1-1-2002

Characterization of Porcine Uterine Estrogen Sulfotransferase

J. G. Kim
USDA-ARS

J. L. Vallet
USDA-ARS

G. A. Rohrer
USDA-ARS

R. K. Christenson
USDA-ARS, christenson@email.marc.usda.gov

Characterization of porcine uterine estrogen sulfotransferase[☆]

J.G. Kim, J.L. Vallet, G.A. Rohrer, R.K. Christenson[*]

*USDA, Agricultural Research Service, Roman L. Hruska US Meat Animal Research Center,
P.O. Box 166, Clay Center, NE 68933-0166, USA*

Abstract

A quantitative trait locus (QTL) for uterine capacity is located on chromosome 8. Comparison of porcine and human genetic maps suggested that the estrogen sulfotransferase (STE) gene may be located near this region. The objectives of this study were to clone the full coding region for STE, compare endometrial STE gene expression between Meishan and White composite pigs during early pregnancy, and map the STE gene. We obtained a clone (1886 bp) containing the full coding region of STE by iterative screening of an expressed sequence tag library. Endometrial STE mRNA expression in White composite gilts was determined by Northern blotting on days 10, 13, and 15 of the estrous cycle; and on days 10, 13, 15, 20, 30, and 40 of pregnancy. STE mRNA expression was elevated ($P < 0.01$) on days 20 and 30 of pregnancy compared to other days of the cycle or pregnancy. Endometrial STE mRNA expression during early pregnancy, determined using real-time RT-PCR, was elevated ($P < 0.01$) on day 20 compared to day 15, decreased ($P = 0.02$) between days 20 and 30, and decreased further ($P < 0.01$) between days 30 and 40 in both Meishan and White composite pigs. Expression of STE mRNA was greater ($P = 0.01$) in White composite pigs compared to Meishan pigs. Using a microsatellite from an STE containing BAC genomic clone, the STE gene was mapped to 65 centimorgans on chromosome 8. Because STE mRNA expression differs between Meishan and White composite pigs, the STE gene may be a candidate for the uterine capacity QTL.

1. Introduction

Estrogen sulfotransferase (STE) catalyzes the addition of sulfate to estrone and estradiol, rendering them both water soluble and inactive. This reaction is thought to play a major role in the control of estrogen levels in target tissues [1]. Porcine endometrial STE (31 kDa) has been isolated from secretory endometrium [2]. In cyclic gilts, uterine STE activity increases during the luteal phase of the cycle [3]. In pregnant sows, endometrial STE activity is elevated during early pregnancy (days 12–28) and declines after day 30 [4]. Elevated endometrial STE activity may contribute to the rise of estrone sulfate in the plasma between days 23 and 30 in pregnant gilts [5]. Measurable quantities of unconjugated estrone and estradiol-17β first appear at days 70–80 of pregnancy and rise to a peak at the time of parturition, and this pattern is followed closely by estrone sulfate [5]. Progesterone treatment induces uterine STE activity in endometrial cultures after estradiol priming [6]. These results indicate that STE has the potential to influence estradiol metabolism in the uterus and, thus, may play a role in regulating the concentrations of unconjugated and sulfated estrogens during early pregnancy. The concentration of active estrogen in the uterine environment during early pregnancy may be important for endometrial function and placental development.

Uterine capacity is one component contributing to litter size in swine [7]. A quantitative trait locus (QTL) for uterine capacity has been identified in a population of half Meishan and half White composite gilts on the long arm of chromosome 8 near 71 centimorgans (cM; 95% confidence interval 53–107 cM) [8]. The effect of substituting a Meishan allele for a White composite allele on uterine capacity was estimated to be approximately 2 pigs per litter [8]. Comparison of pig and human genetic maps indicates that the QTL region is close to the likely location of the STE gene. Thus, because of its location and its potential effect on endometrial function, we selected the STE gene as a candidate for the previously reported uterine capacity QTL.

The full coding region for the STE cDNA sequences of human liver [9], human placenta [10], bovine placenta [11], rat liver [12], and guinea pig adrenal cortex [13] are known. However, the entire coding sequence of porcine endometrial STE cDNA has not been reported. To determine whether the STE gene may account for the effect of the uterine capacity QTL, the objectives of this study were to clone the full coding region for STE, examine the pattern of STE mRNA expression in endometrium collected from White composite and Meishan pigs, and map the STE gene in the porcine genome.

2. Materials and methods

2.1. Isolation of porcine STE cDNAs

Endometrium collected on day 30 of pregnancy from White composite female pigs was snap frozen in liquid nitrogen. Total RNA was isolated from endometrium using the RNeasy kit (Qiagen, Santa Clarita, CA). To obtain a partial cDNA clone of STE, 2 μg of total RNA was used for reverse-transcription (RT) with reverse primer 2 in a reaction volume of 20 μL, and then 1 μL of the resultant product was amplified with all possible pairs of the forward (1–3) and reverse primers (1 and 2) indicated in Table 1. Primer design was based on the known bovine cDNA

Table 1
Primers used in the characterization of the porcine estrogen sulfotransferase

Stage	Primer	Sequence
Initial	Forward	
	F1	TATCCCAAATCTGGTACAACATGGC
	F2	AGAGTTCCTTACCTGGAATGTAGCACTG
	F3	CGGAATGCCAAGGATGTGG
	Reverse	
	R1	TCTTAGTTCGGAACTTCAGGGTAGACC
	R2	GGAAGGGTGAAAGAACAATTTAATCTCC
BAC screening	Forward	
	F4	TGTTCTTCAGCCCCATCCTACTG
	Reverse	
	R4	CTCATTGGGATCATTTATAGACATCTGG
Primer walk	Forward	
	F5	GTTCAAGTTGTTAGAGCCTCATCG
	Reverse	
	R3	CAGAAGGTGAAAGAAAATATCAGGAAGG
	R5	GTGGCAGATGAGACTTCACTATTCTAG
	R6	CAGTTCTGCATTCCAGGTAAGG
	R7	CGATGAGGCTCTAACAACTTGAAC
SB71	Forward	GTCTTTCCATTTATTTCAGTTGATTTG
	Reverse	TCTGCATATTTTTCTGTAGTGATACATC
STE real-time	Sense	GAAAAGTG AATTAGCTCGGCCA
RT-PCR	Antisense	GTCGTCACGGTCCTTCGTTC

