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Novel FSHβ gene SNPs correlation to litter size in Baladi and Zaraibi goats

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Abstract: The objective of the present study is to identify the genetic polymorphism at two loci (exon1 and int2, exon3) in the FSHβ gene in two different Egyptian goat breeds (Baladi and Zaraibi goats) and their correlation with litter size. Using PCR- SSCP technique; 4 genotypes (AA, BB, CC and EE) were detected with the predominance of AA and CC genotype among the two breeds. Several SNPs were reported after sequencing in different genotypes. AA genotype had greater litter size in the two goat breeds. PCR-RFLP for int2, exon3 demonstrated the presence of 2 genotypes (MM and MS) in Baladi goats and only one genotype (MS) in Zaraibi does, with the predominance of MS genotype among the two breeds. One SNP; C 2526 G within the restriction site of the Mnl1 enzyme with no significant effect on litter size. These results revealed that exon1 and int2, exon3 of FSHβ gene could be used for improving breeding programs of goat.

INTRODUCTION

In Egypt economy, the goats play a role as supplier of meat and milk. Since goats have their place in agriculture, ways have to be found to improve their level and efficiency of production and quality of their Products (Farag et al., 2013)

Molecular marker usually does not have any biological effect. Instead, they can be thought of as constant land marks in the genome. They are identifiable DNA sequences, found at specific locations of the genome, and transmitted by the standard laws of inheritance from one generation to the next. They rely on a DNA assay, in contrast to morphological markers that are based on visible traits, and biochemical markers that are based on proteins produced by genes (Ruane and Sonnino, 2007).

Although obvious difficulty in improving litter size of mammalian animals due to low heritability and sex-limited trait, research on genetic mechanism of some kind of mammalian species progresses very well in recent years. The candidate gene approach provides an early breeding tool that could accelerate the improvement of goat reproduction at early time (Zhang et al., 2011).

FSH is a member of the family of pituitary glycoprotein hormones (GPH) that play key roles in fertility. The GPH, which also include CG, LH, and TSH, are heterodimers, each consisting of a common a-subunit (92 amino acids) and a unique b-subunit (111 amino acids in FSH) (Pierce &Parsons ,1981). FSH synthesis and secretion are regulated (both positively and negatively) by protein and steroidal factors from all levels of the hypothalamus-pituitary-gonadal (HPG) axis. FSHβ subunit consists of three exons and two introns. The FSHβ gene is located on the short (p) arm of chromosome 11 at position13, with a total length of 6610 bp (Liu et al., 2009). FSH gene is chiefly responsible for follicular development and could be a potential candidate gene for mammalian reproduction. However, few studies about the polymorphism in FSHβ gene associated with reproduction have been reported in goats (An et al., 2010). Therefore, the aim of this study was to identify genetic polymorphism of FSHβ gene loci in two Egyptian goat breeds and to develop a genetic marker for high prolificacy in breeding programs.

MATERIAL AND METHODS

Animals and Sampling

In the present study, we use eighty blood samples from Egyptian goats of two breeds; Baladi and Zaraibi goats obtained from different private farms. Animals were unrelated individuals following the recommendations spread by ISAG/FAO advisory group on animal genetic diversity (FAO, 1998). The breeding record data for each animal including litter size were collected.

Genomic DNA isolation

Standard salting out method according to Helms (2002) was used for isolation of Genomic DNA from each blood sample. Quality and quantity of DNA were checked electrophoretically on 1% agarose and spectrophotometrically at 260 and 280 nm respectively.

FSHβ genotyping

PCR products of exon1 and Intron2, exon3 loci of the caprine FSHβ gene were amplified using specific pair of primers according to the following

protocol in an automated thermal cycler for 35 cycles of denaturation for 45sec. at 95 °C, different annealing for 45 sec as in table (1) and extension for 45 sec. at 72 °C, with initial denaturation at 95°C for 3 min and final extension for 10 min at 72 °C. Amplification was verified by electrophoresis on 2% (w/v) agarose gel.

