Novel *BsuRI-c.930A>G-FSHβ* Associated with Litter Size Traits on Large White X Landrace Crossbred Sows

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Abstract

The objective of this experiment was to identify the novel single nucleotide polymorphisms (SNPs) on porcine follicle stimulating hormone β subunit (*FSHβ*) genes. Moreover, their association with litter size traits in commercial pigs will be analyzed. 1,155 Large White x Landrace crossbred sows were bled and DNA was extracted. The records included total number of piglet born (TNB), number of piglet born alive (NBA), number of piglets stillbirth (SB) and number of piglets mummified (MM). The known sequence of porcine *FSHβ* (GenBank accession no. D00621.1) was screened homology to known sequences in term of express sequenced tags (ESTs) in public domain, GenBank. The primers were designed for amplified the novel *BsuRI-c.930A>G-FSHβ* fragment which confirmed by PCR-RFLP and nucleotide sequencing then genotyping. The favorable cut homozygous *G/G* allele was highly significant higher than *A/G* allele in terms of TNB and NBA. While, published marker *HaeIII-g.5894A>G-FSHβ* was not significant difference in any litter traits. For haplotype analysis, c.930A>G-*g.5894*A>G of *FSHβ,* unfavorable *GG/AA* haplotype was significantly lower TNB and NBA than other haplotypes. Conversely, this haplotype was significantly higher MM than others. The study concluded that *BsuRI-c.930A>G* and the haplotype of *HaeIII-g.5894A>G - BsuRI-c.930A>G* of *FSHβ* may used for marker-assisted selection on pig breeding program.

Keywords: Litter size traits, Follicle stimulating hormone, Single nucleotide polymorphism, Marker-assisted selection

1. Introduction

Reproductive performance, especially litter size in terms of TNB and NBA are one of the most economically

important traits in pig production. Reproductive success in the pig viewed the number and quality of piglets produced (Kyriazakis and Whittemore, 2006). It is well known that litter size is affected by many factors such as genetics, farm, feeds, season mating boars and fertilization rate, which impact for a large part of the phenotype variance. From a genetic background, litter size might be controlled by numerous genes in complicated physiological metabolisms such as those affecting on ovulation rate, fertilization rate, embryo survival, uterine capacity (Foxcroft *et al*., 2006), fetal survival and pre-weaning losses (Distl, 2007). Nowadays, mean of total number of piglet born is 10.03 ± 2.64 piglets/litter (Imboonta et al., 2007). Litter size is low heritability approximately 0.09 for NBA that affecting slowly changed in genetic drift (Rothschild, 1996; Distl, 2007) and difficult to measure phenotypically by traditional breeding method (Merks *et al*., 2000). Furthermore, litter size trait is sex-limited and is not measurable until sexual maturity (Spötter and Distl, 2006). This breeding method is the-state-of-the-art and setback over 3-5 years or up to 10 generation that gain of about 0.6 piglets within-line selection (Bolet *et al*., 2001).

Polymorphism of candidate genes and their association with litter size was intensively studied in many laboratories all over the world (Ernst *et al*., 2003). The classical molecular marker for litter size traits is *estrogen receptor* gene which SNP of *estrogen receptor* gene associated with TNB and NBA in pigs (Short *et al*., 1997a). Other candidate genes affected litter size traits were *prolactin receptor* (Drogemüller *et al*., 2001)*, retinol-binding protein4* (Linville *et al*., 2001)*, osteopontin* (Short *et al*., 1997b)*, leukemia inhibitory factor* (Spötter *et al*., 2005) *folate binding protein* (Valet *et al*., 2005a) and *erythropoietin receptor* (Vallet *et al*., 2005b).

