Animal (2007), **1:9**, pp 1249–1253 © The Animal Consortium 2007
doi: 10.1017/S1751731107000651

animal

Association of four new single-nucleotide polymorphisms in follicle-stimulating hormone receptor and zona pellucida glycoprotein with reproductive traits in pigs

J. F. Yuan[1,2], O. Jafer[2], N. A. Affara[2], Y. Z. Gong[1], L. G. Yang[1], J. Liu[1], M. Moaeen-ud-Din[1], W. M. Li[1] and S. J. Zhang[1,2†]

[1]*Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education, Huazhong Agricultural University, Wuhan 430070, China;* [2]*Department of Pathology, University of Cambridge, Cambridge CB2 1QP, UK*

(Received 9 November 2006; Accepted 2 July 2007)

*Two new single-nucleotide polymorphisms (SNPs) (C1166T and G1190A) were discovered in the follicle-stimulating hormone receptor (*FSHR*) gene and two (G261A and T302C) in the zona pellucida glycoprotein (*ZP3*) gene. These SNPs were genotyped in three Chinese domestic purebred sow lines (42 Small Meishan, 46 Qingping and 41 Jinhua sows) and three European purebred sow lines (225 Duroc, 195 Large White and 65 Landrace sows) by using SNP chips. Phenotypic data including the functional teat number (i.e. milk-producing teats, TN) and number of piglets born alive per litter (NBA). These traits were tested for association with the genotypes of four SNPs. The association analysis revealed genotype of G261A in the* ZP3 *gene was significantly (*$P < 0.01$*) associated with overall NBA and NBA at later parities (*NBA^{2+}*) but not with NBA at first parity (*NBA^{1}*). There was a significant (*$P < 0.05$*) difference between sows with genotype GG (*14.83 ± 0.18*) and AA (*14.26 ± 0.09*) in TN at position 261 in the* ZP3 *gene. No significant associations were observed for the SNPs in the* FSHR *gene with NBA or TN in our populations. The results showed that the new SNPs in the* ZP3 *gene may be an effective potential marker to be used in conjunction with traditional selection methods.*

Keywords: *FSHR*, *ZP3*, sows, SNPs, reproduction

Introduction

Sow reproductive performance is affected by a number of factors including ovulation rate and embryo survival rate. Current technologies enable scientists to improve the efficiency of traditional selection methods by applying genetic markers through marker-assisted selection. Two main approaches have been successfully considered to choose the candidate genes of reproductive traits. One is based on their physiological roles in reproduction and the other is on their location with regard to quantitative trait loci (QTL) for the reproductive trait of interest (reviewed by Spotter and Distl, 2006). In this study, we have focused on identifying associations between different reproductive phenotypes of pigs with the genotypes at single-nucleotide polymorphisms (SNPs) in two genes, namely the follicle-stimulating hormone receptor (*FSHR*) and the zona pellucida 3 glycoprotein (*ZP3*) genes. *FSHR* in pig is a candidate gene which influences sow reproductive traits (Remy *et al.*, 1995; Zhang *et al.*, 2002). *ZP3* in pig is an extracellular matrix protein which surrounds eggs and early embryos and is critical for normal fertilisation and pre-implantation development (David, 1997). Braundmeier *et al.* (2004) previously reported that zona-binding ability was correlated with average litter size. We have recently reported the association between *ZP3* genes variations and reproductive traits (the effective teat number, TN and number of piglets born alive, NBA) (Yuan *et al.*, 2007) and results showed the significant association between mutation (the 18 bp deletion/insertion from intron2) and NBA. These results have increased our interest in investigating the role of variations in the *FSHR* and *ZP3* genes. Thus, the objective of this study was to look for possible variations of DNA sequence in 10th exon of *FSHR* gene along with two SNPs (G261A and T302C) of the *ZP3* gene, to genotype a number of pig breeds by using SNP chips and to score nucleotide variation that may be associated with various reproductive traits including the effective TN and NBA.

† E-mail: sjxiaozhang@mail.hzau.edu.cn

Material and methods

Pig populations and traits

Genomic DNA was isolated from 612 sows from six purebred lines, which included three Chinese domestic purebred lines (42 Small Meishan, 46 Qingping and 41 Jinhua sows) from the corresponding National Conserved Farmers in China and three European purebred lines (225 Duroc, 195 Large White and 65 Landrace) from Hubei Tianzhong Boar Co. Ltd., Sanli Town, Hubei Province, China. The nutrition of the Chinese breeds was varied depending on the physiological characteristics and maternity status. The management and the health programme followed the European pig breeding company's recommendations. Gilts were mated by artificial inseminations (AI) on the second or later observed oestrus depending upon the body weight (majority were mated at the third oestrus). Phenotypic data were obtained for TN and NBA per litter along with family structure for the dams within breed. The selected sows had at least two or more parities.