Single strand conformational polymorphism (SSCP) for Exon1

Equal volumes of amplified PCR product and denaturing buffer (5x loading dye in formamide in the ratio 1:4) were mixed and heated to 98 °C for 10 min and then cooled on ice for 15 min before loading onto the gel. The mixture was loaded onto 10% polyacrylamide gel with cross linking 39:1 and electrophoresed in 1x TBE buffer at 150 V for 5 min, then at 100 V at room temperature for3hrs to FSHβ Exon1. The gel was stained with 0.2% silver staining (Bassam et al., 1991).

Restriction fragment length polymorphism (RFLP) for Int2, Exon3

PCR-RFLP technique with Mnl1 restriction endonuclease was used to identify the different patterns of FSHB gene (Int2, Exon3) at 37 ◦C for 20 minutes with the following reaction system: a total volume of 12.5 µl containing 8µl PCR products, 1 µl endonuclease, 1.25µl 10X restriction buffer and 2.25 µl dd H2o. The reaction products were elctrophoresed on15% PAGE (polyacrylamide gel electrophoresis) and were subsequently stained with ethidium bromide.

Sequencing

PCR products of different genotypes were separated on 1.0% agarose gels and recovered using DNA gel extraction kit (Vivantis, Malaysia) and sequencing occurred in both directions using an ABI3730xl DNA automatic sequencer (GATC Company, Germany) and the sequences were analyzed using multiple sequence alignment with hierarchical clustering (Corpet, 1988) and Blast in NCBI (National Center of Biotechnology Information).

Statistical analysis

Statistical analysis of the data; Least Square Mean and Standard Errors were carried out according to Snedecor and Cochran (1994). Allele frequencies and mean expected heterozygosities per locus and population were calculated using special statistical program (Assistat).The genotypic frequencies of FSHβ gene and its distribution in two goat breeds were detected by direct counting to estimate Genotype and allele frequencies of genetic variants and chi-square test ($\chi 2$) was used to check whether the populations were in Hardy–Weinberg equilibrium (Zwierzchowski et al., 2010).

RESULTS

PCR amplification

The PCR amplified products of FSHβ different loci (Exon1 and Intron2, exon3) were found to be in the expected molecular size of the designed primers.

SSCP analysis

PCR –SSCP of FSHβ Exon1 of Baladi and Zaraibi goats identified 4 genotypes (AA, BB, CC and EE) (Figure 2).

RFLP analysis

PCR –RFLP of FSHβ Intron2, Exon3 of Baladi goats displayed polymorphism and 2 genotypes (MM and MS) were recorded (Figure 3a) and only one pattern present in Zaraibi goats; MS genotype (Figure3b).

Table (1): primer sequence, amplified regions and product sizes.

Loci	Sequence size (bp)	Annealing temperature	Product size (bp)	Reference
Exon1	F: GATGAAGTCCGTCCAGTT R: TAGACCCTCAGCACCCTC	56°C	202bp	An et al., 2010
Intron2, exon3	F: GTATTCAATCCCTGTCTCA R: GTATTCAATCCCTGTCTCA	54°C	425bp	Zhang et al., 2011

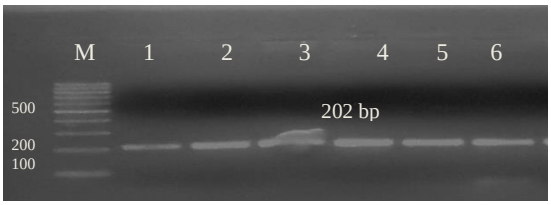


Figure 1a: Agarose gel (2%) showing PCR products of Exon1 of FSHβ gene, lanes 1, 2, 3 for baladi goats, lanes 4, 5, 6 for zaraibi goats and lane M represent the 100bp DNA molecular weight marker.