Bertani *et al*. (2004) identified transcriptional candidate genes for fertility in anterior pituitary gland of pigs. *FSHβ* was found 10% highly expressed between sows selected for ovulation rate and embryo survival compared with the control line. Moreover, *FSHβ* gene was chosen as a direct candidate gene because its functions in follicle maturation (Wang and Greenwald, 1993a, b; Simoni and Nieschlag, 1995). Also, in a direct candidate gene analysis, Li *et al*. (1998), Zhao *et al*. (1999) and Liu *et al*. (2009) found that the genetic polymorphism of intronic *FSHβ* was associated with litter size in Chinese breed pigs. Whereas, no association of these intronic polymorphism with litter size traits in Large White/Landrace composite (Linville *et al*., 2001) and Polish synthetic breed (Korwin-Kossakowska *et al*., 2003) was found. Hence, there must be another nucleotide change which is responsible for its function. The aims of this study were to identify the novel SNP on porcine *FSHβ* genes using *in silico* analysis. Moreover, their association with litter size traits in commercial pigs would be analyzed.

2. Materials and methods

2.1 Animals and data

A totally 1,155 commercial sows (Large White X Landrace or its reciprocal) including 4,162 parities, were bled and recorded in terms of TNB which was calculated as NBA plus SB and MM on 1st parity to 5th parity for association study. Primary data were plotted and 20 sows were selected form extremely tails of population into 2 groups; 10 High-TNB (>15 piglets/litter) and 10 low-TNB sows (<8 piglets/litter) for screening SNPs. Associated study was analyzed between candidate gene and phenotypic traits in term of TNB and NBA and mortality traits including SB and MM. The descriptive statistics was shown as table 1.

Blood samples were collected from jugular vein with 0.5M EDTA anti-coagulant and extracted by using Chelex®. Briefly, 20 μl of blood was lysed twice, centrifuge at 2,000 rpm 1 min, added Chelex® solution over the pellet and incubated overnight. The solution samples were boiled at 95°C for 5 minutes and stored at 4°C until analyzed (Walsh *et al*., 1991).

2.1 Cloning and sequencing

PCR product would be cloned into pGEM-T Easy Vector system. The cloned PCR fragment compared with PCR product obtained from genomic DNA. Three clones with the same size of inserted fragment would be sequenced on the CEQ™ 8000 Genetic Analysis System, Beckman Coulter by using GenomeLab™ DTCS Quick Start Kit (Beckman Coulter, USA).

2.3 PCR-RFLP

In silico SNP analysis was potentially technique that was based on BLAST software which screened homology to known sequences in term of express sequenced tags (ESTs) in public domain, GenBank. Specific primers were designed based on published sequence information of porcine *FSHβ* (GenBank accession no. D00621.1). Similarly, standard PCR was performed in a final reaction volume of 20 μl by using 50.0 ng of genomic DNA sample, 1X NH_4SO_4 buffer, 0.4 μmol of primer; f- acagttttttacaggcctta & r- ctggctgggtccttgtat, 0.5 μl of 0.2

mmol dNTPs, 1.5 mmol $MgCl_2$, 13.4 μl dH_2O and 0.25 unit *Taq* DNA polymerase (Fermentas). The PCR product was checked on 6% polyacrylamide gel electrophoresis. A 2.5 μl of the PCR product of *c.*930A>G-*FSHβ* was digested with 0.25 unit of *Bsu*RI by manufacturing instruction. As well as, PCR of *FSHβ* published marker (Li *et al.*, 1998) was performed as above, the primer; f- gtataccaggtcctaag & r-gtctcgtacaccagctcctt and digested with 0.25 unit of *Hae*III by manufacturing instruction. The PCR-RFLP fragments of each candidate genes were ran on 6% polyacrylamide gel electrophoresis and stained with standardized silver staining protocol.

2.4 Statistic analysis

Allele and genotype frequencies of each candidate genes were determined by the total count of an allele and genotype divided by twice the number of observations. Association of SNP markers with litter size traits were analyzed with general linear model included fixed effect of genotype, parity number and residual as following:

$$Y_{ijk} = \mu + genotype_i + parity_j + e_{ij}$$

Where μ denoted the average normalized of population; $genotype_i$ denoted the fixed effect of genotype *i*; $parity_j$ denoted the fixed effect of parity *j*; and e_{ij} denoted the residual effects of genotype *i*, and parity *j*. Moreover, additive effect (*a*) would be calculated as by comparison of the means of the traits value for homozygote ($a = \frac{1}{2}$ (BB-AA)). The dominance effect (*d*) = alleles A and B would be calculated from the means for three genotypes as follows: *d* = AB-1/2(AA+BB). The estimated effects would be tested by t-test on significant deviation from zero.