sequence (GenBank Acc. M54942) [11]. Amplification with forward primer 3 and reverse primer 1 resulted in a 497 bp PCR product as expected. This product was cloned into pCRII vector (Invitrogen, Carlsbad, CA) and sequenced. Primers based on the resulting sequence were then used to screen the "Meat Animal Research Center (MARC) 2 PIG" porcine expressed sequence tag (EST) library (Fahrenkrug et al., 2002) by PCR. The MARC 2 PIG EST library was derived from reproductive tissues that included ovary, pituitary, and hypothalamus from White composite sows; placenta and endometrium from Landrace X Yorkshire crossbred gilts; and testes from Meishan X White composite crossbred boars. Iterative screening of the EST library revealed a clone (1886 bp) containing the full length coding sequence of STE cDNA. This clone was sequenced in both directions using vector primers that were specific to the SP6 and T7 RNA polymerase binding sites; specific primers based on the initial clone (F2, F3, R1, and R3); and by primer walking (Table 1). Sequence identity and alignment were determined using the GAP procedure of the GCG sequence analysis package (Madison, WI), pairwise BLAST (NCBI), or ALIGN X procedure of Vector NTI Suite 7.0 (InforMax, Inc., Bethesda, MD).

2.2. STE mRNA expression in White composite gilts measured by Northern blotting

Northern blot analysis was performed using 30 μg of total RNA from endometrium of days 10, 13, and 15 cyclic gilts and days 10, 13, 15, 20, 30, and 40 pregnant White composite

gilts ($n = 3$–4 gilts per day) as described previously [14,15]. Total RNA was electrophoresed in 1.5% agarose gels prepared in MOPS (3-[*N*-morpholino] propane-sulfonic acid)/formaldehyde buffer, and the gels were then blotted onto Hybond-N nylon membrane (Amersham Life Science, Buckinghamshire, England). Integrity of RNA and equal gel loading were assessed visually using ethidium bromide stained 18S ribosomal RNA bands. The 1886 bp STE cDNA clone obtained from the EST library was linearized by EcoRV digestion and used as a template to generate radiolabeled RNA probe. Radiolabeled RNA probe was generated with T7 RNA polymerase using the MAXIscript kit (Ambion, Austin, TX) in the presence of [^{32}P]UTP. Membranes were prehybridized for 30 min in ULTRAhyb (Ambion, Austin, TX). Then 1×10^6 cpm/mL of radiolabeled probe was added, and blots were hybridized at 68°C overnight. The membranes were washed once with $2\times$ SSC, 0.1% SDS at 68°C, once with $0.1\times$ SSC, 0.1% SDS at 68°C, and then subjected to autoradiography.

2.3. STE mRNA expression in White composite and Meishan pigs measured by real-time RT-PCR

Endometrium was collected on days 15, 20, 30, and 40 of pregnancy from Meishan or White composite pigs (gilts and sows) and total RNA was isolated from endometrium using cesium chloride gradient purification. Real-time RT-PCR was performed using 100 ng of total RNA ($n = 5$–6 pigs per day) with an ABI Prism 7700 (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. To standardize the procedure, 25, 50, 100, 200, and 400 ng of total RNA from endometrium collected on day 60 of pregnancy was measured for GAPDH and STE mRNA within each assay. TaqMan fluorescent probes for GAPDH and STE were labeled with 6-carboxy-4,7,2′,7′-tetrachloro-fluorescein (TET) and 6-carboxy-fluorescein (FAM), respectively and were used to detect the respective mRNAs. Standard curves were calculated for each mRNA arbitrarily setting 25, 50, 100, 200, and 400 ng for each mRNA as 1, 2, 4, 8, and 16 units, respectively. The linear relationship between the log of the arbitrary units and the threshold cycle (the cycle at which fluorescence could first be detected) was calculated using regression analysis, as suggested by the kit instructions. Threshold cycles for each unknown sample (performed in duplicate) were then used to determine the number of arbitrary units based on the standard curve. STE primers were designed based on the porcine STE clone sequence and the primers are indicated in Table 1. Primers specific for porcine glyceraldehyde-3-phosphate dehydrogenase (GAPDH, GenBank Acc. AFO 17079) were used to measure GAPDH mRNA and the results were included as a covariate in subsequent analysis to correct for differences in RNA sample dilutions. Thermal cycling conditions were 48°C for 30 min and 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min.

2.4. Statistical analysis

Relative expression of STE mRNA was determined by densitometry of Northern blots and results were analyzed by ANOVA using the General Linear Models procedure of the statistical analysis system (SAS Institute, Inc., Cary, NC). The model included effects of status, day of the cycle or pregnancy, and the status-by-day interaction. However, no effect of status (pregnant versus cyclic) or status-by-day interaction was obtained. Therefore, data from

pregnant and cyclic gilts were combined within each day for days 10, 13, and 15, and day effects were evaluated using the following set of orthogonal contrasts: (1) day 10 versus day 13; (2) days 10 and 13 combined versus day 15; (3) day 20 versus day 30; (4) days 10, 13, and 15 combined versus day 40; and (5) days 20 and 30 combined versus days 10, 13, 15, and 40 combined.

Expression of STE mRNA determined by real-time RT-PCR was analyzed by the General Linear Models procedure of the statistical analysis system (SAS Institute, Inc., Cary, NC) after log transformation to decrease heterogeneity of variance between breed–day combinations. In addition, log transformed STE mRNA data were analyzed after correction using the units of GAPDH mRNA as a covariate. The model included effects of day, breed, assay, and the day-by-breed interaction.