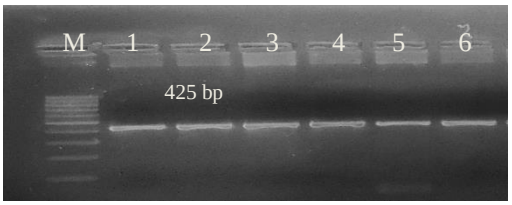


Figure 1b: Agarose gel (2%) showing PCR products of Intron2, Exon-3 of FSHβ gene, lanes 1, 2, 3 for baladi goats, lanes 4, 5, 6 for zaraibi goats and lane M represent the 100bp DNA molecular weight marker.

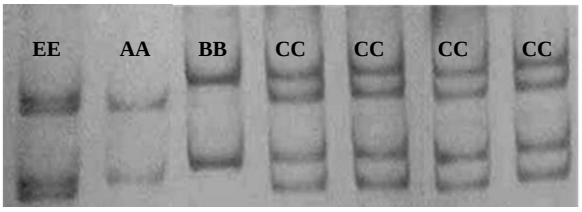


Figure 2a: PCR- SSCP analysis for Baladi goats. Denatured PCR products of FSHβ gene exon-1 separated on 10% native PAGE stained with silver nitrate.

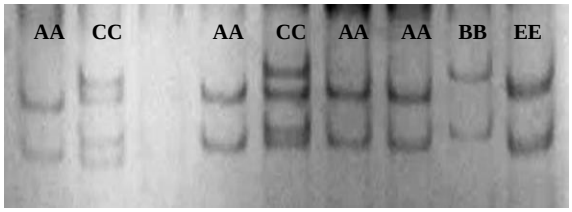


Figure 2b: PCR- SSCP analysis for Zaraibi goats. Denatured PCR products of FSHβ gene exon-1 separated on 10% native PAGE stained with silver nitrate.

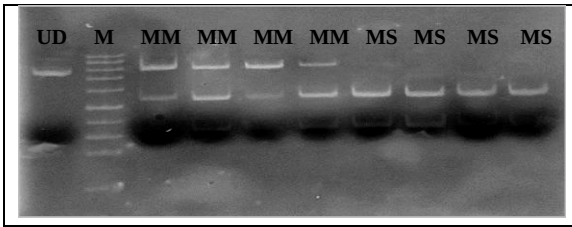


Figure 3a: PCR- RFLP analysis for Baladi goats. Digestion occured by BML1 restriction enzyme and products are separated on 15% native polyacrylamide gel stained with Ethidium Bromide.

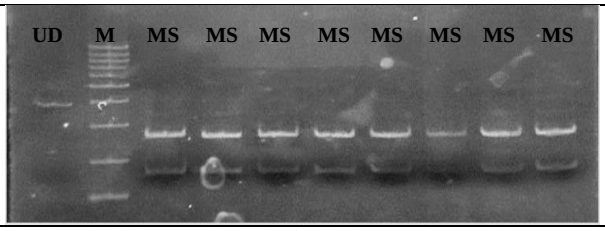


Figure 3b: PCR- RFLP analysis for Zaraibi goats. Digestion occured by BML1 restriction enzyme and products are separated on 15% native polyacrylamide gel stained with Ethidium Bromide.

Genotype and Allele frequencies of caprine FSHβ gene:

Exon1:

Baladi and Zaraibi breeds demonstrated the presence of the 4 genotypes (AA, BB, CC and EE), with the predominance of AA and CC genotype among the two breeds.

A, B, C and E alleles were found in Baladi and Zaraibi breeds with predominance of A allele in Baladi breed and C allele in Zaraibi goats (table 2a).

Intron2, Exon3:

Baladi breeds demonstrated the presence of the 2 genotypes (MM and MS), but Zaraibi breed showed only on genotype; MS genotype with the predominance of MS genotype among the two breeds.