3. Results

In this experiment, *in silico* analysis for screening SNPs that used BLAST software based analysis was used. The *FSHβ* sequence (GenBank accession no. D00621.1) was screened homology to known sequences in public database with BLAST software (Altschul *et al.*, 1997). Totally, 231 SNPs were found comprising 8 SNPs in 5'-UTR, 23 SNPs in exon1, 32 SNPs in exon2, 45 SNPs in exon3 and 123 SNPs in 3'-UTR (Table 2). Only exon3 c.930A>G SNP of *FSHβ* gene was potentially found. Consequently, according to this *in silico* SNP was proved by using PCR-RFLP technique.

Interestingly, *Bsu*RI-*FSHβ* was discovered by *in silico* analysis and proved by PCR-RFLP that did not know about the nucleotide change of this SNP between polymorphic patterns of homozygous uncut and homozygous cut. Consequently, homozygous uncut and homozygous cut of *Bsu*RI-*FSHβ* was sequenced and aligned with BLAST software. Transition of c.930A>G of *FSHβ* was found that confirmed *in silico* analysis.

3.1 Genotype and allele frequencies for the investigated SNP for FSHβ gene

Frequencies for the *FSHβ* gene in 1,155 parent stock of commercial sows including 4,162 parity records were shown in Table 3. Frequency of *A* and *G* allele of *Bsu*RI-*c.930A>G-FSHβ* was 0.81 and 0.19, respectively, while frequency of *Hae*III-*g.5894A>G-FSHβ* (according to Li et al., 1998) was 0.70 and 0.30, respectively. The allele frequencies of these two SNPs were not distributed according to Hardy-Weinberg equilibrium proportion.

3.2 Association study and haplotype analysis

The association between *FSHβ* genotype and litter size traits in terms of TNB, NBA, SB and MM piglets was analyzed as shown as Table 4. For *Bsu*RI-*c.930A>G-FSHβ*, *G/G* genotype of *FSHβ* was highly significant difference than those *A/A* and *A/G* genotype in TNB and NBA. Significantly dominance effect of *Bsu*RI-*c.930A>G-FSHβ* on TNB and NBA was found. While, *Hae*III-*g.5894A>G-FSHβ* gene, according to Li *et al.* (1998), all litter size traits including TNB, NBA, SB and MM were not significant difference in this population.

The haplotype analysis between *c.930A>G-FSHβ* and *Hae*III-*g.5894A>G-FSHβ* on litter size traits was shown in table 5. Higher litter trait was expected, adversely, mortality trait was nil. However, the unfavorable haplotype *GG/AA* was significant different than others haplotype in TNB, NBA and MM, the *GG/AA* was highly significant lower TNB and NBA than other haplotypes, otherwise, was highly significant higher MM than other haplotypes.

4. Discussion

The TNB was approximately 9.2-11.1 piglets/litter and NBA was 8.9-10.2 piglets/litter of 2,400 Landrace x Large White crossbred sow records (Tummaruk *et al.*, 2001). Moreover, 12,599 Thai Landrace sows recorded between's 1993-2005, TNB and SB was 10.03±2.64 and 0.21±0.59, respectively (Imboonta *et al.*, 2007). While, Moeller et al. (2004) stated between's 1997-1999 that TNB was 11.05±1.49 pigs with range 1-25 pigs, and NBA was 9.96±1.38 pigs with range 0-21 pigs.

In present study, 2006-2008, 1,155 Large White x Landrace crossbred sows showed 11.24$\pm$0.05 and 9.91$\pm$0.05 piglets/litter in TNB and NBA, respectively. These parameters exhibited the genetic progress in Thai's swine commercial that tended to be favorable for white breeds and widely used by the commercial to produced F_1 crossbred gilts resulting from imported nucleus herd. Moreover, crossbred gilts tended to younger at puberty, weighted more at farrowing, and produced larger, heavier litters to weaning than the purebred gilts (Cassady *et al.*, 2001). In addition, the main direction of selection conventional method for this founder pigs was the meat deposition in carcass and growth rate, but was not considered in the litter size traits and its related traits.