2.5. Mapping

The RPCI-44 Male Porcine bacterial artificial chromosome (BAC) genomic library (BAC-PAC Resources, Buffalo, NY) was screened using a 1660 bp cDNA probe (84–1743 bp) generated by PCR amplification using forward primer 4 and reverse primer 4 (Table 1) and with the full coding STE cDNA clone obtained from the EST library as a template. A positive BAC clone (76A5) was digested with Sau3AI for 3 h, and the fragments were then treated with calf intestinal alkaline phosphatase for 30 min. Digested and dephosphorylated DNA fragments were precipitated, reconstituted with sterile water, and subcloned into SK vector previously digested with BamHI. Colonies were replicated onto membranes and then screened for microsatellites using a $(GT)_{14}$ oligonucleotide probe. GT probe was endlabeled with $[\gamma^{32}P]dATP$ and the membranes were hybridized with Rapid-hyb (Ambion, Austin, TX) containing 1×10^6 cpm/mL of radiolabeled probe at $58°C$ overnight. Membranes were washed once with $2\times$ SSC, 0.1% SDS at $42°C$, and once with $0.1\times$ SSC, 0.1% SDS at $58°C$ for 15 min, and were then exposed to film. A positive clone containing a microsatellite (SB71; GenBank Acc. AF406989) was sequenced using M13 vector primers. The size of the repeat region was 22 dinucleotide repeats. A pair of primers (Table 1) specific to the microsatellite marker were designed to amplify the region. Primers were used to amplify genomic DNAs collected from the MARC swine reference population [16]. Data were analyzed using CRI-MAP [17].

3. Results

3.1. Isolation and characterization of porcine STE cDNA

The full coding region for the porcine STE cDNA and the predicted amino acid sequence for putative porcine STE cDNA (GenBank Acc. AF389855) are shown in Fig. 1. The STE cDNA contained 1886 bp with a coding sequence of 885 bp that encodes 295 amino acids. Compared to the nucleotide sequence of porcine STE, the bovine [11], human [9], and rat [12] STE were 88, 84, and 76% identical, respectively. The 5′ and 3′, untranslated regions (UTR) of the porcine STE cDNA were 273 and 725 bp, respectively. The size of the porcine 5′ (273 bp) UTR was longer than that of the previously reported bovine placenta STE cDNA

```
   1   GAAACCTGAGTGGGGATTTGAAGAAAAGTGAATTAGCTCGGCCAGTTTCTTTTAGAGGATCATCCAAACAGTTTCACAGCACCTGTTCTTCAGCCCCATC   100

 101   CTACTGAGCTGGTCTTGGCAAGTGACACCCAACGTGGGGCAGGCAGGCATGAAGAACGAAGGACCGTGACGACAACAGAATTTTTATTCACTAGAAGTGC   200

 201   TGTGGAGCCCTGAGAAGAAGGGAGGAATTTTGGGTGACTACGCTAAAGAAATCCTGCAGTATATCACTCCATGATGAATTCTTCCAAATCAGCCTATTTA   300
                                                                             M  N  S  S  K  S  A  Y  L       9
                                                                                *
 301   GATTACTTTGGCAGAATCCATGGAATTCTACTGTATAAAAAATTTATCGAATATTGGAATGATGTGGAGACATTTGAGGCAAGACCAGATGACCTTGTCA   400
       D  Y  F  G  R  I  H  G  I  L  L  Y  K  K  F  I  E  Y  W  N  D  V  E  T  F  E  A  R  P  D  D  L  V  I   43

 401   TTGTCACCTATCCCAAATCTGGCACAACGTGGGTTAGTGAAATTGTGTACATGATTTATACAGAGGGCGATGTGGAAAAGTGCAAAGAAGATACCATTTT   500
        V  T  Y  P  K  S  G  T  T  W  V  S  E  I  V  Y  M  I  Y  T  E  G  D  V  E  K  C  K  E  D  T  I  F   76

 501   TAATCGAATTCCTTACCTGGAATGCAGAACTGAAAATGTAATGAATGGAGTAAAACAATTAAAACAGATGGCATCTCCTAGAATAGTGAAGTCTCATCTG   600
         N  R  I  P  Y  L  E  C  R  T  E  N  V  M  N  G  V  K  Q  L  K  Q  M  A  S  P  R  I  V  K  S  H  L   109

 601   CCACCTGAGCTTCTTCCAGTCTCGTTTTGGGAAAAGAACTGTAAGATCATCTATGTTTGCCGGAATGCCAAGGATGTGGTCGTTTCTTATTACTATTTCT   700
       P  P  E  L  L  P  V  S  F  W  E  K  N  C  K  I  I  Y  V  C  R  N  A  K  D  V  V  V  S  Y  Y  Y  F  F   143

 701   TTCTAATGGTGACTGCTAATCCGGATCCTGGTTCTTTCCAAGATTTTGTGGAGAAGTTCATGGATGGAGAAGTTCCTTATGGTTCCTGGTATAAACATAC   800
        L  M  V  T  A  N  P  D  P  G  S  F  Q  D  F  V  E  K  F  M  D  G  E  V  P  Y  G  S  W  Y  K  H  T   176

 801   AAAAATCCTGGTGGGAAAAGAGAACGAATCCACAAGTGCTGTTTATTTTCTATGAAGACATGAAGGAGAATATCAGAAAAGAGGTGATGAGATTGATAGAA   900
         K  S  W  W  E  K  R  T  N  P  Q  V  L  F  I  F  Y  E  D  M  K  E  N  I  R  K  E  V  M  R  L  I  E   209

 901   TTTCTGGGAAGGAAGGCATCAGATGAGCTTGTGGACAAGATTATAAAACATACTTCATTCCAAGAGATGAAGAACAATCCATCTACCAATTACACAACAC   1000
       F  L  G  R  K  A  S  D  E  L  V  D  K  I  I  K  H  T  S  F  Q  E  M  K  N  N  P  S  T  N  Y  T  T  L   243
                                                            *              *
1001   TTCCAGATGAAGTCATGAACCAAAAAGTATCTGCCTTTATGAGAAAGGGGATTGCAGGAGACTGGAAGAATTACTTTACAGTAGCCCTGAATGAGAAATT   1100
        P  D  E  V  M  N  Q  K  V  S  A  F  M  R  K  G  I  A  G  D  W  K  N  Y  F  T  V  A  L  N  E  K  F   276

1101   TGACATTCACTATGAGCAGCAAATGAAGGGGGTCTACACTGAAGTTACGAACAGAGATCTAGGAAAGTTTTTGTGGTTACATCTGAG**ATTAAA**GTGTTATC   1200
        D  I  H  Y  E  Q  Q  M  K  G  S  T  L  K  L  R  T  E  I  Stop                               295

1201   ACTTCTTACCTCTGTTATTTCAGACTGGTGGAGAATGAATCAGGCTAAAAAGATCATGGTTGAGTTCATGTGATTTTTGAGATCTTCATATCAAAAACAA   1300

1301   GAAAGATTTTTTTACCTTGTTATCTGTTTTAATTTTGTTTCCTTTTTTTACAAATATTGCAAAAATCTATAACCTGTGAGAATGATGTGGGAACACACAAA   1400

1401   TTGTTCAAGTTGTTAGAGCCTCATCGAAAATAACCAAGCATTCCAAATTCTTTAATTGTGTGATTACTTTTTTCCATTTATTGGTACATTTAGTATATCC   1500

1501   AACATACATATATATACATATACACATAAATATATATTAATAACTGAACATGGATTTTGAAAACCTTGTTGGAAAAAATTAAAGAATAATTAAATATGAG   1600

1601   GGCATAACTAGATCTATTATTTCCTTCCTGATATTTTCTTTCACCTTCTGCAAAATGGCTTAATTTGGAATTTATTTAGTCTAATGTAAATGTGAGGCA   1700

1701   TAACAGAACTGATTTCCAGATGTCTATAAATGATCCCAATGAGTGTTATTTATGCATAAATATCTCCAAATGATTTGAAATATTTATGTTCTAGAGTTCT   1800

1801   TTCTGTTCTATAACAATACAATACAATGTTATAAATGTAAGCTCTGTAA**ACTAAA**GTTATTTTTAATGTAAAAAAAAAAAAAAAA   1886
```