DNA extraction and PCR-SSCP

Pig DNA was extracted from peripheral leucocytes using standard procedures as reviewed by Sambrook and Russell (2001). PCR primers (forward primer: 5′ ctctgaccttcatccaa 3′; reverse primer: 5′ agtatagcagccacaga 3′) were designed from 10th exon of *FSHR* gene (AF025377). The reaction reagent was amplified by PCR and carried out by using 20 to 40 ng genomic DNA as template based on the methods described by Lee *et al.* (1992). PCR conditions were 94°C for 10 min, followed by 94°C for 40 s, annealing temperature 57.0°C for 50 s and 72°C for 45 s for 30 cycles and extending at 72°C for 10 min.

SSCP (single-strand conformation polymorphism) analysis was performed on the basis of the methods described by Lee *et al.* (1992). SSCP gel electrophoresis was carried out in 10% polyacrylamide gel (39 acrylamide: 1 bisacrylamide) that were made up in 1× TBE (Tris borate EDTA) buffer, which contained 5% glycerol. Heat-denatured PCR products (2 μl) mixed with 6 μl of sequence-stop solution containing formamide were applied to the gel and electrophoresis was carried out at a constant power of 140 V for 16 to 20 h.

SNP discovery by sequencing of PCR products

PCR products that showed different bands with SSCP were purified by using Qiagen columns (Qiagen, Shanghai, China), according to the manufacturer protocol (Qiagen catalogue no. 282010). Purified products were sequenced by using an ABI 377 automatic sequencer for identification of SNPs.

Microarray based genotyping of SNPs in the different pig breeds

PCR for microarray-based genotyping was carried out in 10 μl reaction volumes containing 10 to 30 ng of porcine genomic DNA, 1 μl dNTPs (250 μmol/l each), 10 pmol of each primer, 1 μl of 10× buffer and 0.1 μl of dynazyme DNA polymerase (Dynazyme). Amplification conditions were 5 min at 94°C, followed by first to 22nd cycles of 30 s at 94°C, 30 s at 65°C, 30 s at 72°C and touchdown to 52°C by decreasing the annealing temperature by 1°C every cycle, and finally 23 cycles of 30 s at 94°C, 30 s at 52°C, 30 s at 72°C and a final extension of 10 min at 72°C.

The genotyping of the samples was performed according to the protocol (Ji *et al.*, 2004) with some modification. Briefly, samples were amplified in a 96-well plate by using primers that covered the SNP region. The average PCR product was 100 bp. After purifying the PCR product by using QIAquick columns (Qiagen), the products were resuspended in 3× SSC to a final concentration of 300 ng/μl and transferred to 384-well plates (Applied Biosystems, Foster City, CA, USA; no. 4309849).

The purified products were printed on GAP slides (Corning no. 40005) using a BioRobotics MicroGrid II arrayer (Genomic Solutions, Ann Arbor, MI, USA) and baked for 2 h at 80°C. There are 9000 spots of 3000 samples due to three repeats of one sample on slide. After baking the slides, the bound DNA was denatured by 2 min incubation in distilled water at 95°C. The slides were then transferred into a bath of 95% ethanol at room temperature, rinsed, and then spun dry in a clinical centrifuge. Slides were stored in a closed box at room temperature until used.

Hybridisation mixes consisted of Cy3- and Cy5-labelled oligonucleotide probes capable of detecting the two different SNP alleles mixed in equimolar amounts. The hybridisation mix contained 2 μl of salmon testis DNA, 10 μl of 20× SSC, 6 μl of 1 μmol each labelled probes, 2 μl of 10% SDS and 14 μl distilled water in 40 μl volume. Hybridisation was conducted in a moist sealed hybridisation chamber (TeleChem, Sunnyvale, CA, USA) under a cover slip at 37°C for 30 min. After hybridisation, the slides were rinsed and washed at room temperature with 5× SSC−0.1% SDS, 5× SSC and distilled water (5 min for each wash), and subsequently dried by centrifugation at 600 rpm for 5 min.

The hybridised slides were scanned using the Axon 4100A scanner (Molecular Devices, Sunnyvale, CA, USA). Analysis and genotype calling of the images acquired by the scanner were performed using Blue fuse software (Bluegnome, Cambridge, UK).

