 h at 37°C, followed by a 10-min incubation at 75°C to inactivate the DNase. The RNA was reverse transcribed in the presence of polythymidine oligonucleotide primers (Oligo-dT_{18}) and Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLVRT; Life Technologies, Inc.). The synthesized complementary DNA (cDNA) was stored at -20°C until for fluorescent real-time quantitative PCR analysis.

Quantification of mRNA expression : Fluorescent real-time quantitative RT-PCR was used to determine differences in mRNA expression abundances for *PPARγ*, *GnRH* receptor (*GnRH-R*), *LH* receptor (*LH-R*), *FSH* receptor (*FSH-R*), estradiol receptor beta (*ES-R*) and aromatase (*Arom*) between anestrous and estrous sows. The quantitative analysis of PCR was carried out in DNA Engine Opticon 2 fluorescence detection system (MJ Research, Watertown, MA, USA) according to optimized PCR protocols and DyNAmo SYBR Green qPCR kit (Finnzymes Oy., Keilaranta, Espoo, Finland), in which SYBR Green I (SGI) was a double-stranded DNA-specific fluorescent dye. The PCR reaction system (20 μl) contained 10 μl DyNAmo SYBR Green qPCR mix, 5 μl primer (0.3 μmol/L forward and 0.3 μmol/L reverse), 2 μl cDNA template (<10 ng/μl), and 3 μl double distilled water. For the PCR reaction, the following experimental run protocol was used: enzyme incubation (50°C for 2 min), denaturation program (95°C for 10 min), amplification and quantification program repeated 36 times (94°C for 20 s,

different annealing temperature for different target genes for 20 s, 72°C for 20 s with a single fluorescence measurement), melting curve program (65-95°C with a heating rate of 0.1°C per second and a continuous fluorescence measurement) and finally 72°C for 10 min. The annealing temperatures for *PPARγ*, *GnRH-R*, *LH-R*, *FSH-R*, *ES-R*, *Arom* and β-*actin* were 56.0, 58.5, 62.0, 60.9, 59.0, 56.7 and 57.5°C, respectively. All samples were measured in triplicate. For all of the experiments, controls without templates were included.

To amplify *PPARγ*, *GnRH-R*, *LH-R*, *FSH-R*, *ES-R*, *Arom* and β-*actin* cDNA fragments, the sequences of PCR primers pairs were used: forward 5'-GCCAACTCTAA GCCACTAACAT-3', reverse 5'-GACACAGGCTCCACTT TGAT-3' for *PPARγ* (317 bp, AJ006756); forward 5'-GCTGCCGATTTACTGGTTAC-3', reverse 5'-CCCGAAG ACGAACACGAG-3' for *GnRH-R* (535 bp, AY542892); forward 5'- TGGGCTATGACTTCCT TAG-3', reverse 5'-CCCTGTCTGCCAGTCTATG-3' for *LH-R* (239 bp, NM_ 214449); forward 5'-GCAACAAATCTATTTAAGGCAA GA-3', reverse 5'-GATGCTCACCT TCATGTAGCTG-3' for *FSH-R* (674 bp, AF025377); forward 5'-GCTACGCAA GTGCTACGAAGT-3', reverse 5'-CGCCAGACAAGACCA ATCAT-3' for *ES-R* (459 bp, Z37167); forward 5'-CATGAG GGTCTGGATAGGTG-3', reverse 5'-GCATGAGGGTCAA CACGTC-3' for *Arom* (323 bp, SSU92245); forward 5'-TTCCTGGGCATGGAATCC TG-3', reverse 5'-CACCTTC ACCGTTCCAGTTT-3' for β-*actin* (515 bp, AY550069). The different source of cDNA was used as template for *PPARγ* (hypothalamus, pituitary and whole ovary), *GnRH-R* (pituitary), *LH-R* (follicle cell), *FSH-R* (follicle cell), *ES-R* (whole ovary), and *Arom* (follicular cell).

The relative standard curve methods were used for quantification of gene expression. The quantification was normalized to an endogenous RNA control β-*actin* (i.e., house-keeping gene), and standard curves were plotted for each target and the endogenous genes. Each of the cDNA fragments of the target gene were purified using DNA wizard cleanup kit (Promega, Madison, Wis., USA) and cloned into plasmids for use as standards in quantifying gene expression level. A standard graph of the cycle threshold (Ct) values obtained from serial dilutions (10^2, 10^3, 10^4, 10^5, 10^6, and 10^7 copies/well) of the plasmid. Fluorescence signal was acquired in each cycle in order to determine the cycle threshold or the fluorescence baseline at which fluorescence rose above background for each sample. The Opticon Monitor 2 software produced a best-fit fluorescence baseline and the standard graph. For each experimental sample, the amounts of mRNA of each target gene and β-*actin* were determined from the Ct plotted on the respective standard curves. The mean values of the replicate wells run for each sample, subsequently, the quantity of each target gene was divided by β-*actin* to obtain a standardized value for each transcript.

Statistical analysis

Six paired full-sib sows, i.e. six estrous and six anestrous, were taken in the final statistical analysis. The last one exhibited estrous in C-group, was omitted from the statistical analysis because her full-sib in R-group was observed being estrous on d 9 after weaning.