Fig. 1. The nucleotide sequence and predicted amino acid sequence for putative porcine estrogen sulfotransferase are shown. Two AT-rich regions and five ATTTA sequences are underlined. A potential polyadenylation signal of ACTAAA is shown in bold letters and underlined. Potential alternative polyadenylation signals of ATTAAA are shown in bold letters. Three possible N-linked glycosylation sites are indicated with an asterisk (∗).

(212 bp) [11]. Sequence identity for a small region of the 5′ UTR of porcine (1–64 bp) and bovine (129–191 bp) STE cDNAs was 84%. The 3′ UTR of the porcine STE cDNA contained a polyA tail indicating that it was complete. The sequence identity of the 3′ UTR of porcine STE with that of bovine STE was 74%. Five ATTTA sequences, a motif previously reported to increase the degradation of mRNA [18], were located in the 3′ UTR of pig STE cDNA (Fig. 1). There were several conserved regions in the 3′ UTR that were 100% identical between porcine and bovine sequences (Fig. 2), including two ATTTA sequences, a 32 and a 28 bp region.

The amino acid sequence of porcine STE was 83, 73, and 69% identical to the bovine [11], human [9], and rat [12] STE, respectively. Potential *N*-glycosylation sites occurred at amino acid residues 2, 235, and 239 (Fig. 1). Potential *N*-glycosylation sites at 235 and 239 are conserved in bovine [11] and human sequences [9], however only the potential *N*-glycosylation site at 239 is conserved in the rat [12]. Alignment of the encoded amino acid sequences of porcine, bovine, human, and rat STE indicated several highly conserved regions (Fig. 3). Region I, located near the amino terminus, contained the consensus sequence YPKSCTxW (AA 46–53). This consensus sequence is identical in the pig, bovine, and human, but contained one mismatch in the rat. Region IV, located near the carboxyl terminus, contained the consensus sequence RKGxxGDWKNxTF (AA 257–269) again with one mismatch in the rat. Finally another conserved region (AA 226–240) was located between regions III and IV. Porcine, bovine, and human sequences were identical in this region, but there was again one mismatch in the rat.

3.2. Expression of the STE gene in the endometrium

Least-square means of densitometry units (±standard error means) for STE mRNA in endometrium during the estrous cycle and pregnancy in White composite gilts are illustrated in Fig. 4. A representative autoradiograph of a Northern blot of endometrial total RNA probed with ^{32}P-labeled STE cRNA along with an ethidium bromide stained gel showing the matching 18S ribosomal RNA bands is also illustrated (Fig. 4). The size of the porcine STE mRNA, determined using 18S and 28S RNA bands as references, was approximately 2.6 kb. There were no significant differences in STE mRNA expression in the endometrium of days 10, 13, and 15 pregnant and cyclic gilts. Although STE mRNA expression on day 13 of pregnancy appeared to be elevated, it was not significantly higher than cyclic gilts. This was primarily because only one of four pregnant gilts on day 13 had elevated STE mRNA expression (not shown). STE mRNA expression in the endometrium increased significantly ($P < 0.01$) from days 15 to 20, remained elevated on day 30, and decreased significantly ($P < 0.01$) from days 30 to 40.