M and S alleles were found in Baladi breeds with predominance of M allele, on the other hand, absence of allele S in Zaraibi goats was observed (table 2b).

Sequencing

Exon1:

Alignment and Chromatogram results of AA and BB genotypes indicate identity of nucleotide sequence except the substitution of A773 G in AA genotype that led to change in amino acids from Lysine (Lys) in BB genotype to Glutamic acid (Glu) in AA genotype. While, EE and CC genotypes indicate presence of other two SNPs; C 698 G that lead to change in amino acid from Serine to Threonine in CC genotype and A 745 C that led to changed glutamine to lysine in EE genotype (Figure 4a,b).

Figure (4): Alignment &Chromatogram results of all sequences of different FSHβ exon 1 genotypes in goat breeds.

Table (2b): Allelic and Genotypic Frequencies of FSHβ gene Intron 2, exon 3 in different two goat breeds.

Breed	Alleles			Genotypes			
	N	M	S	N	MM	SS	MS
Baladi goats	2	0.7	0.3	2	0.4	0	0.6
Zaraibi goats	1	1	0	1	1	0	0

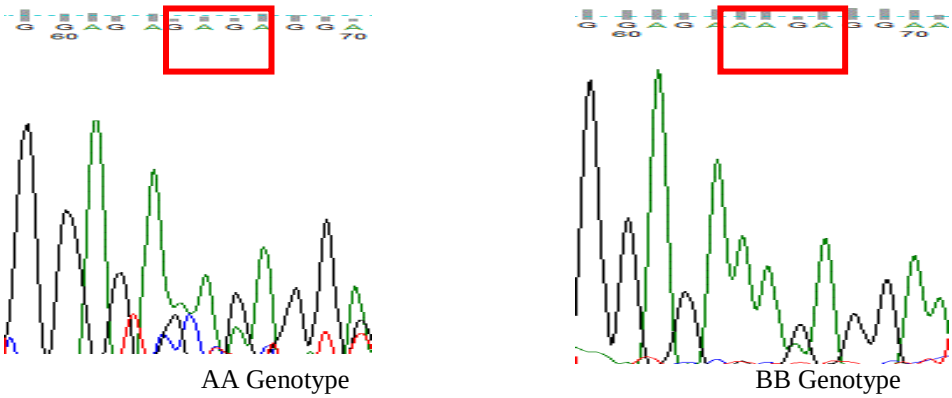
Association of polymorphism with litter size in the goat breeds

Exon1: Baladi and Zaraibi breeds with AA genotype had greater litter size than that with other genotypes (table 3a).

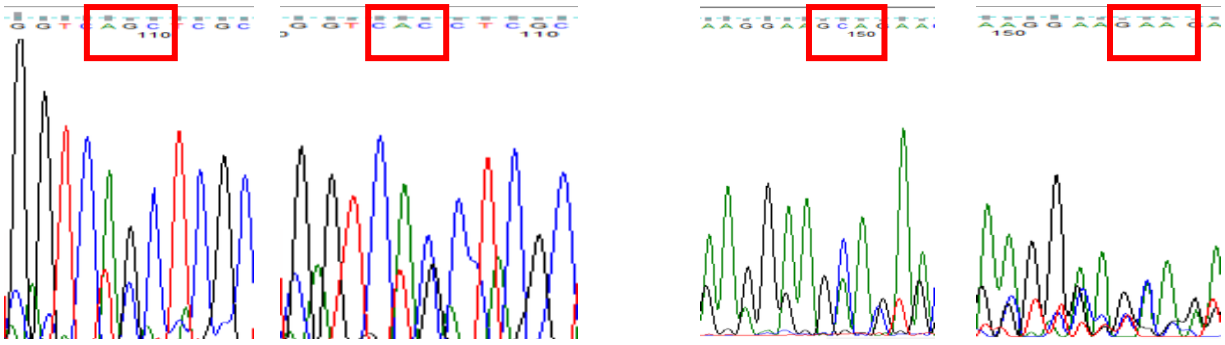
Intron2, exon3: Baladi breed showed a high litter size in MS pattern while Zaraibi breed obtained only MS pattern (table 3b).