The peak amplitude and frequency of reproductive hormones were not analyzed in here because the peak of LH, FSH, E_2 and P_4 were not observed obviously in R-group sows at the same time. Thus, the average value of blood samples in 8-h intervals at the same day was thought as a mean concentration in this day for each hormone profile of sows. The average value of blood samples in 15-min intervals was divided into two periods, the first 3 h recorded as "first", and the second 3 h as "second".

Statistical analyses were performed by SPSS Version 10.0 (SPSS, Inc., Chicago, IL, USA). After computing descriptive statistics (mean, std. Deviation, std. Error and range), paired samples t-tests were used to compare mean values of the paired full-sibs based on their statistical significance (2-tailed). At the same time, the procedure of bivariate correlations were run to test the correlations (Pearson correlation coefficient, 2-tailed significance) of the mRNA expressions of *PPARγ* in the hypothalamus-pituitary-ovary axis and the changes for body weight and backfat thickness during and after lactation, and the mRNA expressions of *GnRH-R*, *LH-R*, *FSH-R*, *ES-R* and *Arom* in different physiological status (estrus vs. anestrus), and the blood profiles sampled before slaughter. Finally, Linear regression analyses with enter method were run to demonstrate the linear regression relationships between expressions levels of *GnRH-R*, *LH-R*, *FSH-R*, *ES-R* and *Arom* and expression levels of *PPARγ* in hypothalamus-pituitary-ovary axis. Statistical significance was assumed at $p<0.05$ and 0.01.

RESULTS

Of the 18 primiparous sows that commenced the experiment, 7 groups of two full-sibs, i.e., 14 animals were successfully completed their 4wk lactation and periods of weaning to slaughter. Of the 4 sows that were removed from the experiment, one had farrowing difficulties and was unable to sustain the litter because of poor milk production, and two sows removed from the experiment because of the diarrhea occurred in their piglets, and another one because of the mastitis, perhaps caused by the piglets biting wound. Therefore, the number of sows that were imposed to statistical was 12, i.e., 6 groups of two full-sibs. One of 7 groups was removed because that one of the full-sibs in R-

Table 1. Measurements of body weight (BW) and backfat thickness (BFT) during and after lactation for C-group (high energy intake) and R-group (low energy intake) sows [1]

Item	C-group	R-group	Paired-samples t-test	
			SE	P-value
BW (kg)				
wk0 (Start of lactation)	150.72	151.96	1.26	0.113
wk1	148.45	149.60	1.32	0.149
wk2	142.45	137.84	1.66	0.001
wk3	134.68	126.52	2.02	0.002
wk4 (on weaning day)	130.05	118.38	2.45	0.001
On slaughtered day of estrus [2]	135.53	124.37	2.15	0.001
On slaughtered day of anestrus [3]	-	131.50	2.32	0.054
BW loss during lactation (kg)	20.66	33.59	1.12	0.000
BW gain postweaning (kg)				
Weaning to estrus [2]	5.48	5.99	0.92	0.475
Weaning to anestrus [3]	-	13.12	1.17	0.000
BFT (mm)				
wk0 (Start of lactation)	26.84	27.03	0.73	0.284
wk1	25.77	25.44	0.80	0.168
wk2	25.27	24.64	1.00	0.034
wk3	24.65	23.58	0.81	0.005
wk4 (on weaning day)	23.86	22.49	0.94	0.002
On slaughtered day of estrus [2]	24.83	23.38	0.72	0.002
On slaughtered day of anestrus [3]	-	24.03	0.69	0.020
BFT loss during lactation (mm)	2.98	4.54	0.871	0.000
BFT gain postweaning (mm)				
Weaning to estrus [2]	0.97	0.89	0.61	0.480
Weaning to anestrus [3]	-	1.54	0.68	0.001

[1] Six sows in C-group occurred estrus at d 5 to 6 (131.4±4.2 h) after weaning, the values were the average of the C-group or R-group sows at the same day for each paired full-sib animal.

[2] Means on the day when C-group sows exhibited estrus and were slaughtered.

[3] BW or BFT in R-group sows on d 12 after weaning compared with those in C-group sows on the day being estrus.

group appeared estrus on day 9 after weaning.

Ovarian follicle development

Examination of the ovaries revealed that the total follicles number and the percentage of small follicles (≤3 mm in diameter) in anestrous sows were more than those in estrous paired sows (average of left and right ovary, 26.2 vs. 22.5, 67.4 vs. 32.4%). However, the percentage of large follicles (≥5 mm in diameter) or middle follicles (3-5 mm in diameter) in anestrous sows were much lower than those in estrous paired sows (average of left and right ovary, 12.1 vs. 42.4, 20.4 vs. 25.1%). Follicles and corpora lutea didn't exceed 14 mm in diameter both for C-group and R-group sows, which indicated that ovary cysts didn't occur in this present study. And according to the category method by Chung et al. (2002), the anestrous status here belonged to the inactive ovary.

