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# Polymorphism of candidate genes associated with reproductive performance in crossbred pigs

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ABSTRACT

This investigation was done to analyze the polymorphism of 5 SNPs in candidate genes associated with reproductive performance in crossbred pig population by PCR-RFLP. The results revealed that out of 5 SNPs genotyped, 2 were polymorphic and 3 were monomorphic. Genotypic frequency of crossbred pigs were CC (100%) for ESR1; GG (21%), GT 65%) and TT (14%) for Prei3; CC (100%) for FSHβ; AA (100%) for OPN and CC (21%), CT (63%) and TT (16%) for CDK20 SNPs, respectively. Allelic frequencies for these SNPs {ESR1 (c.1227C>T), Prei3 (T802G), FSHβ (c.930A>G), OPN (c.425G>A) and CDK20 (T96C)} were 1.00 (C), 0.53 and 0.47 (G/T), 1.00 (C), 1.00 (A) and 0.52 and 0.48 (C/T), respectively. The least squares analysis revealed that both polymorphic SNPs had non-significant effect on litter traits. The values of PIC, heterozygosity and allelic diversity indicated that crossbred population under investigation was of intermediate diversity for both polymorphic SNP loci.

**Keywords**: Crossbred pig, Litter Size, PCR-RFLP, SNP

Profitability of pig enterprises primarily depends on reproductive performance (Singh and Khanna 2000; Neopane 2005). Reproductive traits especially litter size and weights are extremely important for reducing the cost of pork production (Campbell *et al*. 2003; Das *et al*. 2005). Improvement in reproductive performance hence is one of the key interests in pig breeding programs. Rapid genetic improvement in litter traits through selective breeding has proven to be difficult due to low heritability estimates and sex limited attributes (Hanenberg *et al*. 2001; Distl 2007). These biological limitations can partially be overcome with the use of new molecular genetics tools, integrating true genotypic information with phenotypic data (Visscher *et al*. 1998).

A number of candidate genes have been identified as potentially relevant to reproductive performance in pigs. For example, ESR1 is amajor gene for prolificacy in pig breeds and its association with litter size was reported by Horogh *et al*. (2005). Niu *et al*. (2006) studied the polymorphism of Prei3 (T802G) in seven pig breeds and suggested that pre-implantation protein 3 (prei3) is one of the promising candidate gene for litter size in pigs. Pripwai and Mekchay (2011) identified a novel SNP at BsuRIc 930A>G in porcine follicle stimulating hormone (FSHâ) gene having significant association with litter size in commercial pigs. Kumchoo and Mekchay (2015) identified SNP at c.425G>A in OPN gene having significant association with litter size in commercial Thai Large White pigs. Liu and Xia (2011) studied SNP at T96C in exon 4 of CDK20 gene and noticed its significant association with litter size in Large White and Landrace pigs.

The objective of this study was to screen candidate SNPs and test their association with reproduction traits in a crossbred pig population.

## MATERIALS AND METHODS

*Animals, sample collection and phenotypic information:* A total of 100 female crossbred (75% Landrace and 25% Indigenous) pigs, maintained at Swine Production Farm, Livestock Production and Management Section, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India; a unit of ICAR-All India Coordinated Research Project on Pigs were screened for candidate SNPs associated with litter traits. This centre is situated at an altitude of 564 feet above the mean sea level, 28° N latitude and 79° E longitudinal. The temperature of this place touches both the extremes (4–5°C in winter and 40–45°C in summer) and relative humidity ranges between 15 to 85 percent. These pigs were reared under similar feeding and management conditions throughout the experimental period. Blood from all animals (5 ml/pig) under sterile condition was collected from the anterior vena cava in a sterile 15 ml polypropylene centrifuge tube containing EDTA (0.5 ml/10 ml blood). Traits under study were litter size at birth (LSB) and weaning (LSW) and litter weight at birth (LWB) and weaning (LWW).

*DNA extraction:* Genomic DNA was isolated from the blood samples as per the standard protocol (Sambrook and

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Table 1. Details of SNPs along with primer sequence, annealing temperature (AT), restriction enzyme (RE) and amplicon size (AS) of each primer used in the study

| Gene (SNP) | Sequence (5′ to 3′) | AT (°C) | AS | RE | Reference |
|---|---|---|---|---|---|
| ESR1 (c.1227C > T) | F: CCTCCATGATCAAGTGCATCTTCTR: CAGCCAGGTCACTTACTGTCCAG | 55.5 | 138 | PvuII | Gloria *et al.* (2007) |
| Prei3 (T802G) | F: GTTTTAGTAAGTTTTGATTGGTCCGR: AAACCCCTGTTCCTCATTCTTG | 53 | 294 | MspI | Niu *et al.* (2006) |
| FSHâ (c.930A>G) | F: ACAGTTTTTTACAGGCCTTAR: CTGGCTGGGTCCTTGTAT | 55 | 930 | BsuRI | Pripwai and Mekchay (2011) |
| OPN (c.425G>A) | F: TCCGAGGAAGCTGATCGCGR: GATTTTGACCTCAGTCCGT | 55 | 188 | HinfI | Kumchoo and Mekchay (2015) |
| CDK20 (T96C) | F: AC ACC AGC TCC GGG AGC AR: CCT CGG GCC AGC TCA AGA | 61 | 204 | AIu I | Liu and Xia (2011) |

Russell 2001). DNA concentration and purity (A260/A280 ratio) for each sample was assessed using a spectrophotometer. The measured DNA samples were stored at –80°C until further analysis.

