# Oviduct-specific Glycoprotein 1 Locus is Associated with Litter Size and Weight of Ovaries in Pigs

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**ABSTRACT :** Oviduct-specific glycoprotein 1 (*OVGP1*) is implicated in playing a role in fertilization and early embryo development. In this study, we have obtained the sequence of intron 9 of *OVGP1* gene in swine. Comparative sequencing of Meishan (a native Chinese breed) and Large White pig breeds revealed an A/T substitution at position 943. A PCR-*Eco*R*I*-RFLP assay was developed to detect this mutation. Polymorphism analysis in Qingping animals showed that pigs with BB genotype had lower number of piglets born alive (NBA) in multiple parities than pigs with AA ($p<0.05$) and AB genotype ($p<0.01$). In Large White×Meishan (LW×M) $F_2$ offspring, the weight of both ovaries (OW) of the *BB* genotype was significantly lighter than that of *AB* ($p = 0.05$) and *AA* ($p<0.01$) genotypes. Analysis of the data also revealed that the mutation locus affected these two traits mostly by additive effects. These studies indicated that the polymorphism was associated with NBA and OW in two distinct populations and further investigations in more purebreds or crossbreds are needed to confirm these results. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 5 : 632-637)***

**Key Words :** Oviduct-specific Glycoprotein 1, Litter Size, Female Reproductive Tract Components, Pigs

## INTRODUCTION

Mammalian oviduct designs a suitable microenvironment for fertilization, early embryonic development and delivery of a viable embryo to the uterus (Boatman et al., 1997). *OVGP1* is a high molecular weight glycoprotein belonging to the chitinase protein family and presented in the oviduct (Agarwal et al., 2002). It is synthesized from the oviductal epithelial cells and secreted into the oviductal lumen during estrogen dominance in the human (Arias et al., 1994; Bhatt et al., 2004). In human and baboons, this glycoprotein is specifically expressed within the oviduct during the perovulatory phase of the menstrual cycle and several investigators indicated that synthesis of the *OVGP1* was controlled by ovarian steroids (Rapisarda et al., 1993; O'Day-Bowman et al., 1996). *OVGP1* can attach itself to the ovulated oocyte and early embryo during their transit in the oviduct (Malette et al., 1995; O'Day-Bowman et al., 1996). Several reports showed that *OVGP1* adhered to the zona pellucida (ZP) of the ovulated egg thereby enhancing the binding of sperm to the ZP and the speed of sperm penetration within the oviduct (O'Day-Bowman et al., 1996; Boatman et al., 1997), while the blocking of *OVGP1* with antibodies significantly decrease species-specific sperm binding onto oocytes *in vitro* (Schmidt et al., 1997). *OVGP1* in the perivitelline space or endocytosed by the pre-implantation embryo may regulate differentiation during the morula to blastocyst transition (Boatman et al., 1997). All these researches suggested that *OVGP1* might have a role in the early reproductive events occurring within the oviduct. Due to the physiological function of *OVGP1* during fertilization, *OVGP1* is a functional candidate gene for reproductive traits in mammal.

Variations in *OVGP1* gene could be used as genetic markers to select animals for breeding. However, it has not been previously reported how a genetic variant of this gene affects litter size in pigs. The purpose of our research was to determine whether the single nucleotide polymorphisms (SNP) of the ninth intron in porcine *OVGP1* gene would be associated with female reproductive traits.

## MATERIALS AND METHODS

### Experimental animals

In this study, 44 Meishan pigs (a native Chinese breed), 36 Large white pigs, 66 Line DIV pigs, 127 Large white× Meishan resource family $F_2$ offspring were bred and raised at the genetic nucleus station owned by Huazhong Agricultural University. Twenty-one Tongcheng pigs and 30 Erhualian pigs were derived from Tongcheng (Tongcheng, Hubei Province) and Houqiao (Xishan, Jiangshu Province) research farms in China, respectively.

The purebred Qingping animals used in this study were born and raised in Qingping research farm of Hubei province in China. At farrowing, total number born (TNB) and the number of piglets born alive (NBA) were recorded.