4.1 SNPs discovery techniques

There are two different strategies are employed to detect markers for piglet number and its related traits; linkage analyses and direct candidate gene. These approaches were employed to detect genes influencing piglet number and its related traits. Candidate gene approaches identified via their physiological or functional role in the reproductive system in the pig, while linkage analysis detected genomic regions that responded the trait of interest as known as positional candidate (Distl, 2007). All of them were focus on SNPs that associated with economically traits as well as piglet number and its related traits. Normally, SNPs detection referred to the identification of allelic variants of single or low-copy-number gene sequences. Direct sequencing of specific sequence regions is still the most reliable and accurate method of SNPs detection, but it is expensive and labor intensive. PCR-based technique, however, was used to analyze that offers the powerful advantage of allowing one to conveniently pre-screen large numbers of unknown sample of which only implicated variants would need to be directly sequenced (Armersham, 1998).

In silico SNP analysis was potentially technique that is based on BLAST software that screened homology to known sequences in term of express sequenced tags (ESTs) in public domain, GenBank (Marth *et al.*, 1999; Savage *et al.*, 2005 and Kerstens *et al.*, 2009). The main advantages of using EST source were that markers closely associated with or directly in the coding region, could be identified that possible leaded to amino acid substitutions or perhaps leaded to functional differences which could be associated with phenotypic effect in the population. Approximately 40% of this approach was newly candidate SNPs of which cost effectiveness (Picoult Newberg *et al.*, 1999). Consequently, candidate SNPs was validated with RFLP and sequencing approach, respectively, that confirmed true positive results (Marth *et al.*, 1999). However, simulation by Kerstens *et al.* (2009) in 1.2 Gb of pig genome sequence, 98,151 SNPs were identified in which one of the sequences in the alignment represented the polymorphism and 6,374 SNPs in which two sequences represent an identical polymorphism.   To benchmark the SNP identification method, 163 SNPs, in which the polymorphism was represented twice in the sequence alignment, were selected and tested on a panel of three purebred boar lines and wild boar. Of these 163 in silico identified SNPs, 134 were shown to be polymorphic in animal panel. In addition, an economically example of *in silico* method was the variation in *POU1F1* gene that found 23 polymorphic sites of porcine *POU1F1* within the intron (Song *et al.*, 2007). Moreover, SNP of *Secreted phosphoprotein 1* (*SPP1*) or *Osteopontin* (*OPN*) at position g.3836A>G represented an interesting DNA-marker to study phenotypic effects (Murani *et al.*, 2009).

4.2 FSHβ polymorphisms discovery and association

FSHβ was chosen as the candidate gene because *FSHβ* was physiological role on the reproductive in pigs. The function of *FSHβ* included follicular development and sex steroid production necessary for fertility in female (Layman and McDonough, 2000). These functions were supported by studies involving knockout of the *FSHβ* gene ligand and the *FSH* receptor, as well as by human gene mutations (Layman *et al.*, 2002).

Porcine *FSHβ* genomic DNA sequence composted of 10,172 bp comprising 3 exons where exon1 was nucleotide position 5,664-5,696 exon2 at 6,614-6,810 and exon3 at 6,811-8,366 that encoded 129 amino acids at nucleotide position 6,652-6,810 and 8,367-8,597 (accession no. D00621.1). Known D00621.1 sequence was aligned with ESTs from GenBank (Altschul *et al.*, 1997). Exon2 of *FSHβ* was aligned with 16 ESTs that found 32 polymorphic sites. 31 polymorphic sites were only 1 of 16 ESTs, while 1 polymorphic site was 6 of 16 ESTs that *Pvu*II-c.128T>C-*FSHβ* was found. Moreover, exon3 was aligned with 16 ESTs that found 45 polymorphic sites. The *Bsu*RI-c.930A>G-*FSHβ* polymorphism was found 6 of 16 ESTs. These polymorphic sites were validated and confirmed by using PCR-RFLP and with DNA sequencing, respectively. In this study, the novel polymorphism of *Bsu*RI-c.930A>G-*FSHβ* was found that associated with TNB and NBA in sows. However, the allele frequency of *Bsu*RI-c.930A>G-*FSHβ* in our study could not be compared with results published by other authors (Ernst *et al.*, 2003). Classical polymorphism of *FSHβ* was discovered by Ellegren *et al.* (1994) that microsatellite *FSHβ* located on *SSC*2. Rohrer *et al.* (1994) reported a PCR-RFLP marker located within the first intron of *FSHβ* gene. Li *et al.* (1998) reported an association of *FSHβ* gene in the first intron and litter size. Zhao