Body weight and backfat thickness

The results of paired-samples t-test analysis showed that the BW and the BW loss in R-group sows were significantly lower ($p<0.01$) than those in C-group sows since the wk-2 after farrowing (Table 1). The same trends were observed for the thickness and the loss of backfat in C- and R-group sows. The different levels of energy intake daily during and after lactation caused the BW and BFT difference ($p<0.01$) between the C-group and the R-group sows on the day when sows in C-group exhibited estrus (estrous vs. anestrous, 135.53 vs. 124.37 kg for BW, 24.827 vs. 23.382 mm for BFT), which resulted in more BW and BFT loss in anestrous sows than those in estrous sows by 62.47 and 52.18% ($p<0.01$), respectively.

Hormone levels

The obvious peak and the frequency of LH, FSH and E_2 were only observed in C-group sows (data not shown). The peaks of average concentrations daily of E_2, FSH and LH occurred approximately on d 4, 5 and 6, respectively (Figure 1). Serum E_2 and LH levels in R-group sows were higher on d 1 and d 2 postweaning, while were lower on d 3 to d 6 than those in C-group sows after weaning. Serum FSH concentration in R-group sows was lower than that in C-group paired full-sib sows, whereas serum P_4 concentration in R-group sows was higher than that in C-group sows throughout the postweaning. Considering the last blood sample collected before slaughter, the serum

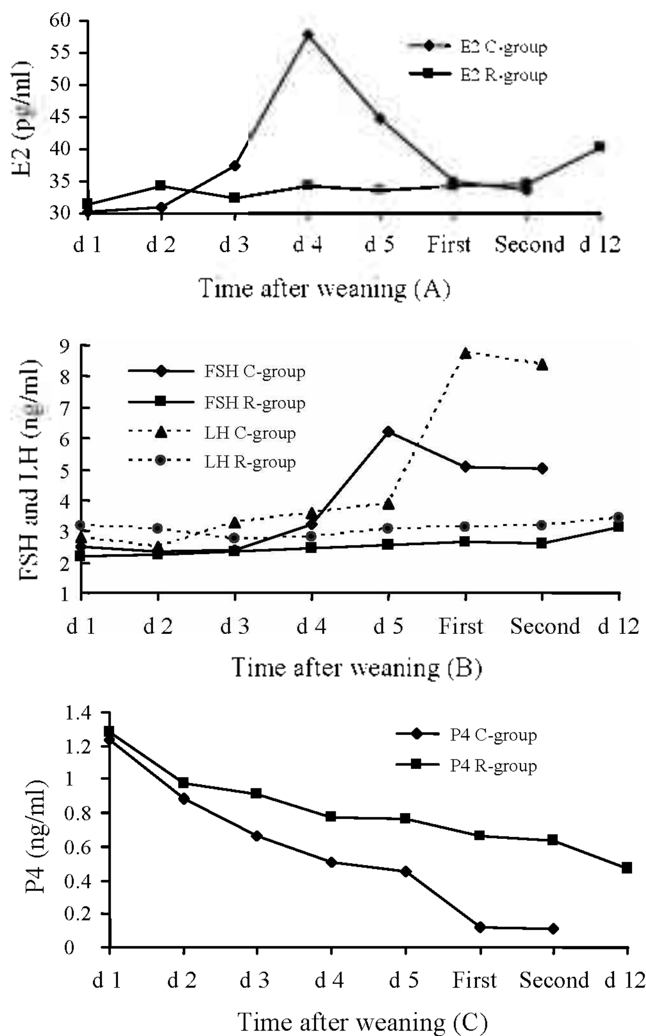

Figure 1. Serum estradiol (E_2) (A), FSH and LH (B), and progesterone (P_4) (C) concentrations of sows in C-group (high energy intake) and R-group (low energy intake) after weaning. The "first" means the average value of the first 3 h, and "second" of the second 3 h of the 15 min interval for 6 h blood sampling.

concentrations of FSH, LH and 15d-PGJ_2 of estrous sows in C-group were significantly higher ($p<0.01$) than those of their full-sibs in R-group at the same time, and also higher than those of their full-sibs in R-group before slaughter. However, the serum concentrations of E_2 and P_4 of estrous sows in C-group were both lower than those at the same time and lower than those before slaughter of their anestrous full-sibs in R-group.

Table 2. The mRNA expression differences of *PPARγ*, GnRH-R, FSH-R, LH-R, ES-R and Arom between C-group and R-group sows[1]

Item	C-group sows	R-group sows	Paired-samples t-test SE	Paired-samples t-test P-value
Hypothalamus				
PPARγ	0.402	0.422	0. 063	0.537
Pituitary				
PPARγ	2.377	1.930	0.097	0.005
GnRH-R	4.620	2.193	0.108	0.001
Ovary				
PPARγ	3.122	6.418	0.257	0.000
FSH-R	3.978	3.125	0.173	0.013
LH-R	7.005	3.733	0.435	0.001
ES-R	8.075	3.598	0.271	0.000
Arom	11.976	3.798	0.351	0.000

[1] The values are expressed as a relative ratio of the amount of target gene copies to the amount of β-actin (housekeeping gene) copies.

Expression abundance of mRNA

The results of mRNA expression abundances of estrous and anestrous paired sows were shown in Table 2. The expression of *PPARγ* in hypothalamus was not significantly different between estrous and anestrous paired sows. In pituitary, the expression abundance of *PPARγ* and *GnRH-R* in the anestrous sows were lower ($p<0.01$) than those in estrous sows. In ovary, the mRNA expression abundances of *FSH-R* ($p<0.05$), *LH-R*, *ES-R* and *Arom* ($p<0.01$) in estrous sows were higher than those in their paired anestrous full-sibs. On the contrary, the *PPARγ* mRNA abundance ($p<0.01$) in estrous sows were markedly lower than that in the paired anestrous full-sibs.

