Preliminary study of the RBP4, EGF and PTGS2 genes polymorphism in pigs and its association with reproduction traits of sows*

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Genes of retinol-binding protein 4 (RBP4), epidermal growth factor (EGF) and prostaglandin-endoperoxide synthase 2 (PTGS2) were examined as candidate genes for reproductive traits in sows. The material included a total of 300 sows of the Polish Landrace (n=86), Polish Large White (n=64) and commercial Line 990 (n=150). Included in the study was the total number of piglets born (TNB), number of piglets born alive (NBA), number of piglets on day 21 of age (NP21), number of piglets weaned (NPW), litter weight on day 21 of age (LW21), litter weight at weaning (LWW) and farrowing interval (FI). Calculations were based on records available for two farrowings (1st and 2nd) from each sow.

The RBP4 genotype was found to have a significant effect on LW21 and LWW (P≤0.05 and P≤0.01, respectively). No significant effect of this genotype was identified for litter size traits (TNB, NBA). The effect of EGF genotype was found significant on TNB (P≤0.01) and NBA (P≤0.05). The A/A animals showed the highest reproductive efficiency compared to those of B/B and A/B genotypes. No significant effects of the PTGS2 genotype were found due to a total absence of one of the homozygous genotypes at this locus.

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Molecular genetics technology is leading to the discovery of individual genes having a significant effect on traits of interest in pigs. The identification of such major genes and the underlying mutations can enhance rates of genetic improvement in the short term [Dekkers 2004] through the application of marker-assisted selection (MAS), which will increase as more associations between markers (genes) and traits are identified [Rothschild 1998]. Development of porcine genome maps offers also an opportunity to identify individual genes controlling reproduction. This technology seems to be especially promising for fertility traits such as litter size, as they are characterized by a low heritability [Drogemüller et al. 2001]. Over the past decade, a variety of genes named “candidate genes”, have been investigated for their possible association with reproductive traits in pigs [Islaer et al. 2002].

In this study genes RBP4, EGF and PTGS2 were selected as potential candidate genes for reproduction due to their integral role in several reproductive pathways.

In the organism the retinol acid (vitamin A) is derived from endogenous retinol, which is delivered to target cells by retinol-binding protein 4 (RBP4). The availability of retinol to conceptus is limited to that which can be transported from maternal capillaries across several boundaries of epithelium to the uterine lumen [Roberts et al. 1993]. Brief and Chew [1985] showed that supplementing the diet of pregnant sows with vitamin A can increase litter size. Assuming that conceptuses signal the uterine secretion of RBP4 and that they express high levels of RBP4 around day 12 of pregnancy, this indicates an important function of retinol and RBP4 at this stage [Trout et al. 1991]. The transport and buffering ability of RBP4 at this critical time of pregnancy makes RBP4 a strong candidate gene for litter size in pigs [Rothschild et al. 2000].

Epidermal growth factor (EGF) is produced by conceptus. In foetus, EGF stimulates growth and proliferation of skin epithelium, whereas in neonate EGF stimulates the pulmonary maturation [Hadley 1996].

Prostaglandin-endoperoxide synthase 2 (PTGS2) also known as cyclooxygenase 2 (COX2), was chosen as it is the rate-limiting enzyme in the formation of prostaglandins [Lim et al. 1997] and is also involved in the synthesis of thromboxanes and prostacyclin. A null mutation was described [Wilson et al. 2002] producing multiple reproductive failures in mice - oocyte maturation was not complete and the first polar body was usually not extruded. An increased expression of cyclooxygenase 2 by the filamentous conceptus was associated with an increased content of prostaglandins (particularly prostaglandin E2) found in uterine luminal fluid.

The objective of the current study was to examine the associations between the polymorphism of genes RBP4, EGF, PTGS2 and sow reproductive traits.

Material and methods
Animals

The animals examined were owned by the National Research Institute of Animal Production, Pig Hybridization Centre in Pawłowice and a single farm co-operating with the University of Technology and Agriculture in Bydgoszcz and comprised Polish Landrace (n=86), Polish Large White (n=64) and commercial Line 990 (n=150) sows. The sows from different breeds were kept in different herds. Two first litters from each sow were evaluated for the following reproduction traits: total number of piglets born (TNB), number of piglets born alive (NBA), number of piglets on day 21 of age (NP21), number of piglets weaned (NPW), litter weight on day 21 (LW21), litter weight at weaning (LWW) and farrowing interval (FI).