PCR -RFLP analysis: Five SNPs of porcine genes [ESR1 (c.1227C > T), Prei3 (T802G), FSHâ (c.930A>G), OPN (c.425G>A) and CDK20 (T96C)] showing association with litter traits in previous literature were taken in the present study. Genotyping of SNPs was done by PCR-RFLP procedure. Detailed information about SNPs along with primer sequence, annealing temperature, restriction enzymes and amplicon size of each primer is given in Table 1. The working solutions of both forward and reverse primers were prepared to obtain a final concentration of 10 pmol of each primer. Final reaction mix (25 µl) comprised of forward primer (1.0 µl), reverse primer (1.0 µl), Dream Taq Green buffer (2.5 µl), dNTPs mix (0.5 µl), Taq polymerase (0.2 µl), DNA template (2 µl) and nuclease free water (17.8 µl). PCR was carried out in a 25 µl reaction volume, which was kept constant for all reactions using thermo cycler (Bio-Rad, USA).

The optimization of appropriate annealing temperature with respect to each primer was determined by gradient PCR. The PCR conditions involved initial denaturation at 95°C for 5 minutes, followed by 40 cycles with denaturation at 94°C for 1 minute, annealing at temperature between 52.5 and 62.0°C for 45 seconds to specifically amplify a target region 1 and 2 and extension at 72°C for 1 minute followed by a final extension at 72°C for 5 minutes. The restriction enzyme (RE) digestion of PCR product was carried out in 0.2 ml tube with a total reaction mixture of 23 µl by overnight incubation at a temperature, specified by enzyme manufacturer. The digested products were thereafter kept at -20°C till further study. The amplified and digested DNA fragments of SNPs were separated on 3.5% agarose gel. The genotype of the individual was determined for each polymorphism by analyzing the size of the fragment in RFLP.

*Statistical analysis:* Heterozygosity, polymorphic information content (PIC), allelic diversity and Hardy Weinberg equilibrium were estimated using proc allele module of SAS 9.3 software. The association analyses between SNPs and litter traits were performed through PROC GLM module of SAS 9.3 using following model:

$$y_{ij} = \mu + g_i + e_{ij}$$

where $y_{ij}$, observation of litter trait on $j^{th}$ pig in $i^{th}$ genotype; µ, overall mean; $g_i$, effect of $i^{th}$ genotype; $e_{ij}$, random error ~NID (0, $e^2$).

## RESULTS AND DISCUSSION

In the present study, out of 5 SNPs genotyped, 2 were polymorphic {Prei3 (T802G) andCDK20 (T96C)} and 3 were monomorphic {ESR1 (c.1227C > T), FSHâ (c.930A>G) and OPN (c.425G>A)}. The allelic and genotypic frequencies at different SNP sites are shown in Table 2. Least squares means of litter traits across the genotypes at different SNP sites are shown in Table 4. The PIC, heterozygosity, allelic diversity and $\chi^2$ values for different SNPs are given in Table 3. The value of PIC, heterozygosity and allelic diversity revealed that crossbred population under investigation was of intermediate diversity for Prei3 (T802G) and CDK20 (T96C) SNP loci. Furthermore, forces were in operation over the period to maintain the population with both alleles. The $\chi^2$ value for HWE further indicated that the population was under Hardy Weinberg equilibrium for the Prei3 (T802G) and CDK20 (T96C) SNPs.

*ESR1* (*c.1227C > T*) *SNP:* The length of amplified PCR

Table 2**.** Allelic and genotypic frequency at different SNP sites in crossbred pigs

| Gene ( SNP) | Allele | Allelic | Frequency genotypic | Genotype frequency |
|---|---|---|---|---|
| ESR1 (c.1227C > T) | C | 1.00 | CC | 1.00 |
| Prei3 (T802G) | G | 0.53 | GG | 0.21 |
| | T | 0.47 | GT | 0.65 |
| | | | TT | 0.14 |
| FSHâ (c.930A>G) | C | 1.00 | CC | 1.00 |
| OPN (c.425G>A) | A | 1.00 | AA | 1.00 |
| CDK20 (T96C) | C | 0.52 | CC | 0.21 |
| | T | 0.48 | CT | 0.63 |
| | | | TT | 0.16 |