Analysis of real-time RT-PCR data indicated that there were significant day ($P < 0.01$) and breed ($P = 0.01$) effects. One Meishan pig on day 30 of pregnancy had an unusually high level (708 units) of STE mRNA and was eliminated from the analysis as an outlier. STE mRNA expression in the endometrium of both Meishan and White composite pigs increased ($P < 0.01$) from days 15 to 20, decreased ($P = 0.02$) between days 20 and 30, and decreased further ($P < 0.01$) from days 30 to 40 (Fig. 5). STE mRNA expression was higher in White composite pigs than Meishan pigs throughout the days measured.

```
        1                                               50 51                                        100
pSTE3   .....GAAAG TTTTTGTGGT TACATCTGAG ATTAAAGTGT T.....ATCA CTTCTTACCT CTG..TTATT TCAGACTGGT GGAGA..... .....ATGAA
bSTE3   .....gaagg ..gttttctt aacatcggag attaaattgt tctttcaccc ttcctcacct tct..tcatt ttaagctagt agata..... .....atgaa
hSTE3   gaaggtcttt ctttacttaa catatctgat attaaagatt tcttttcatt attctccact ttttcttatt ttagattgct agaaaagaca taatcatgga


        101                                             150 151                                      200
pSTE3   TCAGGCTAAA AAGATCATGG TTGAGTTCAT GTGATTTTTG AGATCTTCAT ATCAAAAACA AGAAAGAT.. ...TTTTTT.......... ..........
bSTE3   ttatac..aa aagatcatga tcaggttcac atgattattg agatcttagt atgaaagaga gatttttttc ctgtgatta.
hSTE3   ttatgttgac attttctttt taaatttttg tttaactttt tttttttttt tttgagacag agtctcactc tgttgcctag gctggaggac agtggcacaa


        201                                             250 251                                      300
pSTE3   .......ACC TTGTTATCTG TTTTAATTTT GTTTCCTTTT TTACAAATAT TGCAAAAATC TATAACCTGT GAGAATGATG TGGGAACACA CAAATTGTTC
bSTE3   .......tct ctttaatatt ttctttcctt tcttcttttaa ttataaatat tacacagagc tataacctat gagaatgatg taggtacaca caaattgttc
hSTE3   tcatggctga ttgcagcctt gacctccttg actcaattga tcctcccatc tcagcctccc aagtagctag gactacagac atgtgcaacc atgtttggct


        301                                             350 351                                      400
pSTE3   AAGTTGTTAG AGCCTCATCG AAAATAACCA AGCATTCCAA ATTCTTTAAT TGTGTGATTA CTTTTTT.CC ATTTATTGGT ACATTTAGTA TATCCAACAT
bSTE3   aagttgttag agcctcagta aaaataacca gacattccaa attatataac tttgtgtcta cttttttccc atttattagt atatttgg.. ..........
hSTE3   aattttttta atgttttttt gtagagatga ggtcttatta tattgtccag gctggtcttg aattcctggg ctcaagcttc ccaagtagct gcaacaacag


        401                                             450 451                                      500
pSTE3   ACATATATAT ACATATACAC ATAAATATAT AT.TAATAAC TGAACATGGA TTTTGAAAAC CTTGTTGGAA AAAATTAAAG AATAATTAAA TATGAGGGCA
bSTE3   .....tatat acatatacct ttaaatacat at.taataac tgtacatgga atttgaaaac cttgacaaat agaactga.. aaagaataaa catgagtgta
hSTE3   gcacacacca ccatgctcaa ctaattttat ttctatttttt tgtatagaca ggggcttgct atagtgtcca ggctggtctg aaacccttga gctcaagtga


        501                                             550 551                                      600
pSTE3   TAACTAGATC TATTATTTCC TTCCTGATAT TTTCTTTCAC CTTCTGCAAA ATGGCTTAAT TTGGAATTTT ATTTAGTCTA ATGTAAATGT GAGGCA.TAA
bSTE3   taactagatg tattatttct tggctgatat tatctttca. Ttaatgcaaa atgacttaac ttggaattta ccttagttta gtgtaaattt tcagcatttt
hSTE3   tcttcccaca ccagcctccc aaaatactgg gattacaggc ttgagcctcc atgcctggcc caggtaacat gtttattgag ctgtacatgc atatgagaaa


        601                                             650 651                                      700
pSTE3   CAGAACTGAT TTCCAGATGT CTATAAATGA TCCCAATGAG TGTTATTTAT GCATAAATAT CTCCAAATGA TTTGAAAATAT TTAT..GTTC TAGAGTTCTT
bSTE3   cagaactgat ttccagatgt ctataaataa ttccaatgag tacaatttac attttaatat ctttagctaa tttaaaatcg ttat..gctt tagggttcct
hSTE3   taagaaactt ttttttccta ctatcatctc ttaaattttg ttttcttttt cttttgcttc ctcttcttct tttctatttt ttataaatat catgcacaac


        701                                             750 751                                      800
pSTE3   TCTGTTCTAT AACAATACAA TACAATGTTA TAAATGTAAG CTCTGTAAAC TAAAGTTATT TTTAATGTAA AAAAAAAAAA AAAAA..... ..........
bSTE3   tctatcctat .........a cacaatgtaa tgaaagtaag atctgtaaag ttttaatgaa tgtgacatat accagtaaaa taaaacaaaa gca.......
hSTE3   tataacctat gggaatgatg tagtaacaca gattattcat cttgttagag ttgtattaaa aataaacaag catttcaaat taaaaaaaaa aaaaaaaaaa
```