Molecular test

Genomic DNA was isolated from blood leukocytes according to Kawasaki [1990] using the WIZARD Kit. PCR-RFLP technique was used to genotype sows at the RBP4, EGF and PTGS2 loci. Primer sequences for amplifying the PCR products and reaction conditions followed those described by Rothschild et al. [2000], Mendez et al. [1999] and Gladney et al. [1999]. Some of the reaction conditions were modified according to authors' own experience.

The polymorphism of RBP4 was identified using restriction enzyme MspI while that of PTGS2 using the MseI restriction enzyme. In case of EGF only the PCR product was identified.

Statistical

The relationships between the genotypes of candidate genes and reproductive traits were evaluated with the LSM (SAS, GLM) procedure. The models included the fixed effects of RBP4, EGF, PTGS2 genotypes of the given sow, the breed of the sow, parity, year and season of farrowing, number of boar as well as covariate of additive and dominance effect of RYR1 genotypes (HALA and HALD). The RYR1 genotype was introduced in the models due to its known strong effect on reproduction traits, demonstrated in various populations. Additionally, the farrowing interval was included in the models as covariate for traits TNB, NBA and NP21.

Results and discussion

Effects of the RBP4 genotype

The designed primers were located in exon 2 and exon 4 what made it possible to receive a 550 bp-long fragment of the gene RBP4. In this study both alleles – 1 and 2 – were represented (Photo 1). For whole tested population the frequency of individual alleles at this locus amounted to 0.68 and 0.32 for allele 1 and 2, respectively.

The results of the association analyses are presented in Table 1. No significant effect of RBP4 genotype was identified for the TNB, NBA and FI. A significant effect
was observed for LW21 (P ≤ 0.05) and LWW (P ≤ 0.01) – in both cases the values for 2/2 genotype were higher than those for genotype 1/1. As regards NP21 and NPW the animals of genotype 2/2 showed slight and not significant tendency for the higher reproductive efficiency.

The gene *RBP4* was studied as a possible candidate gene affecting litter size due to its relationship with embryo development. Yelich *et al.* [1997] stated that most embryonic
losses in pigs occur between day 10 and 18 of gestation, concurrent with trophoblast elongation. RBP4 as a major protein produced by the conceptus may play a role in trophoblast elongation [Harney et al. 1990]. Harney et al. [1993] showed that from day 10 to 12 there is an increasing RBP4 expression in gravid porcine endometrium. Their results confirm the important role of this vitamin A transport-protein in the uterine and conceptus physiology during the establishment of pregnancy. Therefore, owing to the integral role of RBP4 at the time of high embryonic mortality rate [Drogemüller et al. 2001] the gene RBP4 was investigated as a candidate gene for litter size. The fact that in the present study its polymorphic variants had no significant effects on TNB and NBA is, compared to results of other studies, somewhat unexpected. Messer et al. [1996] and Ollivier et al. [1997] reported effects associated with gene RBP4 to be 0.52±0.45 for litter size in French Large White sows. Rothschild et al. [2000] using records obtained for 2500 litters, also reported an additive effect associated with RBP4. Allele 1, mentioned by Rothschild as favourable, had a higher frequency while the differences between genotype 1/1 and 2/2 reached approximately 0.55 piglets/litter for TNB and 0.26 piglets/litter for NBA in some commercial lines of pigs. In turn, Drogemüller et al. [2001] reported that the frequency of allele 1 also was the highest, but the RBP4 locus showed no significant effect on litter size in the synthetic line examined. Linville et al. [2001] presented similar results.

Effects of the EGF genotype

In the present study two fragments of gene EGF were received with the PCR-RFLP method: for allele A a 1527 bp-long fragment was amplified, while for allele B – a 652 bp-long fragment (Photo 2). For whole tested population the frequency of individual alleles at this locus amounted to 0.30 and 0.70 for allele A and B, respectively.

The EGF genotype had a significant effect on TNB (P≤0.01) and NBA (P≤0.05). The A/A animals showed the highest reproductive efficiency compared to those of B/B and A/B genotypes. Simultaneously, B/B sows had more numerous litters (TNB) as compared to animals with genotype A/B. A similar situation was observed as regards NPW. However, the difference proved to be significant only between sows of genotype A/A and A/B (P≤0.01) or B/B and A/B (P≤0.05). No significant difference was observed between homozygotes (Fig. 1).