Correlations between *PPARγ* mRNA level and body weight and backfat thickness

The data in Table 3 showed that the BW at weaning was not associated with *PPARγ* mRNA abundances ($p>0.05$), but mRNA levels of *PPARγ* in ovary negatively related to the BW at estrous ($p<0.05$), and mRNA levels of *PPARγ* in hypothalamus positively related to the gain at weaning-to-

Table 3. Correlations between expression levels of *PPARγ* mRNA in hypothalamus-pituitary-ovary axis, and body weight and backfat thickness during lactation and at weaning-to-estrous intervals (WEI)[1,2,3]

Item	Body weight (BW) At weaning	At estrous	Loss during lactation	Gain at WEI	Backfat thickness (BFT) At weaning	At estrous	Loss during lactation	Gain at WEI
$PPAR_h$	-0.084	0.019	0.082	0.679[a]	-0.269	-0.289	0.29	-0.139
$PPAR_p$	0.456	0.473	-0.809[a]	0.184	0.737[a]	0.802[a]	-0.856[A]	0.115
$PPAR_o$	-0.561	-0.691[a]	0.939[A]	0.212	-0.89[A]	-0.905[A]	0.946[A]	-0.202

[1] $PPAR_h$, $PPAR_p$ and $PPAR_o$ means mRNA expression abundance in hypothalamus, pituitary and ovary, respectively.
[2] Data used in analysis included all estrous and anestrous paired full-sib sows. The WEI of R-group sows means the interval of WEI of their paired full-sibs in C-group.
[3] Superscript small letter and capital letter mean significance of coefficients at 0.05 and 0.01 levels, respectively.

Table 4. Linear regression models of GnRH-R, FSH-R, LH-R, ES-R and Arom mRNA expressions and correlation coefficients of mRNA expression between these genes and PPARγ in hypothalamus-pituitary-ovary axis [1,2,3]

Item	Constant	Independent variable coefficients in models			Efficiency of models			Coefficients of correlations		
		$PPAR\gamma_h$	$PPAR\gamma_p$	$PPAR\gamma_o$	R square	F value	P value	$PPAR\gamma_h$	$PPAR\gamma_p$	$PPAR\gamma_o$
Dependent variables										
GnRH-R	7.289	0.885	-0.348^a	-0.733^a	0.824	12.49	0.002	-0.151	0.871^A	-0.807^a
FSH-R	-1.976	-2.466	2.713	0.147^a	0.731	7.258	0.011	-0.201	0.768^a	-0.749^a
LH-R	2.311	-0.955	2.88	-0.576	0.863	16.735	0.001	-0.098	0.583	-0.907^A
ES-R	11.941	-7.705	1.1	-1.112^A	0.939	41.098	0.000	-0.293	0.817^a	-0.96^A
Arom	10.289	-7.018	4.133	-1.763^A	0.93	35.657	0.000	-0.191	0.775^a	-0.957^A

[1] $PPAR_h$, $PPAR_p$ and $PPAR_o$ means mRNA expression abundance in hypothalamus, pituitary and ovary, respectively.

[2] Superscript small letter and capital letter mean significance of coefficients at 0.05 and 0.01 levels, respectively.

[3] Linear regression models of GnRH-R, for example = $7.289+0.885\times PPAR\gamma_h-(-0.348)\times PPAR\gamma_p-(-0.733)\times PPAR\gamma_o$.

Table 5. Correlations between expression levels of *PPARγ* mRNA in hypothalamus-pituitary-ovary axis and blood levels of FSH, LH, E_2, P_4, 15d- PGJ_2 at time before slaughter[1,2]

Item	FSH	LH	E_2	P_4	15d- PGJ_2	E_2:P_4	15d- PGJ_2:P_4
Period 1 [3]							
$PPAR_h$	-0.247	-0.217	0.021	0.211	-0.405	-0.261	-0.225
$PPAR_p$	0.827^a	0.817^a	-0.009	-0.616	0.534	0.817^a	0.848^A
$PPAR_o$	-0.759^a	-0.771^a	0.516	0.481	-0.681^a	-0.912^A	-0.919^A
Period 2							
$PPAR_h$	-0.274	-0.204	0.487	0.283	-0.436	-0.265	-0.247
$PPAR_p$	0.815^a	0.819^a	-0.324	-0.609	0.625	0.822^a	0.851^A
$PPAR_o$	-0.822^a	-0.792^a	0.736^a	0.563	-0.806^a	-0.926^A	-0.905^A

[1] $PPAR_h$, $PPAR_p$ and $PPAR_o$ means mRNA expression abundances in hypothalamus, pituitary and ovary, respectively.

[2] Superscript small letter and capital letter mean significance of coefficients at 0.05 and 0.01 levels, respectively.