Table (2a): Allelic &Genotypic Frequencies of FSH gene exon 1 in different goat breeds.

Breed	Alleles						Genotypes					
	N	A	B	C	E		N	AA	BB	CC	EE	
Baladi goats	4	0.325	0.175	0.3	0.2		4	0.325	0.175	0.3	0.2	
Zaraibi goats	4	0.3	0.15	0.375	0.175		4	0.3	0.15	0.375	0.175	



a) Genotype AA compared to BB genotype:



b) Genotype CC compared to EE genotype:

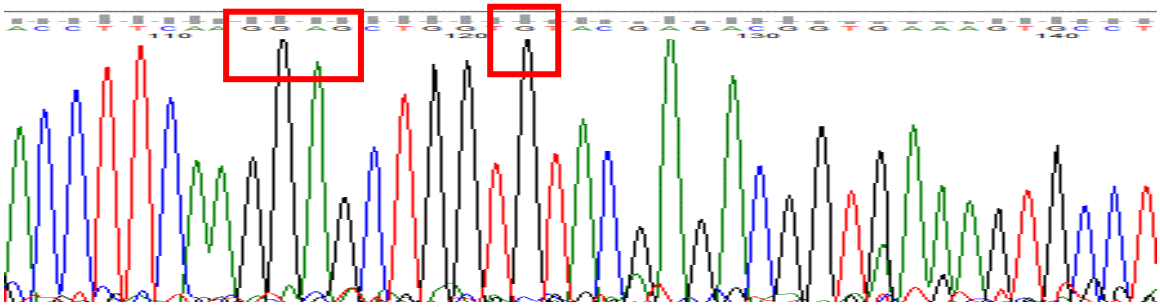


Fig .4c: Sequencing analysis between genotypes of PCR-RFLP.

Table (3a): Association between FSHβ Exon 1 genotypes and Litter Size in different goat breeds.

Breeds /Genotypes	**Genotypes**							
	AA		BB		CC		EE	
	LSM	±SE	LSM	±SE	LSM	±SE	LSM	±SE
Baladi goats	2.55[a]	0.14	2.33	0.24	2.28	0.16	1.83 [b]	0.31
Zaraibi goats	2.50 [a]	0.29	1.50 [b]	0.22	1.75 [b]	0.17	1.60 [b]	0.20

a,b Different superscript letters of mean indicate significant differenceat p<0.05.

Intron2, exon3: Sequences analyzed using multiple sequence alignment revealed the presence of one SNP; C 2526 G within restriction site of the enzyme that led to change in amino acids from Cysteine (Cys) to Serine (Ser). (Figure 4c). Figure (4c): chromatogram results of FSHβ Intron 2, exon 3 genotypes in goat breeds.

Table (3b): Association between FSHβ Intron-2 Exon-3 genotypes and litter size in different goat breeds.

Breeds	**Genotypes**			
	MM		MS	
	LSM	±SE	LSM	±SE
Baladi goats	2.30 [a]	0.14	2.50 [a]	0.13

a,b Different superscript letters of mean indicate significant difference at p<0.05.

DISCUSSION

FSH is the essential hormone for follicle development, which is responsible for the proliferation and survival of follicular somatocytes, and the cyclic recruitment of ovarian follicles into development from early antral stage through maturation to ovulation (McGee and Hsueh, 2000). So, the mutation in goat FSHβ gene perhaps leads to the alteration in the capability of gene expression and subsequently affects the FSH concentration and regulation sensitivity, which results in variation in the ovulation rate and superovulation responses.

Polymorphism of FSHβ Exon1locus

The present study reported the presence of four genotypes (AA, BB, CC and EE) in FSHβ subunit gene Exon 1 in Baladi and Zaraibi breeds with the predominance of AA and CC genotype among the two breeds and AA genotype had greater litter size than other genotypes.