The results presented justify the choice of the gene EGF as a candidate gene. It was identified as a candidate gene on positional (chromosome 8) as well as physiological basis. Earlier studies have provided evidence that on chromosome 8 are located QTLs affecting several reproductive traits in pigs [Wilkie et al. 1999, Rathje et al. 1997, Rohrer et al. 1999, King et al. 2003]. According to Wollenhaupt et al. [2002] the EGF receptor system is involved in regulating the proliferation and differentiation in oviductal and endometrial tissues. However, in the study by Linville et al. [2001] the association between polymorphism in gene EGF and reproductive traits could not be estimated because only two genotypes occurred in the tested population – the effect of A/A homozygote which proved most favourable in the present study could not be
Effects of the PTGS2 genotype

The 1550 bp-long fragment (spanning exon 3 and exon 4) was amplified. At this locus both alleles were present (Photo 3), but no homozygous A/A animal was detected.

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The frequency of individual alleles at this locus amounted to 0.2 and 0.8 for A and B allele, respectively. Preliminary data concerning gene PTGS2 as a candidate gene reported by Gladney et al. [1998], showed opposite results - allele A occurred with a higher frequency (0.68). In turn, Linville et al [2001] showed allele A to be favourable at the PTGS locus. Moreover, in their study the homozygote B/B was not found.

No significant effect of genotypes PTGS2 was observed on litter size and piglet weight (Tab. 2), but animals of the B/B genotype showed no significant tendency to a lower efficiency of reproduction. TNB, NBA and NP21 were significantly affected by both the sow’s breed and successive farrowing (parity), while NPW, PW21 and PWW only by successive farrowing. The FI was significantly affected by the sow’s breed. These results are similar to those recorded by Linville et al [2001], who had not found any significant association between the polymorphism within the gene PTGS2 and reproduction traits. Also consistent with the present results are those obtained by Cas-sady [1999] who performed a complete genome scan for QTLs affecting reproduction traits in an F2 population of Nebraska lines and found evidence (P<0.05) of the presence of a QTL affecting ovulation rate on chromosome 9. The PTGS2 locus is located on chromosome 9. However, the QTL for ovulation rate found by Cassady [1999] was approximately at 1 cM position, whereas PTGS2 has been mapped between markers S0295 and S0114, which are located at the positions 100 and 123 cM, respectively, to the USMARC linkage map [Gladney et al 1999]. It may be possible that the position of this gene is the reason for which candidate gene studied here does not have large effect on reproduction traits in the population analysed.

To conclude, significantly associated with genotypes at locus RBP4 appeared only traits connected with the weight of piglets. The allele frequency and significant relationships between traits and genotypes were different from those reported by other authors. The results obtained in the present study demonstrate the difficulties in confirming the effect of candidate gene when testing various genetic groups of pigs.
The study presented here of candidate gene PTGS2 has equivocally validated the results of genome-wide QTLs searches. There has been a relatively small correspondence between candidate genes and QTL locations, identified for litter size or its component traits. This lack of correspondence between them could be due to the fact that the effect of some candidate genes is too small to be detected for the sample size employed in this research, to the lack of segregation of the candidate genes alleles in some populations, or to the fact that the originally observed candidate gene effects may, in some cases, have been detected due to chance [Kirpatrick 2002]. However, sometimes the results of association between gene and traits of interest confirm the results of the QTL analysis. The EGF gene was identified as a candidate gene both on positional (chromosome 8) and physiological basis. The present study provides evidence supporting the assumption that on porcine chromosome 8 is located a QTL for prolificacy.

In the current study all the nucleotide polymorphisms analysed in the porcine genes RBP4, EGF and PTGS2 were found in introns. Although the SNPs in introns do not alter directly any functionally important amino acid residue, they may prove useful as markers for functional SNP via linkage disequilibrium mapping. In addition, some introns play a role in regulating gene expression and thus their constituent SNPs may be directly related to a functional variation [Jiang et al. 2002].

The results presented in this paper are only part of a major research project, but they show that the genes analysed should be further examined as possible candidate genes. A more conclusive proof of the effect of those genes on litter size would necessitate the examination of a large sample population. Further investigations will comprise data obtained from genotyping about 800 sows and at least four parities for each sow. It gives possibility of breed-specific statistical analysis on different genetic background.
This should render it possible to conclude about genes which have a major impact on the improvement of reproductive efficiency.

REFERENCES


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300 macior ras pbz, wbp i linii syntetycznej 990 (odpowiednio n = 86, 64 i 150) Na podstawie danych pochodzących z dwóch pierwszych miotów, analizowano zależność między genotypami badanych białek a poziomem wybranych cech związanych z liczebnością i masą miotu, a także okresem międzymiotu. Istotne zależności stwierdzono między genotypami RBP4 a masą miotu i między genotypami EGF a liczebnością miotu. W obrębie genu PTGS2 nie zaobserwowano żadnych istotnych zależności. Jednak w przypadku PTGS2, w testowanym materiale stwierdzono występowanie homozygot tylko jednego typu.