Statistical analysis

Genotype frequencies for the *FSHR* and *ZP3* SNPs were estimated within the six purebred breeds. The χ^2 test was employed to study Hardy–Weinberg equilibrium in each population by using Statistical Packages for the Social Sciences (2002). The following linear model (Groeneveld, 1990) was employed for the analysis of variance in six purebreds:

TN records: $Y_{ij} = \mu + X_i + W_j + XW_{ij} + e_{ij}$,

NBA records: $Y_{ijk} = \mu + X_i + W_j + S_k + F_l + XW_{ij} + XF_{il} + XS_{ik} + WF_{il} + XWFS_{ijkl} + e_{ijkl}$,

first-litter (NBA[1]) records: $Y_{ij} = \mu + X_i + W_j + S_k + XW_{ij} + XS_{ik} + XW_{Sijk} + e_{ijk}$,

https://doi.org/10.1017/S1751731107000651 Published online by Cambridge University Press

second and subsequent litters (NBA^{2+}) records:

$$Y_{ijk} = \mu + X_i + W_j + S_k + F_l + XW_{ij} + XS_{ik} + XF_{il} + WF_{jl} + XWFS_{ijkl} + e_{ijkl},$$

where Y was observed trait; X_i was fixed effect of genotype i; W_j was fixed effects of breed j; S_k was fixed effect of sire term k; F_l was fixed effect of litters l; XW_{ij} was fixed interaction effects of genotype i with breed j; XF_{il} was fixed interaction effects of genotype i with litter l; XS_{ik} was fixed interaction effects of genotype i with sire term k; WF_{jl} was fixed interaction effects of breed number with litter l, $XWSF_{ijkl}$ was fixed interaction effects genotype i, breed j, sire term k and litter l; e was error. The multivariable analysis of variance with approximate F statistic is provided as well as the univariate analysis of variance for each dependent variable. After an overall F test of the genotype effect had shown significance ($P < 0.05$), a GLM procedure was applied to evaluate differences among least-squares means for genotype groups.

Results

Discovery of new SNPs in the FSHR *and* ZP3 *genes*

Two new non-synonymous SNPs were discovered in the *FSHR* gene by sequencing PCR products: one SNP (C1166T) was located at position 1166 in the first transmembrane region, and resulted in a substitution of isoleucine (Ile) to threonine (thr) at residue 377. The other SNP (G1190A) was located at position 1190 in the topological domain, and resulted in a substitution of valine (Val) for alanine (Ala) at residue 396.

Allele frequencies

Table 1 shows the allele frequencies for these four SNPs in six purebred lines. The TT^{1166} genotype frequency for the *FSHR* gene position 1166 SNP (C1166 T) was higher than the CC^{1166} genotype frequency except for Qinping population. The position 1190 SNP (G1190A) of the *FSHR* gene showed a lower GG^{1190} frequency than it was expected from the Hardy–Weinberg equilibrium.

The frequency of the AA genotype at position 261 SNP (G261A) of the *ZP3* gene was higher than the GG genotype frequency of our studied population except for Landrace. Moreover, no GG^{261} genotype was found in Duroc and Jinhua populations. The overall frequency of the TT genotype at position 302 SNP (T302C) of the *ZP3* gene was higher than the CC genotype frequency except for Duroc population.

Association of SNPs with TN

TN association analysis was done by using the established model based on breed effect, genotype effect, interaction effects and random factors. The analyses showed that only breed effect and the genotype for position 261 SNP (G/A) of the *ZP3* gene had a significant difference ($P < 0.05$), and TN of sows with the GG genotype (14.83 ± 0.18) was significantly more than that of sows with the AA genotype (14.26 ± 0.09) ($P < 0.05$). However, no significant difference was observed for the other three SNPs in the *ZP3* and *FSHR* gene (Table 2).