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Received September 12, 2005; Accepted January 19, 2006

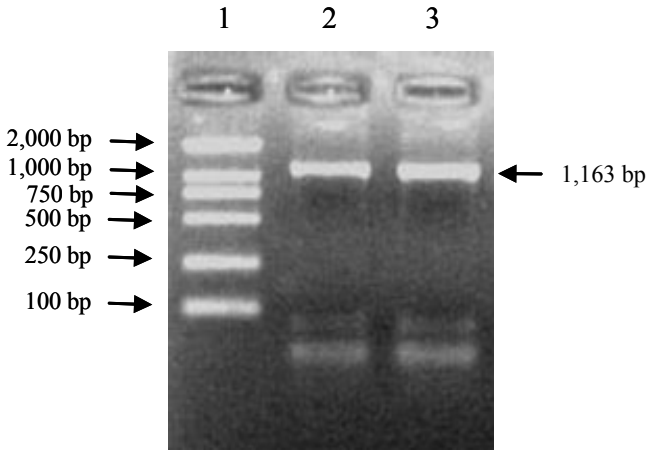


**Figure 1.** PCR amplified products of intron 9 of *OVGP1* gene. Lane 1: DL 2,000 markers; Lane 2, 3: PCR products in Meishan pigs.

The pig resource family was established by mating three Large White boars to seven Meishan sows. Five males and twenty-three females in the $F_1$ generation were selected for intercrossing randomly. All the $F_2$ offspring were slaughtered for testing, including 127 gilts with reproductive tracts components records. Reproductive tract characteristics, including length of uterine horns (LU), uterine weight (UW), length of oviduct (LO) and weight of both ovaries (OW) were recorded. LU was measured according to the method of Lin (1992).

**DNA preparation**

Genomic DNA was isolated from white blood cells as described by Xiong et al. (1999). After isolation, DNA pellet was dissolved in TE buffer and was stored at -20°C.

**Cloning and sequencing of the amplified fragment of intron 9**

Primer pair 1 (Forward primer: 5'-AGTGGTTCCCT ATCTGCCT-3'. Reverse primer: 5'-ACATCATCCAGGTC CAAAGT-3') was developed to amplify a region for the porcine *OVGP1* gene predicted to encompass parts of exons 9, 10 and intron 9 by comparison with the human *OVGP1* cDNA (GenBank accession number: NM 002557) and swine *OVGP1* mRNA (GenBank accession number: NM 214070). PCR was performed in 25 μl reactions containing: 1×PCR buffer, 1.5 mM $MgCl_2$, 250 μmol/L dNTP, 5 pm of each PCR primer, 2 U *Taq* DNA polymerase (Biostar International, Toronto, Canada), and 200 ng genomic DNA as template. PCR was run in the GeneAmp PCR system 9600 Thermocycler (Perkin Elmer, Foster City, CA, USA) as follows: 94°C for 4 min followed by 35 cycles of 94°C for 1 min, 63°C for 50 s, 72°C for 1 min, and final extension of 10 min at 72°C. The purified PCR products were cloned into the pMD18-T vector and were sequenced by Sangon Company (Shanghai, China). The sequencing results of different pig breeds were compared by using CLUSTALX software.

**Detection of PCR-*Eco*RI-RFLP**

Primers 2 were designed based on sequencing result to amplify a 326 bp fragment from intron 9. Forward primer: 5'-AGTGGTTCCCTATCTGCCT-3'. Reverse primer: 5'-ACATCATCCAGGTCCAAAGT-3'. PCR amplification (20 μl final volume) was performed using 250 ng genomic DNA, 200 μmol/L dNTP, 5 pm of each primer, and 1 U *Taq* DNA polymerase with the reaction buffer supplied by the manufacturer. The conditions for PCR are as follows: 94°C for 4 min; 33 cycles of 94°C for 30 min, 63°C for 30 s, and 72°C for 1 min. For the PCR-RFLP assays, 8.5 μl of PCR products were digested with 5 units *Eco*RI (TaKaRa, Tokyo, Japan) in 1×digestion buffer added in a total volume of 10 μl, following digestion for 4 h at 37°C. Digested products were separated by a 8% polyacrylamide gel electrophoresis gels electrophoresis (PAGE) in 1×TBE buffer and stained with silver. Allelic forms of porcine *OVGP1* intron 9 were identified as B (digested by *Eco*RI into 176 bp and 150 bp fragments) and A (a 326 bp fragment undigested by *Eco*RI).

**Statistical analysis**

The association between genotype and reproduction traits was performed with the least square method (GLM procedure, SAS version 8.0). The model used to analyze the data from Qingping pigs included effects of the *OVGP1* SNP genotypes and line as fixed effects. The Qingping pig was included in the model as a random. NBA in multiple parities of Qingping individuals were averaged from 2-7 parities. The model used to analyze the data from F2 gilts included effects of the *OVGP1* SNP genotypes and season of slaughter as fixed effects and $F_2$ individuals as a random effect.