Fig. 2. The 3′ untranslated regions (UTR) of putative estrogen sulfotransferase (STE) cDNA sequences of porcine (pSTE3), bovine (bSTE3), and human (hSTE3) are shown. The potential polyadenylation signals of ATTAAA, AATAAA, or ACTAAA are underlined. The conserved two ATTTA sequences, a 32 bp and a 28 bp region between porcine and bovine sequences are underlined in both sequences. Two AT-rich regions of porcine and human STE cDNAs, which do not align, are italicized and underlined.

```
                                                  Region I
  1                                                                                             100
p MNSSKSAYLD YFGRIHGILL YKKFIEYWND VETFEARPDD LVIVTYPKSG TTWVSEIVYM IYTEGDVEKC KEDTIFNRIP YLECRTENVM NGVKQLKQMA
b -S---PSFS- ---KLG--PM ------QFHN --E------- ---------- ---L---IC- --NN------ ---V----V- ----S--H-- K-----NE--
h ---.ELD-YE K-EEV----M --DFVK--DN --A-Q----- ---A------ ---------- --K------- ---V------ F----K--L- ------DE-N
r -ET-MPE-Y- V--DF--F-M D-R-TK--E- ----L------ -L-------- S--I----D- --K------- ---AL----- D----N-DLI --I----EKE

  101                      Region II                                                 Region III              200
p SPRIVKSHLP PELLPVSFWE KNCKIIYVCR NAKDVVVSYY YFFLMVTANP DPGSFQDFVE KFMDGEVPYG SWYKHTKSWW EKRTNPQVLF IFYEDMKENI
b ---------- VK-------- -------LS- ---------- FLI-----I- --D------- ---------- --FE------ --SK------ L---------
h ------T--- -----A---- -D-----L-- -----A--F- ------AGH- N----PE--- ---Q-Q---- -----V---- --GKS-R--- L----L--D-
r ------T--- AK---A---- -------L-- ---------- ----IMKSY- N-K--SE--- ---E-Q---- ---D-V---- --SK-SR--- M-------D-

  201              ↓                    ↓                                Region IV               295
p RKEVMRLIEF LGRKASDELV DKIIKHTSFQ EMKNNPSTNY TTLPDEVMNQ KVSAFMRKGI AGDWKNYFTV ALNEKFDIHY EQQMKGSTLK LRTEI
b -----K-L-- ---------- ---------- ---------- ---------- ---P-----D V-----H--- -------M-- ---------- F--K-
h ----IK--H- -E--P-E--- -R--H----- ---------- ------I--- -L-P------ T-----H--- -------K-- -----E---- F----
r -R--VK---- -E-DP-A--- -R--Q----- ------C--- SM--ETMIDL ---P------ V-----H-PE --R-R-EE-- Q----DCPV- F-AEL
```

Fig. 3. A comparison of the predicted amino acid sequence for putative porcine (p) STE with those of bovine (b), human (h), and rat (r) STE sequences is shown. The four highly conserved regions are underlined and the consensus sequences within these regions are indicated in bold letters. Region I is located near the amino terminus with the consensus sequence of YPKSGTxW (AA 46–53). Region II consensus sequence is RNx(K,R)DxxVSx(Y,W)x(F,L). No consensus sequence is known for region III. Region IV is located near the carboxyl terminus with the consensus sequence of RKGxxGDWKNxFT (AA 257–269). The beginning and the end of a highly conserved 15-AA region (HTSFQEMKNNPSTNY) are indicated by ↓.
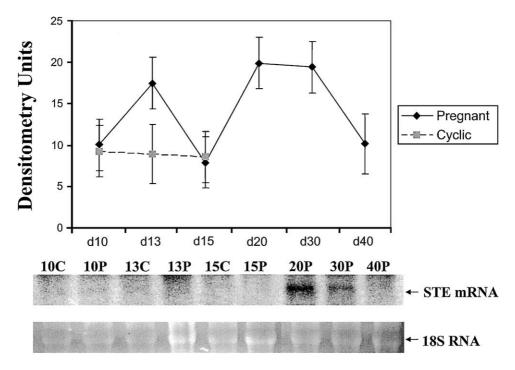
Fig. 4. Least-square means (±standard error means) of densitometry units after Northern blotting for estrogen sulfotransferase (STE) mRNA in endometrium of White composite gilts during the estrous cycle and pregnancy are illustrated. STE mRNA expression increased significantly ($P < 0.01$) during days 20–30 of pregnancy. A representative autoradiograph of a Northern blot and a photograph of an agarose gel showing the matching 18S ribosomal RNA bands are also shown. The size of the porcine STE mRNA, determined using 18S and 28S RNA bands as references, was approximately 2.6 kb. Gels were loaded with total cellular RNA (30 μg) from endometrium of cyclic (C) gilts on days 10, 13, and 15, and pregnant (P) gilts on days 10, 13, 15, 20, 30, and 40. Arrows indicate STE mRNA band and 18S ribosomal RNA bands.
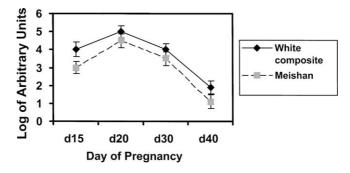
Fig. 5. Least-square means (±standard error means) of log transformed arbitrary units from real-time RT-PCR for estrogen sulfotransferase (STE) mRNA in endometrium of Meishan and White composite pigs during early pregnancy are illustrated. STE mRNA expression increased significantly ($P < 0.01$) from days 15 to 20 of pregnancy, decreased ($P = 0.02$) between days 20 to 30, and decreased ($P < 0.01$) between days 30 and 40.

3.3. Mapping of the STE gene

Amplification of the genomic DNA from the MARC pig reference population [16] using primers based on a microsatellite marker developed from a BAC containing the porcine STE gene revealed six alleles (including one null allele, not amplified) for SB71. Allele assignments were made for 130 meioses and resulted in a maximum logarithm of odds ratio (LOD) score of 36.12, based on the segregation of the alleles observed. The STE gene was assigned to chromosome 8 based on two-point analysis and mapped to position 65 cM on chromosome 8 after multi-point analysis.

4. Discussion

This is the first report of the full coding region of porcine STE cDNA. The amino acid sequence of porcine STE more closely resembles bovine STE than human or rat STE. Expression of STE mRNA in the endometrium was elevated ($P < 0.01$) on day 20 of pregnancy compared to day 15. STE mRNA expression was higher ($P < 0.01$) in White composite pigs than Meishan pigs from days 15 through 40. Finally, the STE gene was mapped to 65 cM on chromosome 8, which is within the previously identified uterine capacity QTL [8].

The porcine STE mRNA observed by Northern blotting (2.6 kb) is larger than the cDNA (1886 bp) determined by sequencing. This discrepancy could be due to either 5′ or 3′ UTR sequences that have not been identified. However, the porcine and bovine (1812 bp) STE cDNAs are similar in size and Northern blotting indicated that bovine STE mRNA was a single transcript estimated to be between 1.5–3.0 kb [11].