et al. (1998) found the insertion is a retroposon of 292 bp siting in intron I at the site between + 809 and + 810 base. Li *et al*. (2000) identified *FSHβ* allele in Chinese pigs that had an 11 bp deletion in 3'-UTR in anterior pituitary glands. Recent study, Li *et al*. (2009) reported microsatellite *FSHβ* associated with the number of litter per year, but not yet with litter size and its related traits and Liu *et al*. (2009) fund insertion in the first intron of *FSHβ*.

The results reported in this study provide important evidence in favor of the presence of new alleles of the *FSHβ* gene associated with litter size traits. We observed a low number of sows with *G/G* genotype in the *Bsu*RI-c.930A>G-*FSHβ* gene or generally low presence of *G* allele in our population that was mutant type. The favorable *G* allele of novel *Bsu*RI-c.930A>G-*FSHβ* in 3'-UTR was exhibited in the high-piglet number sows as well as TNB and NBA in Large White x Landrace crossbred sows. *G/G* homozygote sows produced on average 0.76 and 0.87 piglets more than did *A/G* sows for TNB and NBA, respectively. Adversely, the published marker, *Hae*III-g.5894A>G-*FSHβ* (Li *et al*., 1998), found no associative effect between genotype and litter traits in this population. Moreover, no additive and dominance effect was found. Although large differences in allele frequencies were found for genetic marker within *Bsu*RI-c.930A>G-*FSHβ* locus, influences of potential genetic drift were confounded with those results because the additive and dominance effects of the allele did not differ from zero for any trait (Bertani *et al*., 2004). The results of *Hae*III-g.5894A>G-*FSHβ* failed to confirm population wide linkage disequilibrium between the *Hae*III-g.5894A>G-*FSHβ* polymorphism and litter size traits suggesting that the degree of linkage disequilibrium probably varies between populations (Gibson *et al*., 2002). Zhao *et al*. (1998) found a retroposon in intron 1 of *FSHβ* that non-retroposon homozygote sows produced 2.53 piglets more than retroposon homozygote sows for TNB on the 1st parity. On the contrary, recent study reported inversely association between piglet number and *HaeIII-FSHβ* locus in Polish pig line (Korwin-Kossakowsak *et al*., 2003), in Large White (Wang *et al*., 2006; Humpolicek *et al*., 2007). Microsatellite 5'-flanking *FSHβ* affected number at weaning, litter weight at weaning, average individual weight at weaning in Large White x Chinese Meishan F$_2$ (Li *et al*. 2008). The fluctuated result of association study on porcine *FSHβ* gene may be allele effects differed between lines or populations or the genetic background, it can be explained by a high number of low-effect genes influencing the litter size (Drogemüller *et al*., 2001). The results can be explained by a high number of low-effect genes influencing the piglet number. Moreover, the animal genome is a complex set that interacted on different levels of biological organization, it seems difficult to find direct marker that proofed these effects (Matousek *et al*., 2005).