Both additive and dominance effects were also estimated using REG procedure of SAS version 8.0, where the additive effect was denoted as -1, 0 and 1 for *AA*, *AB* and *BB*, respectively, and the dominance effect represented as 1, -1 and 1 for *AA*, *AB* and *BB*, respectively (Liu, 1998).

## RESULTS

**Amplification, cloning and sequencing analysis of intron 9**

The primer pair 1 produced fragments with genomic DNA from Meishan and Large White pigs. Products of PCR were detected by electrophoresis on 1% agarose gel (Figure 1). Sequence analysis of PCR products revealed that a new *Eco*RI polymorphism was identified at 943$^{th}$ position where the nucleotide A was substituted by nucleotide T.

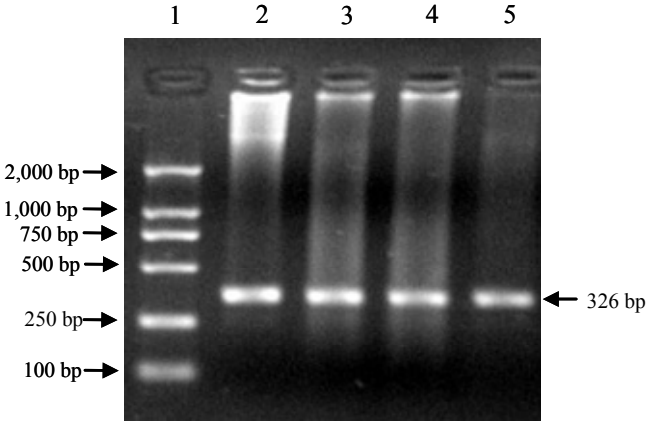


**Figure 2.** PCR amplified products of intron 9 with primer pair 2. Lane 1: DL 2,000 marker; Lane 2-5: PCR products.

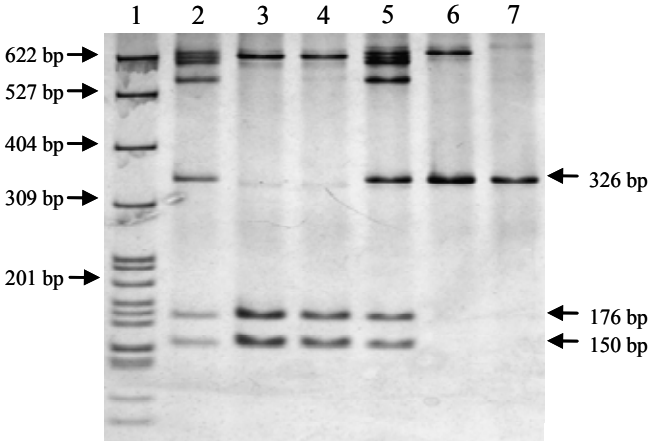


**Figure 3.** The PCR-RFLP results of intron 9 with primer pair 2. Lane l: pBR322 DNA/*Msp*I marker; Lane 2, 5: genotype AB; Lane 3, 4: genotype BB; Lane 6, 7: genotype AA.

**Table 1.** Abbreviations of names for traits

| No. | Abbreviations | Names | Unit |
|---|---|---|---|
| 1 | TNB | Total number born | piglet |
| 2 | NBA | Number born alive | piglet |
| 3 | UW | Uterine weight | g |
| 4 | OW | Weight of both ovaries | g |
| 5 | LU | Length of uterus horn | cm |
| 6 | LO | Length of oviduct | cm |

**Polymorphism in intron 9**

A 326 bp fragment was amplified with primer pair 2. PCR was performed on DNA of seven pig breeds and 124 $F_2$ offspring. Results of amplification were shown in Figure 2.

The A/T substitution can be detected by PCR-*Eco*RI-RFLP. The PCR products were digested with restriction enzyme (*Eco*RI) and the fragments were separated by electrophoreseing on an 8% PAGE. Three genotypes were presented: *AA* (326 bp), *AB* (326 bp+176 bp+150 bp), *BB* (176 bp+150 bp) (Figure 3).

