Interactions of the bovine placental lactogen and prolactin receptor genes are associated with fertility traits in cattle

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Decline in fertility of high-producing dairy cattle has become a global challenge to the dairy industry. Because of low heritability and complexity, it is difficult to find genetic markers for fertility traits in cattle. Here, we report the use of an in vitro fertilization (IVF) system and candidate gene approach to test genetic associations of a single-nucleotide polymorphism (SNP) in bovine placental lactogen (bPL), and its interactions with SNPs in the prolactin receptor (PRLR) and growth hormone receptor genes with fertility traits in an IVF system. The associations suggest a possible involvement of genetic interactions between bPL and PRLR in the fertilization and embryonic development processes, and the potential for application in a marker-assisted selection program.

Keywords: bovine placental lactogen, candidate gene, fertility, epistasis

Implications

Fertility of the modern high-producing dairy cow has been decreasing for the last 30 to 50 years and has become a major concern of farmers and the dairy industry worldwide. Many reasons account for this reduced reproductive efficiency, but the most important component seems to be a reduction in embryonic survival and fertilization rates. As such, there is an urgent need to identify the genetic factors responsible for the decline in embryo survival. Identifying these factors would enable reduction in the frequency of the deleterious alleles at these loci by marker- or gene-assisted selection.

Introduction

There is growing concern with the decline in reproductive efficiency in high-producing dairy cows (Royal et al., 2008). A variety of reasons have been proposed to account for infertility in cattle, including genetic factors, nutrition, physiology and management (Lucy, 2001; Veerkamp and Beerda, 2007). It is of particular interest to identify polymorphisms among specific genes and/or interactions between them that are associated with infertility in cattle, as this not only helps to elucidate the biology of infertility, but also provides genetic markers for use in marker-assisted selection.

Although heritability is generally low, there is substantial genetic variation for fertility traits (Veerkamp and Beerda, 2007), enabling possible identification of genetic factors. However, owing to the complex nature of fertility, it is very challenging to find significant associations between genetic polymorphisms and fertility traits. An in vitro fertilization (IVF) system has the advantage of a unified environment and well-isolated components of the embryonic development process. Indeed, using an IVF system developed in our laboratory, single-nucleotide polymorphisms (SNPs) in several genes have been successfully shown to be associated with fertilization rate and early embryonic survival rate (Khatib et al., 2008a and 2008b). Importantly, SNP–SNP interactions between biologically interacting genes in the same signaling pathway were also found to contribute significantly to fertilization and early embryonic development (Khatib et al., 2009).

Placental lactogen or chorionic somatomammotropin hormone 1 (CSH1), is a polypeptide hormone found in the placenta of mammals. It is structurally and functionally related to prolactin (PRL) and growth hormone (GH), two pituitary-derived hormones. Bovine placental lactogen (bPL) shares about 50% and 24% amino acid sequence homology with PRL and GH, respectively (Anthony et al., 1995). However, no specific bPL receptor has been identified to date, yet it has been shown to be able to act through PRL receptor (PRLR) and GH receptor (GHR) (Byatt et al., 1992, Anthony et al., 1995). bPL has a wide range of biological
activities in vivo, including mammogenesis, ovarian steroidogenesis and fetal growth (Byatt et al., 1992). Nonetheless, the roles of bPL in fertility are less clear. Importantly, SNPs in GHR and PRLR, and their interactions with a STAT5A (signal transducer and activator of transcription 5A) SNP, have been shown to be associated with early embryonic survival rate (Khatib et al., 2009). The functional roles of bPL and its interacting genes make bPL a good candidate gene for fertility traits. The objectives of this study were to identify SNPs in the bPL gene and to test associations of SNPs or SNP–SNP interactions with fertility traits in the IVF system.

Material and methods

Phenotypic data were collected as described earlier (Khatib et al., 2008b). Briefly, oocytes were aspirated from ovaries obtained from a local abattoir. A total of 6586 matured oocytes from 382 ovaries were fertilized with frozen-thawed semen samples from 12 bulls. Two reproductive traits were considered. The fertilization rate of oocytes for each ovary was calculated as the number of cleaved embryos 48 h after combining of semen and oocytes divided by the total number of fertilizations attempted. Embryos were cultured for 7 days until the blastocyst stage, before they were morphologically graded as ‘normal’ or ‘degenerative’. The survival rate of embryos was defined as the number of normal embryos divided by the total number of embryos produced.