Two of the polymorphisms, *Bsu*RI-c.930A>G-*FSHβ* and *Hae*III-g.5894A>G-*FSHβ*, were considered to be the most interesting because of an associated with litter characteristics and because these two polymorphisms, assembled together in haplotypes, specified the nine individual haplotypes revealed in the *FSHβ* gene in the Large White x Landrace sows. Individual effects were analyzed and significant differences were detected for *Bsu*RI-c.930A>G-*FSHβ*. However, because of the significant linkage disequilibrium between *FSHβ* polymorphisms, the haplotypes were tested instead of the individual polymorphisms and found to have a highly significance effect on TNB and NBA (Ciobanu *et al*., 2004). Moreover, increasing the number of marker genes or haplotype raised the positive detection rate more than single marker gene alone. These results proved that using multiple markers or haplotype indeed improved the positive detection rate. The detection rate indicated the sensitivity of using the multiple markers for detection (Sher *et al*., 2005). Three particular haplotypes; *AA/AA*, *AA/AG* and *AG/AG*, within the *FSHβ* gene are highly frequent in the population and do not differ in their effects on MM, even though they carry different alleles. This might indicate the effect of other SNP linked to the two SNPs considered in this study or some degree of epistasis among the SNP within the same chromosome (Schenkel *et al*., 2005).

A QTL mapping approach normally uses families created form crosses based on divergent lines. Indeed, this approach has been used in relation to litter size traits using segregation analysis (de Vries *et al*., 2000). A number of pig populations are now being used created for the purposes of searching for litter traits QTLs including crosses between the following breeds: wild boar, Large White, Meishan, Landrace and Duroc. The QTL scan for plasma FSH level in pigs identified several chromosome regions, independent of the *FSHβ*, influencing this trait that located on chromosome 3, 8, 10 and X (Linville *et al*., 2001 and Rohrer *et al*., 2001). A major genetic effect of litter size has been located on SSC 7 (Milan *et al*., 1998), SSC 8 (Wise *et al*., 2001) and on SSC 11 (Cassady *et al*., 2001). Furthermore, mRNA differential display of granulosa cell culture with FSH treatment has been also mapped on porcine that localized on various porcine chromosomes (Clouscard-Martinato *et al*., 1998). ESR was located on SSC 1 (Rothschild, 1996) and *LEPR* was located on SSC 6 (Wilkie *et al*., 1999) those have an influence on litter size in pigs. *FSHβ* was located to 2p12-16 by physical mapping (Mellink *et al*., 1995) and mapped on SSC2 by linkage mapping (Rohrer *et al*., 1994). The genome scans for regions where significantly

affected plasma FSH in boars showed that located on chromosome 3, 10 and X, while no evidence has been found that any loci located on chromosome 2 affecting FSH level (Rohrer *et al*., 2001). Rathje *et al*. (1997) stated the QTL for ovulation rate on chromosome 8. Short *et al*. (1997b) reported association between microsatellite and OPN gene that located in the same region of the chromosome 8 and litter traits. Recent study, a 5% genome-wide significant QTL was detected at 88 cM on SSC 15 for NBA, which also showed suggestive effect on TNB, four suggestive QTL were detected on SSC 6, 7, 8 and 15 for TNB, NBA or SB, and no QTL was found for MM (Li *et al*., 2009). Moreover, mRNA differential display of *FSH-regulated* gene was localized on SSC 7 where the QTL related to litter size was found (Milan *et al*., 1998; Clouscard-Martinato *et al*., 1998). Thus, an unknown gene must stimulate changes in *FSHβ* mRNA profiles. Pomp *et al*. (2001) proposed that *FSHβ* locus itself has not yet been implicated as a QTL.

This *FSHβ* candidate gene study concluded that polymorphism of novel *Bsu*RI-c.930A>G-*FSHβ* was found that associated with TNB and NBA. However, animal genome is a complete set realized through a branch network of the hormonal interactions of hypothalamic-pituitary-ovarian-uterine axis. The polymorphisms in the gene studied may not directly affect the trait. These polymorphisms could be markers linked with causative mutation within the gene or a closely linked gene. It would be of interest to examine the association study and haplotype analysis in other populations and to extend research with a large number of animals to confirm the results of this study. Moreover, it should be focused at the analysis of high number of genes in order to identify gene interaction network and to describe metabolism of gene activities.

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Table 1. Descriptive statistics of commercial sow population

traits	N	Mean±SE	SD	Range
TNB	4162	11.24±0.05	3.06	1-21
NBA	4162	9.91±0.05	3.04	0-19
SB	4162	0.92±0.02	1.17	0-13
MM	4162	0.42±0.02	1.18	0-15

TNB = total number of piglets born, NBA = number of piglets born alive, SB = number of piglet stillbirth and MM = number of piglet mummified.