**Frequencies of allele and genotype of different pig breeds**

Allele frequencies for the PCR-*Eco*RI-RFLP were studied in seven different populations (Table 2). Allele A was the predominant allele in all pig populations and allele B was found only in Chinese indigenous pig breeds, such as Meishan, Qingping, Tongcheng, Erhualian pigs. There were three genotypes (AA, BB and AB) existed in all Chinese native pig populations except Erhualian animals. Chi square ($\chi^2$) testing showed highly significant difference in genotype frequencies between Large White pig population and Chinese native pig breeds (Meishan breed and Tongcheng breed) (Table 3), indicating clearly genetic differentiation at the locus between Chinese and Western pigs, whereas the differences among Chinese Erhualian, Meishan and Tongcheng pigs were significant as well.

**Association analysis**

Results of tests for *OVGP1* genotypes and litter size traits on Qingping pigs were given in Table 4. Pigs with *BB* genotype had less NBA than pigs with *AA* genotype ($p<0.05$) and AB genotype ($p<0.01$), with additive effect -0.897 piglet. Significant association with NBA of multiple parities in Qingping animals was found, but no significant conclusion could be made on other litter size traits. The results also indicated that AB sows showed the highest and BB sows showed the lowest litter size values in the later and subsequent parities. Table 5 summarized the association of porcine *OVGP1* genotypes with the female reproductive trait components on LW×M pigs. The results showed that the OW of BB gilts were significantly lighter than AB gilts

**Table 2.** Distribution of *Eco*RI-RFLP genotype and allele frequencies among different pig populations

| Populations | N | Genotype and frequency | | | Allele frequency | |
|---|---|---|---|---|---|---|
| | | *AA* | *AB* | *BB* | *A* | *B* |
| Meishan | 68 | 0.37(25) | 0.49(33) | 0.15(10) | 0.61 | 0.39 |
| Qingping | 92 | 0.61(56) | 0.32(29) | 0.08(7) | 0.77 | 0.23 |
| Tongcheng | 21 | 0.38(8) | 0.52(11) | 0.10(2) | 0.64 | 0.36 |
| Erhualian | 30 | 0.97(29) | 0.03(1) | 0.00(0) | 0.98 | 0.02 |
| Line DIV | 66 | 1.00(66) | 0.00(0) | 0.00(0) | 1.00 | 0.00 |
| Large White | 36 | 1.00(36) | 0.00(0) | 0.00(0) | 1.00 | 0.00 |
| Duroc | 15 | 1.00(15) | 0.00(0) | 0.00(0) | 1.00 | 0.00 |

N: Total number of pigs observed.

**Table 3.** $\chi^2$ test results for the genotype frequency distribution among different populations

| Populations | Qingping | Tongcheng | Erhualian | Line DIV | Large White | Duroc |
|---|---|---|---|---|---|---|
| Meishan | 9.260 | 0.375 | 30.224** | 61.456** | 38.812** | 19.682 |
| Qingping | | 3.746 | 13.754 | 33.447** | 19.599 | 8.846 |
| Tongcheng | | | 21.328* | 48.034** | 28.870** | 14.534 |
| Erhualian | | | | 2.223 | 1.218 | 0.511 |
| Line DIV | | | | | 0 | 0 |
| Large White | | | | | | 0 |

* p<0.05; A, B, ** p<0.01.

**Table 4.** Effect of OVGP1 EcoRI-RFLP genotypes on litter size traits in Qingping sows

| Trait | | Least square means±standardized error | | | Additive effect | Dominant effect |
|---|---|---|---|---|---|---|
| | | AA | AB | BB | | |
| N | | 56 | 29 | 7 | | |
| Multiple parities | TNB | 11.003±0.264 | 11.450±0.369 | 10.143±0.812 | -0.328±0.400 | -0.396±0.268 |
| | NBA | 10.775±0.238$^{a}$ | 11.024±0.330$^{A}$ | 8.982±0.667$^{bB}$ | -0.897±0.358* | -0.573±0.240* |

N: Total number of pigs observed.
Letter denoting significant difference between groups: a, b, * p<0.05; A, B, ** p<0.01.

**Table 5.** Effect of *OVGP1 Eco*RI-RFLP genotypes on reproductive tracts components traits in LW×M $F_2$ offspring

| Traits | Least square means±standardized error | | | Additive effect | Dominant effect |
|---|---|---|---|---|---|
| | AA | AB | BB | | |
| N | 39 | 67 | 18 | | |
| UW (g) | 429.615±41.985 | 400.270±35.195 | 454.167±61.800 | 14.038±29.351 | 28.163±19.500 |
| LU (cm) | 108.987±8.022 | 98.821±6.167 | 100.833±11.809 | 4.077±6.894 | 4.525±4.580 |
| OW (g) | 15.719±1.078$^{A}$ | 13.437±0.840$^{AB}$ | 12.156±1.481$^{B}$ | 1.723±0.904 | 0.463±0.622 |
| LO (cm) | 20.473±0.834 | 19.144±0.625 | 21.059±1.230 | -0.218±0.722 | 0.658±0.475 |

N: Total number of pigs observed.
Letter denoting significant difference between groups: a, b, * p<0.05; A, B, ** p<0.01.