[3] Period 1, blood samples of C- and R-group were collected at the same time when the full-sib sows in C-group exhibit standing heat; period 2, blood samples of C- and R-group were collected just before slaughter, respectively.

estrus intervals (WEI) ($p<0.05$). However, the BW loss during lactation was negatively associated with *PPARγ* mRNA levels in pituitary ($p<0.05$) and positively related to *PPARγ* mRNA levels in ovary ($p<0.01$). As for the BFT, the BFT at weaning or at estrus were not related to *PPARγ* mRNA expression in hypothalamus ($p>0.05$), but positively related to mRNA level of *PPARγ* in pituitary ($p<0.05$) and negatively related to mRNA level of *PPARγ* in ovary ($p<0.01$). The significant correlation between the recovery of BFT at WEI and *PPARγ* mRNA abundance was not observed, whereas the BFT loss during lactation was negatively or positively related to mRNA levels of *PPARγ* in pituitary or ovary ($p<0.01$), respectively.

Correlations of mRNA level between *PPARγ* and *GnRH-R*, *FSH-R*, *LH-R*, *ES-R* and *Arom*

The results of linear regression analysis showed that the mRNA levels of *PPARγ* in ovary is significantly related to the expression abundances of *GnRH-R*, *FSH-R*, *ES-R* and *Arom*, and only *GnRH-R* was obviously associated with *PPARγ* expression in pituitary (Table 4). However, *PPARγ* expression in hypothalamus was likely not related to these genes expression. These results were consistent with the analysis results of coefficients of pearson correlation (Table 4), and the mRNA levels of *GnRH-R*, *FSH-R*, *LH-R*, *ES-R* and *Arom* were positively or negatively related to *PPARγ* mRNA level in pituitary or ovary, respectively.

Correlation between *PPARγ* expression and blood hormone level

From the correlation coefficients listed in Table 5, we found the trends of the associations between mRNA abundances of *PPARγ* and blood hormone profiles were very similar in period 1 (blood samples of C- and R-group were collected at the same time when full-sib sows in C-group exhibit standing heat) and period 2 (blood samples of C- and R-group were collected just before slaughter, respectively). The serum FSH and LH levels were positively related to *PPARγ* mRNA abundance in pituitary ($p<0.05$) and negatively associated with *PPARγ* mRNA abundance in ovary ($p<0.05$). The positive coefficient between 15d-PGJ_2 and mRNA level of *PPARγ* in ovary was statistically significant ($p<0.05$). The ratios of E_2 to P_4 and 15d-PGJ_2 to P_4 were related to *PPARγ* expression levels in pituitary (positive) and ovary (negative) ($p<0.05$ or 0.001).

DISCUSSION

That the primiparous sows failed to eat sufficient to meet the demands of lactation may occur spontaneously

under farm conditions (Prunier and Quesnel, 2000). Fortunately, they are able to mobilize their own body reserves to supply the nutrients required for milk production, rather than conserving these reserves to ensure a prompt start to the next reproductive cycle (Mullan and Williams, 1989). Sows having a lower daily caloric intake during lactation lose more weight and backfat than sows fed at a higher level (Bilkei, 1995). van den Brand et al. (2000) found that only body weight loss during lactation was greater in sows fed low energy intake level than that in sows fed the high feeding level, while backfat loss during lactation was not affected by treatments. In agreement with the results of Bilkei (1995), our findings indicated that sows with low energy intake had much more lactation losses in both BW and BFT compared to sows with high energy intake. However, the gains of BW and BFT from weaning to the day when sows with high energy intake exhibited estrus were no obviously statistical significance between estrous and non-estrous full-sib sows. At the same time, we also found that the sows with high energy intake were all estrous within 6 days, whereas six of seven sows with low energy intake didn't exhibit estrus within 12 days after weaning. Bilkei (1995) had the similar results that high feed and caloric intake during lactation significantly shortened the WEI of sows. The percentage of sows that exhibited estrus within 10 d after weaning was lower in sows fed the low energy feeding level than that in sows fed the high feeding level, irrespective of energy source (Bilkei, 1995; van den Brand et al., 2000). Furthermore, Thaker and Bilkei (2005) also found that lactation weight loss exerted a quadratic effect on WEI, and the effect extent of lactation weight loss on subsequent reproduction performance depended on the parities of sows.

The results of study on human indicated ovarian function is extremely vulnerable to an energy imbalance (Ellison, 1990). Strowitzki et al. (2002) reported that a negative energy balance and low amount of body fat have a negative effect on human ovarian function. In the present study, examination of the ovary development revealed that the percentage of large follicles (≥5 mm in diameter) or middle follicles (3-5 mm in diameter) in anestrous sows were much lower than those in sib-pair estrous sows. These findings were similar to the results of Quesnel et al. (1998) who reported the proportion of small (0.4 to 1.0 mm) healthy follicles to the total number of antral follicles was increased in feed-restricted primiparous sows at the expense of the proportion of bigger healthy follicles. van den Brand et al. (2000) also found that the average follicle diameter in sows fed high energy level was higher than that of sows with low feeding level on d 2 after weaning. The higher serum P_4 concentration in anestrous sows was mainly due to the larger size and higher number of corpora lutea in ovary. These results indicated that a decrease in energy intake during lactation resulted in impaired follicle development and persistent corpora lutea, ultimately affected the ovarian function.