Table 2. Distribution of nucleotide substitution types of the *FSH* SNPs

Type	Contig					Total	Percent
	5'	Exon1	Exon2	Exon3	3'		
A/C	-	7	1	2	14	24	10.39
G/T	1	5	2	11	18	37	16.02
A/G	-	3	2	2	15	22	9.52
C/T	1	6	6	9	18	40	17.32
A/T	-	-	8	8	27	43	18.61
C/G	1	2	12	7	21	43	18.61
In/del	5	-	1	6	10	22	9.52
Total	8	23	32	45	123	231	
Percent	3.46	9.96	13.85	19.48	53.25		100

Table 3. Genotype and allele frequency of porcine *FSHβ*

SNPs	Genotype	Genotype frequency	Cumulative frequency	Allele frequency	χ^2
c.*930A>G*	*A/A*	736	736	f(a)=0.81 f(b)=0.19	987 (df=2) P<0.00
	A/G	390	1126		
	G/G	29	1155		
g.5894A>G (Li et al, 1998)	*A/A*	527	527	f(a)=0.70 f(b)=0.30	367 (df=2) P<0.00
	A/G	561	1088		
	G/G	67	1155		

Table 4. Piglet number related trait in parent stock of commercial pigs on parity 1-5 of *FSHβ* gene

Traits	*BsuRI-c.930A>G- FSHβ*			*a*	*d*
	A/A	A/G	G/G		
TNB	11.46+0.07[AB]	11.12+0.10[B]	11.71+0.28[A]	-0.12+0.14	-0.46+0.16**
NBA	9.91+0.07[AB]	9.67+0.10[B]	10.38+0.27[A]	-0.24+0.14	-0.47+0.16**
SB	1.08+0.03	1.02+0.04	0.89+0.10	0.10+0.05	0.04+0.06
MM	0.47+0.03	0.42+0.04	0.43+0.11	0.02+0.05	-0.03+0.06
Traits	*HaeIII-g.5894A>GFSHβ*			*a*	*d*
	A/A	A/G	G/G		
TNB	11.46+0.08	11.28+0.08	11.31+0.20	0.07+0.10	-0.10+0.12
NBA	9.90+0.08	9.81+0.08	9.84+0.20	-0.03+0.10	-0.06+0.12
SB	1.09+0.03	1.02+0.03	1.04+0.08	0.02+0.04	-0.04+0.05
MM	0.46+0.03	0.45+0.03	0.43+0.08	0.01+0.04	0.43+0.08

[A,B] LSMeans+SEM within row with different superscripts differ (P<0.01).

** means highly significant difference.

Table 5. Haplotype analysis of *FSHβ* gene on litter size traits in parent stock of commercial pigs

trait	c.930A>G – *Hae*III-*FSHβ*								
	AA/AA	*AA/AG*	*AA/GG*	*AG/AA*	*AG/AG*	*AG/GG*	*GG/AA*	*GG/AG*	*GG/GG*
TNB	11.37+0.07[A]	11.030+0.10[A]	11.53+0.32[A]	11.22+0.23[A]	10.97+0.10[A]	10.56+0.30[A]	9.33+1.02[B]	11.92+0.39[A]	11.72+0.42[A]
NBA	9.97+0.07[AB]	9.96+0.10[AB]	9.86+0.32[AB]	9.72+0.23[AB]	9.76+0.09[AB]	9.54+0.30[B]	7.33+1.01[C]	10.93+0.39[A]	10.60+0.42[A]
SB	0.98+0.03	0.89+0.04	1.03+0.12	0.97+0.09	0.84+0.04	0.78+0.12	0.78+0.39	0.67+0.15	0.81+0.16
MM	0.42+0.03[B]	0.45+0.04[B]	0.63+0.12[B]	0.53+0.09[B]	0.37+0.04[B]	0.27+0.12[B]	1.22+0.39[A]	0.31+0.15[B]	0.30+0.16[B]

[A,B] LSmean+SEM within row with different superscripts differ (P<0.01).