(p = 0.05) and AA gilts (p<0.01), the additive effect was 1.723 g (p = 0.05). Significant association with OW in $F_2$ gilts was observed as well. No significant association was found for other reproductive tract characteristics.

## DISCUSSION

In this study, the candidate gene approach has been used to demonstrate associations between specific genes and litter size. Using this approach, polymorphisms in some defined genes such as the estrogen receptor (Legault et al., 1996; Rothschild et al., 1996; Short et al., 1997; Li et al., 2004), follicle-stimulating hormone-β (Zhao et al., 1998; Li et al., 2002), prolactin receptor (Vincent et al., 1998) and retinol-binding protein (Rothschild et al., 2000) have all been reported to be associated with litter size in swine. So candidate gene approach is a very efficacious method to find molecular markers.

*OVGP1* gene has been extensively investigated in humans. In human the highest expression of human *OVGP1* at the time of ovulation is consistent with a supportive role in fertilization and early embryo development (Lok et al., 2002). In pigs, it has been reported that *OVGP1* significantly reduced the incidence of polyspermy among pig eggs matured and fertilized *in vitro* (Andrew et al., 2000). In addition, *OVGP1* provided a significant increase in postcleavage development from embryo to blastocyst in swine (Andrew et al., 2000). Therefore, this gene may play an important role in the *in vivo* fertilization process (Rapisarda et al., 1993; Malette et al., 1995; Andrew et al., 2000). Thus, in our study *OVGP1* gene was selected as a candidate gene to be associated with reproductive trait in swine.

The genetic effect on reproductive traits was investigated in Qingping sows and $F_2$ offspring animals. Based on the data representing litter records from Qingping sows, the NBA and TNB in multiple parities were higher for AB sows than for the sows of the other two genotypes, though on association between the *OVGP1* gene locus and TNB was discovered. In view of these, it is tempting to suggest that AB sows have better performance than BB sows for litter size traits in Qingping pigs.

However, our results in $F_2$ offspring showed that piglets with AA genotypes had heavier OW than AB. Association between the marker and the traits may vary across populations, lines or families. This was shown in several studies with the ESR gene for reproductive traits. According to Rothschild et al. (1996) and Short et al. (1997) the B allele was advantageous to the A allele for litter size. Another research showed no statistically significant effect

of the ESR genotype on litter size (Legault et al., 1996). The observed difference between Qingping sows and $F_2$ gilts may be explained that there are variations in the genetic background. In addition, the observed effects might be caused by the linkage of this locus with other quantitative trait locus (QTLs) which contributed to the reproductive traits.

Mutation of nucleotide can alter gene function, either by changing the coding region of the protein, translation or stability of the mRNA or control of transcription of the gene. In the experiments reported here, a polymorphism analyzed in *OVGP1* gene was found in intron 9. Although the SNP in introns do not directly alter any amino acid residue, they may play a role in regulating gene expression and thus their constituent SNPs may be directly related to functional variation (Zhang et al., 2005). Furthermore, it should be taken into consideration that the location of *OVGP1* gene on chromosome has not been reported in the literature, and hence we cannot say whether *OVGP1* is the gene leading to different litter size and OW itself or if it is only a genetic marker linked with other QTLs which contributed to the reproductive traits.

This is the initial step to consider *OVGP1* gene as a candidate gene for litter size. This polymorphism could be a potential genetic marker for reproductive traits, especially for NBA. The presented estimations are based on the data from Qingping breeds and F2 intercross pedigrees, however, in this study the populations are weak and the number of Qingping individuals or F2 gilts is limited. So, analyzing more animals is necessary to confirm the association between the *OVGP1* genotype and reproductive traits in Qingping pigs or other purebreds and crossbreds.

## ACKNOWLEDGMENTS

We are gratefully to Dr. Y. J. Shao for her cooperation. This work was supported financially by the Key Project of Chinese Ministry of Education (104132), Innovation Fund of Huazhong Agricultural University, the National High Technology Development Project (No.2002AA211041) and the National “973” Project of P. R. China (No.G2000016105).

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