Association of SNPs with NBA

Association analysis between genotypes and NBA was conducted by using the established model based on breed effect, genotype effect, litter effect, sire terms within line, interactions among effects and random factors. F-test showed breed effect, litter effect, genotype effect, interaction effects of genotype with litter number, and interaction effects of genotype with breed were significant for the position 261 SNP (G261A) of *ZP3* gene. Thus these effects were considered when we evaluated least-squares means in genotype effect at this position. Sows with GA genotype in NBA had more piglets (9.84 ± 0.16) than that of the GG genotype (8.78 ± 0.25) ($P < 0.01$), and the AA genotype (9.22 ± 0.13) ($P < 0.01$). In addition, sows with GA genotype in NBA^{2+} had more piglets (9.94 ± 0.19) than that of the GG genotype (8.81 ± 0.34) ($P < 0.01$), and the AA genotype (9.38 ± 0.18) ($P < 0.05$), whereas no significant difference was observed in NBA^{1} (Table 2). The same procedure were used at the position 302 SNP (T302C) of the same gene, GLM results showed NBA^{2+} of sows with CC genotype had significantly ($P < 0.05$) more piglets

Table 1 *Allele frequency distribution in three Chinese domestic purebreds and three Europe purebreds*

	Allele frequency (%)							
	FSHR (C1166 T)		*FSHR* (G1190A)		*ZP3* (G261A)		*ZP3* (T302C)	
Breed	C	T	G	A	G	A	T	C
Small Meishan	20.2	79.8	17.6	82.4	39.8	60.2	84.5	15.5
Qinping	51.3	48.7	2.8	97.2	29.7	70.3	66.2	33.8
Jinhua	38.0	62.0	37.2	62.8	15.9	84.1	36.7	63.3
Duroc	20.5	79.5	32.4	67.6	5.9	94.1	56.3	43.7
Large White	21.9	78.1	37.2	62.8	42.4	57.6	78.9	21.1
Landrace	36.3	63.7	50.0	50.0	55.2	44.8	75.0	25.0

https://doi.org/10.1017/S1751731107000651 Published online by Cambridge University Press

Table 2 *Least-squares mean and standard error for TN and NBA affected by genotype*

Position	Genotype	No. of sows	TN	NBA	NBA[1]	NBA[2+]
FSHR (C1166 T)	CC	47	14.20 ± 0.19	9.28 ± 0.25	7.87 ± 0.65	10.12 ± 0.40
	CT	203	14.41 ± 0.10	9.35 ± 0.14	8.16 ± 0.32	9.85 ± 0.20
	TT	212	14.41 ± 0.09	9.46 ± 0.13	7.69 ± 0.35	10.10 ± 0.18
FSHR (G1190A)	GG	82	14.53 ± 0.14	8.80 ± 0.32	8.05 ± 0.65	9.82 ± 0.35
	GA	195	14.39 ± 0.11	9.40 ± 0.19	8.16 ± 0.37	9.82 ± 0.20
	AA	247	14.29 ± 0.09	9.29 ± 0.14	7.93 ± 0.32	10.20 ± 0.20
ZP3 (G261A)	GG	55	14.83^{a} ± 0.18	8.78^{A} ± 0.25	8.15 ± 0.66	8.81^{A} ± 0.34
	GA	167	14.38 ± 0.11	9.84^{AB} ± 0.16	8.31 ± 0.35	9.94^{Ab} ± 0.19
	AA	313	14.26^{a} ± 0.09	9.22^{B} ± 0.13	7.61 ± 0.38	9.38^{b} ± 0.18
ZP3 (T302C)	TT	230	14.52 ± 0.10	9.43 ± 0.14	8.18 ± 0.34	9.54 ± 0.18
	TC	266	14.31 ± 0.09	9.18 ± 0.14	7.77 ± 0.36	9.21^{c} ± 0.17
	CC	55	14.45 ± 0.18	9.59 ± 0.17	7.57 ± 0.72	9.77^{c} ± 0.33

[a,b,c]Values in the same column with same superscripts significantly differ at $P < 0.05$; [A,B]Values in the same column with same superscripts significantly differ at $P < 0.01$.

(9.77 ± 0.33) than sows with CC genotype (9.21 ± 0.17). While no significant difference was found among the three genotypes in NBA and NBA[1].

F-test showed only breed effect, litter effect, and interaction effects of genotype with litter number had significance at the position 1166 SNP (C1166 T) and (G1190A) of the *FSHR* gene and no significant difference in genotype effect was found in our tested population (Table 2).

Discussion

At present, candidate genes for reproductive traits in pigs can be chosen by considering the following four factors or a combination of them: (1) the physiological role of a gene in reproduction (Rothschild *et al*., 1996); (2) genes located or closely linked with possible reproductive relative QTL (positional candidate genes); (3) using information on orthologous genes in syntenic chromosomal regions of other species (positional comparative candidate genes) (Haley, 1999); and (4) differential expression of genes in the tissue under specific investigation (Liang and Pardee (1992); reviewed by Spotter and Dist (2006)).