The porcine 5′ (273 bp) and 3′ (725 bp) UTR were similar in size to the bovine placental STE cDNA (212 and 712 bp, respectively) [11]. The lack of homology in most of the 5′ UTR (65–273 bp in the pig) between the porcine and the bovine could be due to insertions or differential splicing in either species. In the human, the 5′ and 3′ UTR sequences of liver STE cDNA [9] reportedly contain only 106 and 55 bp, respectively. Thus, the larger 5′ UTR of the porcine and bovine sequences may contain regulatory components that are lacking in the human STE cDNA sequence.

There were three potential polyadenylation signals (ATTAAA) at 1186, 1578, and 1589 bp in the pig STE cDNA sequence (Fig. 1), which suggests the possibility of alternative polyadenylation signals. The ACTAAA sequence located 15 bp upstream from the polyA tail is likely an actual polyadenylation signal for the porcine STE cDNA (Fig. 1). Potential polyadenylation signals (AATAAA and ATTAAA) are present in the bovine placental STE cDNA at 1124, 1516 and 1798 bp [11]. The first and third putative polyadenylation signals of porcine STE cDNA (Fig. 2) correspond to the positions of putative polyadenylation signals in the bovine. Previous reports suggesting different 3′ UTR for human STE support the possibility of alternative polyadenylation sites in the pig and bovine. The 3′ UTR of the human liver STE cDNA contained only 72 bp [9] with an ATTAAA sequence located 19 bp upstream of the polyA tail. This ATTAAA is conserved in porcine and bovine sequences (Fig. 2). A human STE cDNA from fetal liver and spleen (GenBank Acc. U55764) contained a partial coding sequence and an 801 bp 3′ UTR, thus both are likely products of the same gene. The overlapping portions

of the cDNAs from the two reports were identical. Furthermore, two mRNA transcripts of 1.4 and 1.8 kb were detected in the adrenal gland of guinea pig [13]. Whether alternative forms of porcine STE cDNA will be obtained from other tissues requires further investigation.

The presence of ATTTA sequences or AT-rich regions in the 3′ UTR of cDNAs can render mRNA less stable [18]. Out of five ATTTA sequences in the 3′ UTR of porcine STE cDNA, the first and the fourth ATTTA sequences were conserved between the porcine and bovine sequences (Fig. 2). A 40 bp AT-rich region (36 AT and 4 C) and a 45 bp AT-rich region (38 AT and 7 GC) are present in the 3′ UTR of porcine STE cDNA (Figs. 1 and 2). As polyadenylation at the first potential site would result in a shorter 3′ UTR lacking these sequences, the choice of polyadenylation site could alter susceptibility of STE mRNA to degradation.

The four conserved regions of sulfotransferase (ST) enzymes are present throughout phylogeny [20]. The consensus sequences YPKSGTxW (AA 46–53) of region I near the amino terminus and RKGxxGDWKNxFT (AA 257–269) of region IV near the carboxyl terminus were speculated to be involved in the binding of 3′-phosphoadenosine-5′-phosphosulfate (PAPS), a cosubstrate for the ST reaction of STE and other ST enzymes [19]. A highly conserved 35-AA region spanning from SPROV to YYFFL corresponds to region II [20] with the consensus sequence RNx(K,R)DxxVSx(Y,W)x(F,L), which is important for catalytic activity [21].

Functional significance of region III (AA 162–197) has not been well characterized. A highly conserved 15-AA region (HTSFQEMKNNPSTNY in Fig. 3) between the regions III and IV has not been reported previously. Potentially, any polymorphisms occurring within functionally important regions may affect STE activity and thus change estrogen levels in the endometrium.

Expression of STE mRNA in the endometrium of pregnant White composite pigs measured by Northern blotting increased significantly ($P < 0.01$) from days 15 to 20, remained elevated on day 30, and decreased significantly ($P < 0.01$) from days 30 to 40. Although STE mRNA appeared elevated on day 13 of pregnancy in White composite gilts (Fig. 4), STE mRNA expression was not significantly higher than in cyclic gilts. The pattern of STE mRNA expression determined by real-time RT-PCR was similar to that determined by Northern blot analysis, except that day 30 was somewhat lower than day 20 (Figs. 4 and 5). The significant breed effect ($P = 0.01$) between Meishan and White composite pigs in STE mRNA expression suggests that STE remains a strong candidate for the previously reported QTL. Polymorphisms that influence STE gene expression may influence uterine capacity, and this possibility requires further investigation.

Expression of STE mRNA from days 10 to 40 reported here are generally in agreement with the elevated STE activity previously observed during early pregnancy in sows [4], in that STE activity decreases dramatically after day 30. Our data also agree with a report showing that measurable quantities of estrone sulfate were present in the maternal plasma at day 16, but not at day 9; rose to a peak between days 23 and 30; and fell on day 46 [5]. The estrone sulfate between days 23 and 30 was thought to be synthesized and secreted by the conceptus [5]. Our data suggest that the endometrium may contribute to the rise in estrone sulfate in maternal plasma. High levels of conjugated estrogens during early pregnancy [5] may be due to high STE and low estrogen sulphatase activities in the endometrium [4]. Unconjugated estrogens subsequently rise to a peak at the time of parturition followed closely by estrone sulfate [5], which does not correlate with the low STE and high estrogen sulphatase activities in the endometrium

[4]. One function of STE in the endometrium during pregnancy may be to regulate the tissue responses to placental estrogen. The increase in STE on days 20 and 30 corresponds to the peak in placental estrogen secretion that occurs on day 30 of pregnancy [22]. It has been suggested that the dramatic changes in the secretion of proteins, such as uteroferrin and retinol binding protein [23], may be controlled by the ratio of progesterone to active estrogen present in the endometrium [24]. Thus, the increase in STE on days 20 and 30 may alter the ratio of progesterone to estrogen present in the endometrium, affecting protein secretion. The rate of protein secretion likely affects conceptus growth and development. In addition, it has also been reported that uterine blood flow increased dramatically around this time [25], suggesting that estrogens may be involved in blood flow increases. Thus, STE activity could regulate numerous aspects of uterine and conceptus physiology and thus influence uterine capacity.