In the present study, we firstly confirmed that *PPARγ* were expressed in hypothalamus and pituitary of postweaning sows. The relative expression abundance of *PPARγ* in hypothalamus was very lower and had no obvious difference ($p = 0.537$) between estrous and anestrous paired full-sib sows. However, the relative expression abundances of *PPARγ* in pituitary and ovary were higher and had significant difference ($p<0.01$) between estrous and anestrous paired full-sib sows. These results indicated that *PPARγ* may be involved in regulating the estrous behavior of primiparous sows. To confirm this speculation, we examined the expression of receptors which were related to ovarian function and estrus at the same time, such as *GnRH-R*, *LH-R*, *FSH-R*, *ES-R* and *Arom*. Normally, reproductive function is mediated by *GnRH-R* expressed only on the membranes of pituitary gonadotropes. The density of *GnRH-R* on gonadotropes determines their ability to respond to GnRH (Wise et al., 1984). Therefore, knowledge regarding what regulates the level of *GnRH-R* mRNA is essential to understanding changes in pituitary sensitivity to GnRH and ultimately, to expression of the LH surge (Nett et al., 2002). Regulation of *GnRH-R* gene expression is influenced by a number of factors including gonadal steroids, estradiol, progesterone, inhibin, activin and GnRH itself (Gregg et al., 1991). In the present study, both the linear regression and the pearson correlation analyzing results showed *GnRH-R* expression is also influenced significantly by mRNA expression levels of *PPARγ* in pituitary and in ovary in just inverse ways. With respect to the estrous sows, the increase of *PPARγ* mRNA level may be stimulated the expression of *GnRH-R*, which enhanced pituitary sensitivity to GnRH during the periovulatory period (Nett et al., 2002), and activated the synthesis and secretion of FSH and LH, which increased their serum concentration and ultimately stimulated the growth of follicle cells. Though the mRNA of *PPARγ* was found in hypothalamus, the expression level appeared no obvious effect on *GnRH-R* expression in pituitary, hormones levels and their receptors expression abundances in our results.

PPARγ has been more extensively studied in ovarian tissue and has been detected in mouse (Cui et al., 2002), rat (Komar et al., 2001), sheep (Froment et al., 2003), cow (Löhrke et al., 1998), pig (Schoppe et al., 2002) and human (Lambe and Tugwood, 1996) ovaries. In the present study, the mRNA expression of *PPARγ* was also detected in porcine ovary and lower expression levels were observed in normal estrous sows after weaning, which means the

expression of *PPARγ* is maybe down-regulated in response to the LH surge (Komar et al., 2001; Froment et al., 2003). The results of examination for ovarian development here indicated the higher expression levels of *PPARγ* in ovary of postweaning sows disturbed the growth of follicle cell and the ovarian steroidogenesis, which is consistent with the results of study on mouse (Cui et al., 2002). The production of E_2 in ovary of anestrous sows was very similar with that in estrous full-sib sows at the day when standing heat exhibited. However, the obvious pulse peak of E_2 was not observed in anestrous sows postweaning and contrarily, was found in estrous full-sibs. Decreased serum FSH and LH levels maybe resulted from the higher levels of E_2 at about weaning days in anestrous sows by increasing the sensitive of hypothalamo-hypophyseal axis to the negative feedback effects of E_2 (Almond and Dial, 1990). And failure to return to estrus in primiparous sows may be due, at least in part, to a reduced pituitary responsiveness to GnRH induced by durative changing with less amplitude of E_2 after weaning. In fact, PPARs are able to bind to estrogen response elements-EREs, and can act as competitive inhibitors (Keller et al., 1995). In this present study, we found *PPARγ* can reduce the mRNA expression of estrogen receptor β (*ES-R*) and *FSH-R*, and these two receptors were important to sow reproduction (Xiong et al., 2004a,b). Furthermore, *PPARγ* mRNA level in ovary was negatively related to expression of aromatase, the rate limiting enzyme for the conversion of androgens to estradiol.

The activation of *PPARγ* can influence P_4 production by ovarian cells. Komar et al. (2001) reported that activators of *PPARγ* stimulated P_4 secretion by granulosa cells obtained from eCG-primed immature rats. In cultured bovine luteal cells with the endogenous ligand for *PPARγ*, PGJ_2, P_4 production was increased over a 24 h culture period (Löhrke et al., 1998). Treated with synthetic and natural ligands for *PPARγ*, P_4 production by porcine theca cells was increased (Schoppe et al., 2002). However, the results in here showed that the less 15d-PGJ_2 level companied with the more serum P_4. Except for the experimental method (*in vitro* or *in vivo*), these difference may be due to the higher *PPARγ* mRNA level stimulated by other potential factors. Interestingly, there were not obvious relation between mRNA levels of *PPARγ* and E_2 or P_4 alone, but the notablely positive relationship between mRNA levels of *PPARγ* in pituitary and the ratios of 15d- PGJ_2 to E_2 and 15d- PGJ_2 to P_4, the remarkably negative association between mRNA levels of *PPARγ* in ovary and the ratios of 15d- PGJ_2 to E_2 and 15d- PGJ_2 to P_4 were observed. These results implied that the *PPARγ* expression in pituitary-ovary axis was associated with the interaction of E_2, P_4 and 15d-PGJ_2, at least at ovarian level. On the other hand, hormone receptors in ovary are very important for these hormone physiological effects on ovarian function. The abundances of expression for *FSH-R*, *LH-R* and *ES-R* were negatively associated with mRNA levels of *PPARγ* in ovary. Thus, the higher ovarian *PPARγ* mRNA level in energy-restricted sows may be, at least in mRNA level, related to the postweaning anestrus of primiparous sows by decreasing the sensitivity of ovary to FSH, LH and E_2.