ZP is laid down during oogenesis and increases in thickness as oocytes increase in diameter and it plays important roles during mammalian oogenesis, fertilisation, pre-implantation development (Wassarman, 1999; Wassarman *et al*., 2004; Herrick *et al*., 2005) and passage through the oviduct (Rankin *et al*., 2000). During the stages of pre-implantation development, the embryo remains encased in the ZP, without which the very early embryo is unable to traverse the oviduct (Bronson and McLaren, 1970). Just prior to implantation, the embryo escapes from the zona pellucida by mechanisms not yet fully described. ZP effectuates the individuality of the embryo by preventing aggregation of individual embryos and facilitates survival in the oviduct (Kolbe and Holtz, 2005). Braundmeier *et al*. (2004) previously reported zona-binding ability was correlated with fertility when estimated by average litter size ($r = 0.64$, $P < 0.05$). Thus, the mutations of the *ZP3* gene possibly have an effect on reproduction traits. Unfortunately, the map location of the gene in the pig remains unknown. However, Yuan *et al*. (2007) have discovered 13 new SNPs in the exon1 of *ZP3* gene, and four of them, which have the restriction enzyme position, were chosen to genotype and associate with reproductive traits in the European breeds (Duroc, Large White and Landrace). Here, we selected another two SNPs under the requirement of SNP chips and genotyped in three Chinese domestic purebreds and three European purebreds and analysed the association of these mutations and haplotypes with NBA and TN. Association analysis of SNPs indicated that these two mutations may influence the process of fertilisation and pre-implantation development, and finally may affect the observed phenotypic differences in reproductive traits performance. Certainly, the relationship between mutations and phenotypic data is still poorly understood and needs to be explored by further experimentation.

The follicle-stimulating hormone (FSH) is essential for normal gametogenesis. In females FSH is required for ovarian development and follicle maturation whereas in males FSH determines sertoli cell number and qualitatively normal spermatogenesis. As the receptor of FSH, the FSH-receptor (FSHR) mediates action of FSH on ovarian follicular granulosa cells. Recently, SNPs in the human *FSHR* gene have been identified and severely affects the process of gametogenesis and infertility (Gromoll *et al*., 1996; Ahda *et al*., 2005; Wunsch *et al*., 2005; De Leener *et al*., 2006). Although the *FSHR* gene was first investigated as a potential candidate gene influencing NBA in pig in 1995 (Remy *et al*., 1995), only a few researchers have done further investigation on SNPs of this gene. Zhang *et al*. (2002) performed an association study of SNP located at exon7 in the *FSHR* gene with size of reproductive organ and revealed sows that could provide more piglets with bigger reproductive organs usually carried BB genotype on the site

https://doi.org/10.1017/S1751731107000651 Published online by Cambridge University Press

of *FSHR* gene. Our findings demonstrated the trends that the sows with the AA genotype at position 1190 SNP (G/A) of 10th exon had higher NBA^{2+} than the sows with the GA and GG genotypes, although it was not significant (Table 2). Two other factors could affect our results: (1) the worst performing sows would be screened out after one or two parities, and therefore potentially useful genetic variation for the purposes of the study would be lost; (2) although we could model the effects of breeds, litters, the sire term effect and the interact effects among breeds, genotype and parities successfully, other effects such as nutrition and reproductive diseases were not considered here.

For animal breeding we must get reliable information. Due to the limited number of samples we could not analyse the association between genotype and phenotypic traits within the single breeds. Thus to confirm our finding, further studies are required using more samples and thorough examination of potential association of these four mutations with reproduction traits in all breeds.

Acknowledgments

Financial assistance from 863 High Technology and Development Project of China (2002AA211041, 2006AA10Z195), Hubei project (2003AA211041, 2006CA023), Wuhan project (20067003111-06) and National Project of China (04EFN214 200206) is greatly appreciated.

References

Ahda Y, Gromoll J, Wunsch A, Asatiani K, Zitzmann M, Nieschlag E and Simoni M 2005. Follicle-stimulating hormone receptor gene haplotype distribution in normozoospermic and azoospermic men. Journal of Andrology 26(4), 494–499.

Braundmeier AG, Demers JM, Shanks RD and Miller DJ 2004. The relationship of porcine sperm zona-binding ability to fertility. Journal of Animal Science 82(2), 452–458.

Bronson RA and McLaren A 1970. Transfer to the mouse oviduct of eggs with and without the zona pellucida. Journal of Reproduction and Fertility 22, 129–137.

David PL 1997. Three-dimensional structure of the zona pellucida. Reviews of Reproduction 2, 147–156.

