The interaction of gonadotropin-releasing hormone (GnRH) with its receptor stimulates the up-regulation of the gene for its own receptor. Consequently, GnRHR and GnRH represent a key interaction for regulation of reproduction. Our laboratory is interested in transcriptional regulation of the porcine GnRHR gene and its possible effects on ovulation rate. Towards this end, we have chosen to use lines of swine divergent for ovulation rate. Studies were thus conducted using: 1) the Chinese M breed which has approximately 4-5 more piglets per litter, largely due to ovulation rate; 2) the I line, ovulating 7 more oocytes than control animals; and 3) the C line, an unselected white crossbred line of pigs. We have isolated approximately 5100 bp of the 5’ flanking region of the porcine GnRHR gene for all three lines of swine. Transient transfections into a gonadotrope-derived cell line using vectors containing the full-length sequence for all three lines of swine indicated a significant increase in luciferase (Luc) activity between M and C groups. Further analysis remains to determine the importance of these elements in differential regulation of the GnRHR gene in lines of swine divergent for ovulation rate.

Methods

- Inverse PCR was used to identify additional 5’ flanking sequence from that reported by Jiang et al., 2001.
- Specific primers were utilized to isolate the promoter from genomic DNA for each line of swine.
- Vectors were constructed containing the full-length promoter fused to the cDNA encoding luciferase resulting in a 2 to 4-fold increase in luciferase activity (I and M, respectively) over the c line (P < 0.05). Vectors containing sequential deletions of the full-length promoters for each line were constructed and transfected to identify a breed specific element(s). Differences in luciferase activity between lines were maintained following reduction of the proximal promoters from 5100 to 1900 bp. Sequence analysis of the 1900 promoters from each line revealed numerous putative elements and base changes between M and C groups. To examine the importance of this region in differential expression of the GnRHR gene, the 500 bp between -1900 and -1400 bp of C promoter were replaced with the same sequence from the M promoter, and vice versa. Transient transfections indicated no difference between promoter “swap” constructs and their respective constructs containing native M and C sequence. Further 500 bp deletions of the -1900 promoters from each line indicated that differential activity was lost when promoters were reduced to 1000 bp of 5’ flanking region. Sequence analysis of the -1400 to -1000 bp region upstream of the translational start site revealed multiple elements that may play a role in transcriptional regulation of the GnRHR gene between pig lines, including NF-Y, GATA-1, Sp1, and C/EBP. Thus, further analysis remains to determine the importance of these elements in differential regulation of the GnRHR gene in lines of swine divergent for ovulation rate.

Results

- Vectors with the 500 bp region between M and C lines of swine. Transfections were performed containing luciferase vectors containing the full-length M and C promoters (M5000pGL3 and C5000pGL3), these vectors with the 500 bp region between -1900 and -1400 “swapped” between lines (M/C5000pGL3 and C/M5000pGL3) or promoterless control (pGL3).

Summary

Deletion studies with the 5100 bp 5’ flanking region of the porcine GnRHR gene from three lines of swine divergent for ovulation rate (M, I, and C), as well as “swap” experiments between those lines with the largest differences in promoter activity (M and C), suggested that an element(s) responsible for differential regulation of the GnRHR gene was localized between 1400 and 1000 upstream of the translational start site. Sequence analysis of this region revealed putative cis-acting elements, including binding sites for C/EBP and Sp1. Further deletion studies and mutational analysis of this region are required to elucidate element(s) responsible for differential regulation of the GnRHR gene promoter in lines of swine with divergent ovulation rates. Additionally, electrophoretic mobility shift assays will be performed to identify transcription factors that bind to these elements.