Table 3. Polymorphic information content, heterozygosity and allele diversity value for different SNPs along with test of Hardy Weignberg equilibrium

| Locus | Number of individual | Number of alleles | PIC | Heterozygosity | Allelic diversity | Test for HWE | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | $\chi^2$ | DF | Pr > $\chi^2$ |
| Prei3 | 100 | 2 | 0.3738 | 0.6500 | 0.4976 | 9.3882 | 1 | 0.0022 |
| CDK20 | 100 | 2 | 0.3744 | 0.6300 | 0.4988 | 6.9252 | 1 | 0.0085 |

Table 4. Least squares means of litter traits across the genotypes at different SNP sites

| Locus | Genotype | LSB | LWB | LSW | LWW |
|---|---|---|---|---|---|
| Prei3 | GG | 7.96±0.67 (20) | 7.82±0.52 (20) | 7.09±0.54(20) | 56.17±6.75(20) |
| | TG | 7.02±0.97 (66) | 6.63±0.76 (66) | 6.43±0.78(66) | 63.58±9.77(66) |
| | TT | 10.32±1.12 (14) | 9.37±0.87 (14) | 8.80±0.90 (14) | 67.51±11.19 (14) |
| CDK20 | CC | 8.41±0.69 (21) | 7.76±0.54 (21) | 7.51±0.55 (21) | 68.49±6.90 (21) |
| | CT | 9.92±1.04 (63) | 9.35±0.81 (63) | 8.47±0.84 (63) | 61.13±10.43(63) |
| | TT | 6.96±0.98(16) | 6.71±0.77(16) | 6.35±0.79(16) | 57.65±9.88(16) |

Figures in parentheses indicate number of observations.

fragment was 138 bp, which corroborated the findings of Muñoz *et al.* (2007). In this population, only C allelic variant was observed whereas Muñoz *et al.* (2007) noticed both C and T variants with 54 and 46% frequency in Chinese-European pigs.

*Prei3* (*T802G*) *SNP:* Prei3 (T802G) SNP had three genotypes; GG, TT and GT with fragments size of 183 and 111bp; 294 bp; and 294, 183 and 111 bp, respectively. The size of amplified PCR fragment (294bp) was in accordance with findings of Niu *et al.* (2006). All three genotypes were observed at Prei3 (T802G) SNP locus with 21 (GG), 65 (TT) and 14% (GT) frequency. The frequency of G and T allele at Prei3 (T802G) SNP locus was 53 and 47%, respectively. Frequency of homozygote GG, TT and heterozygote GT in present study did not corroborate with the results of previous investigations. Niu *et al.* (2006) observed higher frequency of TT (91%) genotype and lower frequency of TG (8%) and GG (1%) genotype in Large White pigs. The frequency of G and T allele at this locus also differed than that reported by Niu *et al.* (2006). They observed frequency of G and T allele in Large White pigs as 5 and 95%. The least squares analysis revealed non-significant association of Prei3 (T802G) SNP with all the litter traits. Contrary to present study, significant effect of Prei3 (T802G) SNP was noticed by Niu *et al.* (2006) on litter size at birth.

*FSHβ* (*c.930A>G*) *SNP:* The length of amplified PCR product (930 bp) was similar to the findings of Pripwai and Mekchay (2011). Monomorphism at this SNP locus was however contrary to the findings of Pripwai and Mekchay (2011) in crossbred pigs of Large White and Landrace breeds. They reported that frequency of *A* and *G* allele at FSHâ (*c.930A>G*) *SNP locus* was 0.81 and 0.19, respectively.

*OPN* (*c.425G>A*) *SNP:* The length of amplified PCR fragment (188 bp) corroborated the findings of Niu *et al.* (2008) and Kumchoo and Mekchay (2015). Monomorphism at this locus was however not observed in previous investigations. Kumchoo and Mekchay (2015) observed wild (c.425G) and mutant (c.425A) allelic variants at OPN (c.425G>A) SNP locus with 38 and 62% frequency in Thai Large White pigs. Niu *et al.* (2008) also observed both allelic variants at OPN (c.425G>A) SNP with 20 (c.425G) and 80% (c.425A) frequency in Tibet pig breed.

*CDK20* (*T96C*) *SNP:* Homozygote CC and TT and heterozygote CT genotypes were obtained at CDK20 (T96C) SNP site with fragment size of 136 and 60 bp; 196 bp; and 196, 136 and 60 bp, respectively. The size of amplified PCR product (204bp), frequency of homozygote CC (21%), TT (16%) and heterozygote CT (63%) and frequency of C (52%) and T (48%) allele in this study were similar to the findings of Liu and Xia (2011). They reported that frequency of allele C and T as 57.5 and 42.5% in Large White and 55 and 45% in Landrace pigs. The least squares analysis in their study also revealednon-significant association of CDK20 (T96C) SNP with litter traits.

The inconsistency among results could be attributed to sample size, sampling error, and genotype by environment interaction, linkage, genetic background or population stratification.

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