De Leener A, Montanelli L, Van Durme J, Chae H, Smits G, Vassart G and Costagliola S 2006. Presence and absence of FSH receptor mutations provide some insights to spontaneous ovarian hyperstimulation syndrome physiopathology. Journal of Clinical Endocrinology and Metabolism 91(2), 555–562.

Groeneveld E 1990. PEST user manual (version 3.1) Federal Agricultural Research Centre. Neustadt, Germany.

Gromoll J, Simoni M, Nordhoff V, Behre HM, De Geyter C and Nieschlag E 1996. Functional and clinical consequences of mutations in the FSH receptor. Molecular and Cellular Endocrinology 125, 177–182.

Haley C 1999. Advances in quantitative trait locus mapping. In Jay Lush to genomics: visions for animal breeding and genetics (ed. JCM Dekkers, SJ Lamont and MF Rothschild), pp. 47–59. Iowa State University, Ames.

Herrick SB, Lschweissinger D, Kim SW, Bayan KR, Mann S and Cardullo RA 2005. The acrosomal vesicle of mouse sperm is a calcium store. Journal of Cellular Physiology 202(3), 663–671.

Ji M, Hou P, Li S, He N and Lu Z 2004. Microarray-based method for genotyping of functional single nucleotide polymorphisms using dual-color fluorescence hybridization. Mutation Research 548(1–2), 97–105.

Kolbe T and Holtz W 2005. Differences in proteinase digestibility of the zona pellucida of *in vivo* and *in vitro* derived porcine oocytes and embryos. Theriogenology 63(6), 1695–1705.

Lee HH, Lo WJ and Choo KB 1992. Mutational analysis by a combined application of the multiple restriction fragment-single strand conformation polymorphism and the direct linear amplification DNA sequencing protocols. Analytical Biochemistry 205, 289–293.

Liang P and Pardee AB 1992. Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science 257, 967–971.

Rankin T, Soyal S and Dean J 2000. The mouse zona pellucida: folliculogenesis, fertility and pre-implantation development. Molecular and Cellular Endocrinology 163(1–2), 21–25.

Remy JJ, Lahbib-Mansais Y, Yerle M, Bozon V, Couture L, Pajot E, Greber D and Salesse R 1995. The porcine follitropin receptor: cDNA cloning functional expression and chromosomal localization of the gene. Gene 163, 257–261.

Rothschild MF, Jacobson C, Vaske DA, Tuggle CK, Wang L, Short T, Eckardt G, Sasaki S, Vincent A, Mileham DG and Plastow G 1996. The estrogen receptor locus is associated with a major gene influencing litter size in pigs. Proceedings of the National Academy of Sciences USA 93, 201–205.

Sambrook J and Russell DW 2001. Molecular cloning: a laboratory manual, third edition. Cold Spring Harbor Laboratory Press, USA.

Spotter A and Distl O 2006. Genetic approaches to the improvement of fertility traits in the pig. Veterinary Journal 172, 234–247.

Statistical Packages for the Social Sciences 2002. Version 11.5 for Windows. SPSS Inc., Chicago, IL, USA.

Wassarman PM 1999. Mammalian fertilization: molecular aspects of gamete adhesion, exocytosis, and fusion. Cell 96, 175–183.

Wassarman PM, Jovine L, Litscher ES, Qi H and Williams Z 2004. Egg–sperm interactions at fertilization in mammals. European Journal of Obstetrics and Gynecology and Reproductive Biology 115(Suppl. 1), S57–S60.

Wunsch A, Ahda Y, Banaz-Yasar F, Sonntag B, Nieschlag E, Simoni M and Gromoll J 2005. Single-nucleotide polymorphisms in the promoter region influence the expression of the human follicle-stimulating hormone receptor. Fertility and Sterility 84(2), 446–453.

Yuan JF, Moaeen-ud-Din M, Gong YZ, Peng XL, Yang LG, Feng YP, Liu J, Hu B, Affara NA, Jafe O and Zhang SJ 2007. Genetic effects of the mutations of zona pellucida glycoprotein (ZP3) on pig reproduction. Journal of Animal Breeding and Genetics 124, 144–149.

Zhang SJ, Xiong YZ, Deng CY, Zeng FT, Qiu XP, Xiao SM, Xia Y, Xu JX and Ruan Z 2002. [Study of molecular genetics of the size of reproductive organs and litter size in the sow]. Yi Chuan Xue Bao 29(12), 1073–1077.

https://doi.org/10.1017/S1751731107000651 Published online by Cambridge University Press