DNA was extracted from ovaries through proteinase K digestion of ovary tissue followed by phenol/chloroform extraction and ethanol precipitation. To identify polymorphisms in the bPL gene, three DNA pools, each consisting of DNA from 10 ovaries, were constructed and amplified with PCR primers covering bPL exons; the PCR products were purified and sequenced. The sequence traces were visually inspected for DNA polymorphisms as indicated by multiple peaks at the same position, which were further confirmed by sequencing individual DNA samples. Only one C/T SNP was identified near the 5’ end of the bPL transcript (GenBank accession number NM_181007 position –198 upstream). Genotyping of this SNP was carried out by PCR-RFLP. Briefly, forward primer 5’TCAACTTAGCACGCCCCGCATAGGGTGATACAGGTA3’ and reverse primer 5’GGAATGCTAAGGAGGAAACC3’ were used to amplify a 237-bp fragment, in which the third nucleotide from the 3’ end of the forward primer was mutated from A to G to create a Csp6I recognition site. The PCR program had an initial denaturing step at 94°C for 5 min followed by 28 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s, followed by a final extension at 72°C for 10 min. The PCR products were digested by Csp6I (Fermentas, Glen Burnie, MD, USA) according to the manufacturer’s instructions, and then resolved in 2% agarose gel. The C allele appeared as 35-bp and 202-bp bands and the T allele was one intact band. Genotypes of PRLR (Blott et al., 2003) and GHR (Viitala et al., 2006) were obtained in an earlier study (Khatib et al., 2009) using similar PCR-RFLP approaches but different restriction enzymes.

To test the association between the bPL SNP and fertilization and survival rates, the following generalized linear mixed model was used to model the binary response of successful fertilization of oocytes or survival of embryos up to the blastocyst stage with a linear combination of predictors:

\[ n_{ijk} = b_0 + b_1 bPL_i + \text{Bull}_j + \text{Ovary}_k (bPL_i), \]

where \( b_1 \) is the fixed effect of genotype \( i \) for the bPL SNP, \( \text{Bull}_j \) is the random effect of the \( j \)th bull and \( \text{Ovary}_k \) is the random effect of the \( k \)th ovary, which is nested within genotype of bPL SNP. The response variable was coded as binary (‘1’ for successful fertilization; ‘0’ otherwise) and ‘1’ for normal development to blastocyst stage; ‘0’ otherwise, respectively. A canonical logit link function was used. The model was fitted using GLIMMIX procedure in SAS software version 9.1 (SAS Institute, 2009). The fixed effect of bPL SNP in the model was tested by a type-III F-test (using ‘contain’ for ‘DDFM’ option) with its denominator degrees of freedom computed from the ovary random effect nested within bPL genotype (SAS Institute, 2009). The genotypic effect can be interpreted as effect of mother’s genotype on the fertilization of oocytes or survival of embryos. To test for association between fertilization/survival rate and SNP–SNP interaction between bPL and PRLR, a similar model was used with PRLR and bPL: PRLR interaction as additional fixed effects in the model. Significance for bPL: PRLR interaction in the model was tested similarly by the type-III F-test in SAS software. Association of bPL: GHR interaction with each of the two traits was also tested using the same model.

Results and discussion

The observed fertilization rates for oocytes produced from ovaries with each of the three genotypes of bPL were 0.660 (CC), 0.693 (CT) and 0.665 (TT), whereas the observed survival rates of embryos were 0.346 (CC), 0.336 (CT) and 0.323 (TT). Interestingly, there was a pattern of over-dominance for fertilization rate, which may explain the overrepresentation of heterozygotes in the population (Table 1). However, the differences in fertilization rates or survival rates did not reach statistical significance (Table 1).