In line, several studies reported the polymorphism of FSHβ gene; Liang et al. (2006) found three genotypes in BG (AA, CC and AC) and Jining Grey goats (AA, AB and AC) breeds and the ewes with AA genotype had higher litter size than those other genotypes in Jining Grey goats, An et al. (2010) reported a polymorphic locus in exon 1 and 2 of FSHβ gene were analyzed by SSCP. Their results showed the ewes with EE genotype had higher litter size ($P < 0.05$) than that EF and EE genotypes in BG and SG breeds. Another studies on pigs found that AB genotype had higher litter size than that BB genotype in Suhuai pigs (Qu et al., 2008) and AA genotype had higher litter size than that BB genotype in Laiwu black pigs (Liu et al., 2002). On the other hand, Hua (2006) showed that the FSHβ gene exon1 and 2 of Haimen and Matou goats were non polymorphic. Xu et al. (2003) and Li et al. (2004) reported FSHβ genotypes had no effects on litter size, respectively in Jinhua and Min pigs.

Several SNPs in different genotypes were reported in our study ; A773 G in AA genotype that led to change in amino acids from Lysine (Lys) in BB genotype to Glutamic acid (Glu) in AA genotype. While, EE and CC genotypes indicate presence of other two SNPs; C 698 G that lead to change in amino acid from Serine to Threonine in CC genotype and A 745 C that led to changed glutamine to lysine in EE genotype.

In the same text, Qu et al. (2008) investigated the distributions of insert mutation of FSHβ gene exon1 in Suhuai pigs and An et al. (2010) reported that two mutations (G40A and T148C) in FSHβ gene exon 2 in Xinong and Boer goat breeds. Presence of one SNP; C 2526 G within restriction site of the Mnl1enzyme that led to change in amino acids from Cysteine (Cys) to Serine (Ser) was reported in the present study.

Polymorphism of FSHβ Intron2, exon3 locus

Two genotypes in FSHβ Int2, Exon3 locus were recorded (MM and MS), the two patterns were present in Baladi goats while Zaraibi goats had only MS genotype which is the predominant pattern and having higher litter size among the two Egyptian breeds.

In accordance, Zhang et al. (2011) recorded three genotypes named AA, AB and BB in Boer and Matou and AA genotype had the largest litter size. The authors detected a mutation (A2645G, GenBank Accession no: S64745) located in exon 3 FSHβ subunit gene that caused an amino acid change from glutamine (Gln) to arginine (Arg) at the residue 115, which destroyed the restriction site recognized by endonuclease MnlI.

The different non- synonymous SNPs in exon1 and int2, exon3 of FSHβ subunit gene in Egyptian goats founded in the present study leads to the alteration in the capability of gene expression and subsequently affects the FSH concentration and regulation sensitivity, which could resultd in variation in the ovulation rate, superovulation responses and subsequently, on animal fertility. So, SNPs and their relation to litter size as reproductive trait making FSHβ gene a potential candidate gene for fecundity in goats.

CONCLUSION

FSHβ gene had several SNPs in a different loci (exon1 and int2, exon3) affecting reproductive traits (litter size) that could be used as a genetic marker for goat breeding programs improvement.

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REFERENCES

An, X. P. Han, D. Hou, J. X. Li, G. Wang, Y.N. Li, L. Zhu, G. Q. Wang, J. G. Song Y. X. and Cao, B.Y. (2010). Polymorphism of exon 2 of FSHB gene and its relationship with reproduction performance in two goat breeds. Agric. Sci. China 9: 880–886.

Bassam, B. J. Caetano-Anolles G. and Gresshoff, P. M. (1991). Fast and sensitive silver staining of DNA in polyacrylamide gels. Analytical Biochemistry, 196: 80- 83.