From the present study, we can concluded that the effects of *PPARγ* on functions of pituitary and ovary have not only obviously opposite mechanisms, but also feedback interaction, to modulate the balance of development of follicle cells and ultimately regulate the estrous behavior of primiparous sows after weaning.

IMPLICATIONS

PPARγ was associated with body weight and backfat thickness of primiparous sows during and after lactation, and was directly or indirectly related to hormones reproduction and their receptors expression of postweaning sows. Interestingly, not serum estradiol, progesterone and 15d-PGJ_2 alone, but the ratios of 15d-PGJ_2 to estradiol and 15d-PGJ_2 to progesterone may be related to the *PPARγ* expression, at least in pituitary and ovary level, and can indirectly be used as the indicator that forecast whether the sow will be normal estrous after weaning.

REFERENCES

Almond, G. W. and G. D. Dial. 1990. Estradiol feedback inhibition of luteinizing hormone concentrations in the anestrous sow. J. Anim. Sci. 68:1077-1086.

Asami-Miyagishi, R., S. Iseki, M. Usui, K. Uchida, H. Kubo and I. Morita. 2004. Expression and function of *PPARγ* in rat placental development. Biochem. Bioph. Res. Co. 315:497-501.

Bilkei, G. 1995. Herd health strategy for improving the reproductive performance of pigs. J. Hung. Vet. 10:766-768.

Boyd, R. D., K. J. Touchette, G. C. Castro, M. E. Johnston, K. U. Lee and In K. Han. 2000. Recent Advances in Amino Acid and Energy Nutrition of Prolific Sows - Review. Asian-Aust. J. Anim. Sci. 13:1638-1652.

Capparuccia, L., D. Marzioni, A. Giordano, F. Fazioli, M. De Nictolis, N. Busso, T. Todros and M. Castellucci. 2002. *PPARγ* expression in normal human placenta, hydatidiform mole and choriocarcinoma. Mol. Hum. Reprod. 8:574-579.

Caroll, C. M., P. B. Lynch, M. P. Boland, L. J. Spicer, F. H. Austin, N. Leonard, W. J. Enright and J. F. Roche. 1996. The effects of feed intake during lactation and postweaning on the reproductive performance and hormone and metabolite concentrations of primiparous sows. Anim. Sci. 63:297-306.

Cheng, C. S., H. T. Yen, S. W. Roan, J. F. Wu and J. C. Hsu. 2001. Effect of Dietary Protein and Lysine Levels on Lactating

Multiparous Sows and Litter Performance. Asian-Aust. J. Anim. Sci. 14:77-81.

Chung, W. B., W. F. Cheng, L. S. Wu and P. C. Yang. 2002. The use of plasma progesterone profiles to predict the reproductive status of anestrous gilts and sows. Theriogenol. 58:1165-1174.

Cui, Y., K. Miyoshi, E. Claudio, U. K. Siebenlist, F. J. Gonzalez, J. Flaws, K. U. Wagner and L. Hennighausen. 2002. Loss of the peroxisome proliferation-activated receptor gamma (*PPARγ*) does not affect mammary development and propensity for tumor formation but leads to reduced fertility. J. Biol. Chem. 277:17830-17835.

Desvergne, B. and W. Wahli. 1999. Peroxisome Proliferator-Activated Receptors: Nuclear Control of Metabolism. Endocr. Rev. 20:649-688.

Ellison, P. T. 1990. Human ovarian function and reproductive ecology. Am. Anthropol. 2:933-952.

Froment, P., S. Fabre, J. Dupont, C. Pisslet, D. Chesneau, B. Staels and P. Monget. 2003. Expression and functional role of peroxisome proliferator-activated receptor γ in ovarian folliculogenesis in the sheep. Biol. Reprod. 69:1665-1674.

Gourdine, J. L., H. Quesnel, J-P. Bidanel and D. Renaudeau. 2006. Effect of season, parity and lactation on reproductive performance of sows in a tropical humid climate. Asian-Aust. J. Anim. Sci. 19:1111-1116.

Gregg, D. W., R. H. Schwall and T. M. Nett. 1991. Regulation of gonadotropin secretion and number of gonadotropin-releasing hormone receptors by inhibin, activin-A and estradiol. Biol. Reprod. 44:725-732.

Guan, Y., Y. Zhang, L. Davis and M. D. Breyer. 1997. Expression of peroxisome proliferator-activated receptors in urinary tract of rabbits and humans. Am. J. Physiol. Renal. Physiol. 273:F1013-F1022.

Houseknecht, K. L., B. M. Cole and P. J. Steele. 2002. Peroxisome proliferator-activated receptor gamma (*PPARγ*) and its ligands: A review. Domest. Anim. Endocrin. 22:1-23.

Issemann, I. and S. Green. 1990. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. Nature 347:645-650.