<table>
<thead>
<tr>
<th>Ovary genotype (n)</th>
<th>Fertilization rate (n²)</th>
<th>Survival rate (n³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC (133)</td>
<td>0.660 (2284)</td>
<td>0.346 (1508)</td>
</tr>
<tr>
<td>CT (193)</td>
<td>0.693 (3333)</td>
<td>0.336 (2308)</td>
</tr>
<tr>
<td>TT (56)</td>
<td>0.665 (969)</td>
<td>0.328 (644)</td>
</tr>
<tr>
<td>Association test</td>
<td>0.211</td>
<td>0.892</td>
</tr>
</tbody>
</table>

P values

bPL = bovine placental lactogen gene; SNP = single-nucleotide polymorphism.
1Number of ovaries with the genotype.
2Number of fertilizations attempted.
3Number of total embryos produced.
between bPL and PRLR was also tested for association with
Because of their biological interaction, SNP–SNP interaction
the bPL gene and its interactions with hormone receptors
significant for either trait.

either trait, its interaction with PRLR showed significant
action between the bPL SNP and the SNP in GHR was not
lization and early embryonic development. However, inter-
2009). Interestingly, the genotypic effect of the bPL SNP
had a negative effect on fertilization rate as compared to the

Acknowledgements
The authors thank Rick Monson for help with the in vitro ferti-
1Number of ovaries with the genotype combinations.
1Number of oocytes (for fertilization rate) or embryos (for survival rate) produced from ovaries with the genotype combinations.

References

In addition to single SNP associations, SNP–SNP interactions between genes in the same signaling pathway have also been shown to contribute significantly to the variation in fertilization rate and survival rate (Khatib et al., 2009). Because of their biological interaction, SNP–SNP interaction between bPL and PRLR was also tested for association with fertilization and survival rates. As single SNPs, the SNP in PRLR was not associated with fertilization rate or survival rate, whereas the SNP in GHR was significantly associated with survival rate but not fertilization rate (Khatib et al., 2009). Interestingly, the genotypic effect of the bPL SNP depended on the genotype of PRLR, an indication of genetic interaction or epistasis. For example, the CC genotype of bPL had a negative effect on fertilization rate as compared to the CT and TT genotypes, only when the genotype of PRLR was AG (Table 2). Remarkably, interaction between the bPL SNP and the SNP in PRLR was statistically significant ($P = 0.05$) for fertilization rate and showed suggestive association ($P = 0.10$) with survival rate, suggesting a concerted genetic model involving epistasis between bPL and PRLR for fertilization and early embryonic development. However, interaction between the bPL SNP and the SNP in GHR was not significant for either trait.

In summary, we investigated the association of a SNP in the bPL gene and its interactions with hormone receptors and fertility traits in dairy cattle. Although single gene analysis of bPL did not reveal significant association with either trait, its interaction with PRLR showed significant association with fertilization rate and suggestive association with survival rate. This is a further support of the significant contribution of SNP–SNP interactions between biologically interacting genes to these fertility traits in cattle (Khatib et al., 2009). In addition, it could justify the use of genotypic combination of interacting genes in marker-assisted selection programs to improve fertility in cattle.

Table 2
Fertilization rates of oocytes and survival rates of embryos from ovaries by bPL, PRLR, and GHR genotypes

<table>
<thead>
<tr>
<th></th>
<th>Fertilization rate (n1, n2)</th>
<th>Survival rate (n1, n2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>bPL</td>
<td>PRLR</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>AG</td>
</tr>
<tr>
<td></td>
<td>0.714 (27, 545)</td>
<td>0.600 (25, 417)</td>
</tr>
<tr>
<td>bPL</td>
<td>0.704 (44, 760)</td>
<td>0.727 (66, 1214)</td>
</tr>
<tr>
<td></td>
<td>0.645 (21, 310)</td>
<td>0.782 (16, 330)</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>0.600 (25, 417)</td>
</tr>
<tr>
<td></td>
<td>0.666 (41, 662)</td>
<td>0.724 (11, 167)</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>0.645 (21, 310)</td>
</tr>
<tr>
<td></td>
<td>0.432 (27, 389)</td>
<td>0.316 (25, 250)</td>
</tr>
<tr>
<td></td>
<td>0.297 (21, 200)</td>
<td>0.279 (16, 258)</td>
</tr>
<tr>
<td></td>
<td>0.295 (16, 258)</td>
<td>0.339 (11, 121)</td>
</tr>
<tr>
<td></td>
<td>0.279 (16, 258)</td>
<td>0.339 (11, 121)</td>
</tr>
</tbody>
</table>

bPL = bovine placental lactogen gene; PRLR = prolactin receptor genotype; GHR = growth hormone receptor genotype.