In conclusion, the full coding sequence for porcine STE mRNA is reported. STE gene expression by the endometrium of pregnant gilts suggests that STE may play a role in modulating the effect of estrogen on the endometrium or other tissues during early pregnancy in the pig. The significant breed effect in STE mRNA expression between Meishan and White composite pigs suggests that they may differ in modulating the effect of estrogen on the endometrium, thus affecting uterine capacity. The position of the STE gene in the swine genome and the difference in STE gene expression in Meishan compared to White composite gilts both suggest that the STE gene is a strong candidate for the uterine capacity QTL in swine.

Acknowledgments

References

[1] Luu-The V, Bernier F, Dufort I. Steroid sulfotransferases. J Endocrinol 1996;150(Suppl):S87–97.

[2] Brooks SC, Battelli MG, Corombos JD. Endocrine steroid sulfotransferases: porcine endometrial estrogen sulfotransferase. J Steroid Biochem 1987;26:285–90.

[3] Pack BA, Brooks SC. Cyclic activity of estrogen sulfotransferase in the gilt uterus. Endocrinology 1974;95:1680–90.

[4] Dwyer RJ, Robertson HA. Oestrogen sulphatase and sulphotransferase activities in the endometrium of the sow and ewe during pregnancy. J Reprod Fertil 1980;60:187–91.

[5] Robertson HA, King GJ. Plasma concentrations of progesterone, oestrone, oestradiol-17beta and of oestrone sulphate in the pig at implantation, during pregnancy and at parturition. J Reprod Fertil 1974;40:133–41.

[6] Meyers SA, Lozon MM, Corombos JD, Saunders DE, Hunter K, Christensen C, et al. Induction of porcine uterine estrogen sulfotransferase activity by progesterone. Biol Reprod 1983;28:1119–28.

[7] Christenson RK, Leymaster KA, Young LD. Justification of unilateral hysterectomy–ovariectomy as a model to evaluate uterine capacity in swine. J Anim Sci 1987;65:738–44.

[8] Rohrer GA, Ford JJ, Wise TH, Vallet JL, Christenson RK. Identification of quantitative trait loci affecting female reproductive traits in a multigeneration Meishan–White composite swine population. J Anim Sci 1999;77:1385–91.

[9] Aksoy IA, Wood TC, Weinshilboum R. Human liver estrogen sulfotransferase: identification by cDNA cloning and expression. Biochem Biophys Res Commun 1994;200:1621–9.

[10] Bernier F, Lopez S, Labrie F, Luu-The V. Cloning and expression of cDNA encoding human placental estrogen sulfotransferase. Mol Cell Endocrinol 1994;99:R11–5.

[11] Nash AR, Glenn WK, Moore SS, Kerr J, Thompson AR, Thompson EO. Oestrogen sulfotransferase: molecular cloning and sequencing of cDNA for the bovine placental enzyme. Aust J Biol Sci 1988;41:507–16.

[12] Demyan WF, Song CS, Kim DS, Her S, Gallwitz W, Rao TR, et al. Estrogen sulfotransferase of the rat liver: complementary DNA cloning and age- and sex-specific regulation of messenger RNA. Mol Endocrinol 1992;6:589–97.

[13] Oeda T, Lee YC, Driscoll WJ, Chen HC, Strott CA. Molecular cloning and expression of a full-length complementary DNA encoding the guinea pig adrenocortical estrogen sulfotransferase. Mol Endocrinol 1992;6:1216–26.

[14] Klemcke HG, Vallet JL, Christenson RK, Pearson PL. Erythropoietin mRNA expression in pig embryos. Anim Reprod Sci 2001;6:93–108.

[15] Kim JG, Vallet JL, Christenson RK. Characterization of uterine epidermal growth factor during early pregnancy in pigs. Domest Anim Endocrinol 2001;20:253–65.

[16] Rohrer GA, Alexander LJ, Keele JW, Smith TP, Beattie CW. A microsatellite linkage map of the porcine genome. Genetics 1994;136:231–45.

[17] Green P, Falls K, Crooks S. Documentation for CRI-MAP, Version 2.4. St. Louis, MO: Washington University School of Medicine, 1990.

[18] Shaw G, Kamen R. A conserved AU sequence from the 3′ untranslated region of GM-CSF mRNA mediates selective mRNA degradation. Cell 1986;46:659–67.

[19] Weinshilboum RM, Otterness DM, Aksoy IA, Wood TC, Her C, Raftogianis RB. Sulfation and sulfotransferases 1: sulfotransferase molecular biology: cDNAs and genes. FASEB J 1997;11:3–14.

[20] Varin L, DeLuca V, Ibrahim RK, Brisson N. Molecular characterization of two plant flavonol sulfotransferases. Proc Natl Acad Sci USA 1992;89:1286–90.

[21] Marsolais F, Varin L. Recent developments in the study of the structure–function relationship of flavonol sulfotransferases. Chem Biol Interact 1998;109:117–22.

[22] Knight JW, Bazer FW, Thatcher WW, Franke DE, Wallace HD. Conceptus development in intact and unilaterally hysterectomized–ovariectomized gilts: interrelations among hormonal status, placental development, fetal fluids and fetal growth. J Anim Sci 1977;44:620–37.

[23] Vallet JL, Christenson RK. The effect of estrone and estradiol treatment on endometrial total protein, uteroferrin, and retinol-binding protein secretion during midpregnancy or midpseudopregnancy in swine. J Anim Sci 1996;74:2765–72.

[24] Knight JW, Bazer FW, Wallace HD. Hormonal regulation of porcine uterine protein secretion. J Anim Sci 1973;36:546–53.

[25] Ford SP, Stice SL. Effects of the ovary and conceptus on uterine blood flow in the pig. J Reprod Fertil Suppl 1985;3:83–90.