Keller, H., W. Wahli and M. Perroud. 1995. Signaling cross-talk between peroxisome proliferator-activated receptor/retinoid X receptor and estrogen receptor through estrogen response elements. Mol. Endocrinol. 9:794-804.

Kliewer, S. A., J. M. Lenhard, T. M. Willson, I. Patel, D. C. Morris and J. M. Lehmann. 1995. A prostaglandin J_2 metabolite binds peroxisome proliferator-activated receptor gamma and promotes adipocyte differentiation. Cell 83:813-819.

Komar, C. M., O. Braissant, W. Wahli and T. E. Curry Jr. 2001. Expression and localization of PPARs in the rat ovary during follicular development and the periovulatory period. Endocrinol. 142:4831-4838.

Lambe, K. G. and J. D. Tugwood. 1996. A human peroxisome-proliferator-activated receptor-γ is activated by inducers of adipogenesis, including thiazolidinedione drugs. Eur. J. Biochem. 239:1-7.

Löhrke, B., T. Viergutz, S. K. Shahi, R. Pöhland, K. Wollenhaupt, T. Goldammer, H. Walzel and W. Kanitz. 1998. Detection and functional characterisation of the transcription factor peroxisome proliferator-activated receptor γ in lutein cells. J. Endocrinol. 159:429-439.

Meredith, M. J. 1984. Anoestrus in the pig. Pig News Info. 5:213.

MLC. 1995. Pig Year Book 1995. Meat and Livestock Commission, Milton Keynes, p. 149.

Mullan, B. P. and I. H. Williams. 1989. The effect of body reserves at farrowing on the reproductive performance of first-litter sows. Anim. Prod. 48: 449-457.

National Research Council. 1998. Nutrient Requirements of Swine. 10th Ed. National Academic Press, Washington, DC.

Nett, T. M., A. M. Turzillo, M. Baratta and L. A.Rispoli. 2002. Pituitary effects of steroid hormones on secretion of follicle-stimulating hormone and luteinizing hormone. Dom. Anim. Endocrinol. 23:33-42.

Prunier, A. and H. Quesnel. 2000. Nutritional influences on the hormonal control of reproduction in females pigs. Livest. Prod. Sci. 63:1-16.

Quesnel, H., A. Pasquier, A. M. Mounier and A. Prunier. 1998. Influence of feed restriction during lactation on gonadotropic hormones and ovarian development in primiparous sows. J. Anim. Sci. 76:856-863.

Rozeboom, D. W., R. L. Moser, S. G. Cornelius, J. E. Pettigrew and S. M. El Kandelgy. 1993. Body composition of postpubertal gilts at nutritionally induced anestrus. J. Anim. Sci. 71:426-435.

Schoppe, P. D., J. C. Garmey and J. D. Veldhuis. 2002. Putative activation of the peroxisome proliferator-activated receptor γ impairs androgen and enhances progesterone biosynthesis in primary cultures of porcine theca cells. Biol. Reprod. 66:190-198.

SPSS Inc. 1999. SPSS Base 10.0 for Windows User's Guide. SPSS Inc. Chicago IL.

Strowitzki, T., B. Halser and T. Demant. 2002. Body fat distribution, insulin sensitivity, ovarian dysfunction and serum lipoproteins in patients with polycystic ovary syndrome. Gynecol. Endocrinol. 16:45-51.

Thaker, M. Y. C. and G. Bilkei. 2005. Lactation weight loss influences subsequent reproductive performance of sows. Anim. Reprod. Sci. 88:309-318.

Tontonoz, P., E. Hu and B. M. Spiegelman. 1994. Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. Cell 79:1147-1156.

van den Brand, H., S. J. Dieleman, N. M. Soede and B. Kemp. 2000. Dietary energy source at two feeding levels during lactation of primiparous sows: I. Effects on glucose, insulin, and luteinizing hormone and on follicle development, weaning-to-estrus interval, and ovulation rate. J. Anim. Sci. 78:396-404.

Wise, M. E., D. Nieman, J. Stewart and T. M. Nett. 1984. Effect of number of receptors for gonadotropin-releasing hormone on the release of luteinizing hormone. Biol. Reprod. 31:1007-1013.

Wright, P. J. and J. Malmo. 1992. Pharmacologic manipulation of fertility. Vet. Clin. North. Am. Food Anim. Pract. 8:57-89.

Xiong Yuanzhu, Li Fenge, Lei Minggang, Zheng Rong, Zuo Bo, Jiang Siwen and Deng Changyan. 2004a. The effects of estrogen receptor locus on reproductive tracts components and performance traits in large white meishan F_2 offspring. Asian-Aust. J. Anim. Sci. 17:1223-1226.

Xiong Yuanzhu, Zhang Shujun, Den Changyan, Xiao Senmu, Xu Jianxiang, Xia Yu, Liu Xiaohua, Wang Chunfang and Sun Shulin. 2004b. FSHR gene mutation and its effect on litter size in pigs. Asian-Aust. J. Anim. Sci. 17:179-182.

Ziecik, A. J., K. L. Esbenshade and J. H. Britt. 1989. Effects of a gonadotrophin-releasing hormone antagonist on gonadotrophin secretion and gonadal development in neonatal pigs. J. Reprod. Fertil. 87:281-289.