Molecular Cloning of cDNA for Equine Follistatin and Its Gene Expression in the Reproductive Tissues of the Mare

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ABSTRACT. A cDNA clone encoding equine follistatin was isolated from an equine ovarian cDNA library. Out of 1.2×10^5 independent clones screened, one positive clone was isolated and its cDNA sequence determined. The isolated clone, named EQ-FS-1, contained a complete open reading frame encoding 344 amino acid residues. The similarity of its deduced amino acid sequence to these of other mammalian species was greater than 95%. Although its expression level varied among the tissues examined, follistatin mRNA was detected in the equine uteroplacental tissues, follicles and corpora lutea by Northern blot analysis. *In situ* hybridization revealed that the expression of follistatin mRNA in the equine follicle was restricted exclusively to granulosa cells. When the expression pattern of follistatin mRNA in the equine uteroplacental tissues from mid- to late-pregnancy was examined, it was shown that its expression level tended to decrease after mid-pregnancy. These results suggest that follistatin acts in the reproductive tissues of the mare in maintaining pregnancy.—KEY WORDS: cDNA, equine, follicle, follistatin, placenta.

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The dimeric polypeptides, inhibin and activin, originally discovered as gonad-derived molecules, are capable of suppressing and enhancing the secretion of follicle stimulating hormone (FSH) from the anterior pituitary, respectively [5]. Inhibin is a heterodimer, composed of an α- and β-subunit (βA or βB), while activin is a homodimer of the β-subunit.

In 1987 two groups independently identified a monomeric glycoprotein capable of suppressing secretion of hypophysial FSH but different from inhibin [14, 21]. Ueno *et al.* [21] isolated the substance from porcine ovarian-follicular fluid and Robertson *et al.* [14] isolated it from bovine ovarian-follicular fluid. The substance was named follistatin [21], or FSH-suppressing protein [14]. Later, in 1990, Nakamura *et al.* [9] discovered that follistatin is an activin binding protein and found that its suppressive effect on hypophysial FSH secretion was through its binding to activin [4, 9]. It is now widely accepted that the inhibin-activin system functions in various tissues, including reproductive tissues such as ovary, testis and placenta, and that follistatin also plays important roles by modulating activin-action by its binding property [19].

In recent studies we have isolated cDNA encoding equine inhibin-activin subunits [25, 26] and shown that the mRNA encoding these subunits is expressed in the reproductive tissues of the mare and its fetus [22–24]. These results suggested that the inhibin-activin system plays role in maintaining successful pregnancy of the mare.

In the present study we have cloned a cDNA encoding equine follistatin and examined the expression of follistatin in the reproductive tissues of the mare to estimate the importance of this gene's activity in the inhibin-activin system.

MATERIALS AND METHODS

Tissues: Tissue collections were as described previously [22–24]. Uteroplacental tissues were collected from mares on days 150, 180, 210, 240 and 300 of pregnancy (the normal length of horse pregnancy is about 330 days). Follicles and corpora lutea were taken from a non-pregnant mare during the mid-luteal phase (7 days after ovulation). Adult testes were taken from a 5-year-old stallion. Fetal testes and ovaries were collected on day 210 and 240 of pregnancy, respectively. Other non-reproductive tissues were taken from a pregnant mare on day 150 of pregnancy. Tissue samples were frozen rapidly in liquid nitrogen and stored at −70°C until use.

Extraction of Poly(A)$^+$RNA: Total cellular RNA was extracted from each tissue by the cesium chloride ultracentrifugation method [15]. Poly(A)$^+$RNA was selected with Oligotex-dT30 Super (Takara, Kyoto, Japan).

Reverse transcription-polymerase chain reaction (RT-PCR): A partial fragment of equine follistatin cDNA reverse transcribed from equine pituitary RNA was amplified by PCR using a primer set (forward, 5'-GGGCAGATCCAT

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TGGATTAGCCTAT-3'; reverse, 5'-ACACTGCTGGAC AGTTTACCACTCT-3') according to a previously described method [10]. The conditions used for PCR were as follows: 1 cycle of 94°C for 10 min; 35 cycles of 94°C for 1 min, 51°C for 1 min, 72°C for 1 min; and 1 cycle of 72°C for 10 min. The amplified fragment (370 bp) was subcloned into a plasmid vector, pGEM-T (Promega, WI) for verification of its cDNA sequence. As the cDNA sequence showed a high degree of homology with that of follistatin cDNA of other species [1, 3, 16–18, 20], the amplified cDNA fragment obtained was judged to be part of equine follistatin cDNA. A rat G3PDH (glycerol-3-phosphodehydrogenase) cDNA fragment (983 bp) was also amplified by RT-PCR using the primer set from Clontech (Rat G3PDH Control Amplimer Set, Clontech, CA).