Corpet, F. (1988). Multiple sequence alignment with hierarchical clustering, Nucl. Acids Res., 16: 10881-10890.

FAO, (1998). Food quality and safety systems. A training manual on food hygiene and the Hazard Analysis and Critical Control Point (HACCP) system. Rome, FAO (available at: http://www.fao.org/docrep/W8088E/W8088E00.htm).

Farag, I. M. Abd El-Aziz, K. B. Ahmed, E. S. Abdelsalam, A. Z. E. Soliman, K. h. A. Darwish, A. M. M. and Asmaa Abu Shady, (2013). Polymorphism of BMPR-1B, BMP-15 and Beta casein genes in two of Egyptian domestic goats, Journal of Applied Sciences Research, 9(4): 3199-3211.

Helms, C. (2002). Salting out procedure for human DNA extraction. Lab manual homepage (on line).

Hua, G. H. (2006). Polymorphism analysis of five candidate genes related to reproduction traits and their effects on litter size in four goat breeds. M. S. thesis, Huazhong Agricultural University, Wuhan.

Li, J. Yang, R. Q. Meng, H. and Pan, Y. C. (2004). Study on four candidate genes of litter size in Min pig. Journal of Shanghai Jiaotong University (Agriculture Science), 22: 74-77.

Liang, C. Chu, M. X. Zhang, J. H. Liu, W. Z. Fang L. and Ye, S. C. (2006). PCR-SSCP polymorphism of FSHb gene and its relationship with prolificacy of Jining Grey goats. Hereditas, 28: 1071- 1077.

Liu, J. J., Ran, X. Q. Li, S. Feng Y. L. and Wang, J.F. (2009). Polymorphism in the first intron of follicle stimulating hormone beta gene in three Chinese pig breeds and two European pig breeds. Anim. Reprod. Sci. 111: 369–375.

Liu, S. F., Yan Y. C. and Du, L. X. (2002). Analysis on polymorphisms at FSHβ subunit gene in Laiwu pigs. Journal of Shandong Agricultural University (Natural Science), 33: 403-408.

McGee, E. A and Hsueh, A. J. (2000). Initial and cyclic recruitment of ovarian follicles. Endocr. Rev. 21: 200–214.

Pierce, J. G. Parsons, T. F. (1981). Glycoprotein hormones: structure and function. Annu Rev Biochem 50:465–495.

Qu, L. Li, K. Z. Wang, X. X. Yu, C. J. Qiu, X. S. Zhou, B. Wang J. S. and Huang, R. H. (2008). Effects of polymorphisms of FSHb gene on reproductive performances in Suhuai pigs. Animal Husbandry & Veterinary Medicine, 140: 24-27.

Ruane, G. and Sonnino, A. (2007). Marker assisted selection as a tool for genetic improvement of crops, livestock, forestry and fish in developing countries an overview of the issues, in: marker assisted selection, FAO, pp 494.

Snedecor, G. A. and Cochran, W. G. (1994). Statistical Method. Iowa State Univ. Press, Ames.

Xu, N. Y., Zhang S. Q. and Peng, S. H. (2003). Investigation on the distribution and their effects on reproduction traits of three major genes in Jinhua pigs. Acta Genetica Sinica, 30: 1090- 1096.

Zhang, C. Wu, C. Zeng, W. Huang, K. Li, X. Feng, J. Wang, D. Hua, G. Xu, D. Wen, Q. Yang, L. (2011). Polymorphism in exon 3 of follicle stimulating hormone beta (FSHB) subunit gene and its association with litter traits and superovulation in the goat .,Small Ruminant Research 96 (2011) 53–57.

Zwierzchowski, L. Siadkowska, E. Opradek, J. Flisikowski, K. and Dymnicki, E. (2010). An association of C/T polymorphism in exon 2 of the bovine insulin like growth factor 2 gene with meat production traits in Polish Holistein-Friesian cattle. Czech J. Anim. Sci.; 55: 227-233.