Northern blot analysis: Northern blot analyses were performed as described previously using 3 micrograms of poly(A)$^+$RNA extracted from each tissue [22–24]. The ApaI/SacI fragments from the plasmid containing partial cDNA of both equine follistatin and rat G3PDH (see above) were used as probes. They were labeled with [α-^{32}P]dCTP (3000 Ci/mmol, Amersham-Pharmacia, Tokyo, Japan) using a Megaprime DNA labeling kit (Amersham-Pharmacia). As spectrophotometrical measurement of the amount of poly(A)$^+$RNA is not necessarily accurate, for a quantitative analysis of the expression of follistatin mRNA in the uteroplacental tissues of the mare, autoradiograms of Northern blots of both follistatin mRNA and G3PDH mRNA were analyzed by quantitative densitometry of appropriately exposed films using an image scanner and NIH Image software (NIH, ver. 1.62). Data were expressed as the amount of follistatin mRNA relative to G3PDH mRNA.

In situ hybridization: The plasmid vector containing the partial equine follistatin cDNA fragment was linearlized and digoxigenin (DIG)-UTP labeled cRNA probes (antisense and sense) were synthesized using T7/SP6 RNA polymerase (DIG RNA Labeling Kit, Boehringer Mannheim, Germany). *In situ* hybridization was performed on cryostat sections of the equine follicle (5 μm thick) as described previously [22, 23].

cDNA cloning of equine follistatin: Using the [α-^{32}P]dCTP labeled partial fragment of equine follistatin cDNA as a probe, around 1.2×10^5 plaques from the equine follicle cDNA library constructed in λZAPII (Stratagene, CA) [26] were screened as described previously [25, 26].

RESULTS AND DISCUSSION

As the presence of follistatin in the pituitary has been described in the rat [4], we initially performed RT-PCR on the RNA sample from equine pituitary. The predicted size of the PCR product was 370 bp and the size of the product we obtained was in agreement with this. A DNA sequencing analysis on this product revealed that the sequence had a high degree of homology (>90%) with follistatins of other mammalian species [1, 3, 16–18, 20]. Therefore, we concluded that the fragment obtained was a partial fragment of equine follistatin cDNA.

Using this fragment as a probe, we examined the level of expression of follistatin mRNA in various equine tissues (Fig. 1). Among the tissues examined, although the levels varied, a significant signal showing the presence of follistatin mRNA was detected in the uteroplacental tissues, follicles and corpora lutea. The size of the transcript (2.7 kb) in these equine tissues was quite similar to that reported for rat follistatin (2.6 kb) [16], and suggested that the gene for follistatin is highly conserved among species. In the present study, Northern blot analysis could not detect follistatin mRNA in the pituitary despite the partial fragment of equine follistatin cDNA being initially isolated from this tissue by RT-PCR. This might be due to the lower sensitivity of the Northern blot analysis in comparison with RT-PCR.

The result obtained by *in situ* hybridization analysis

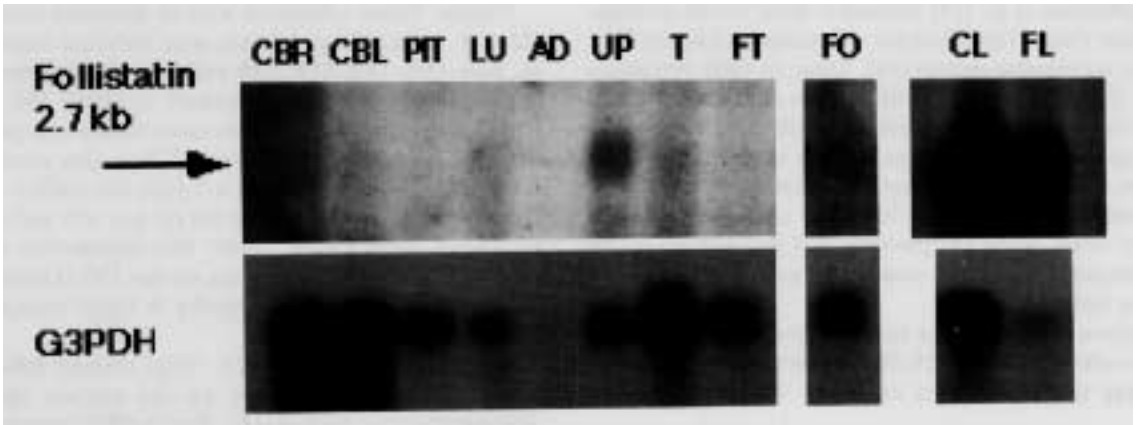

Fig. 1. Northern blot analysis of the expression of follistatin mRNA in equine tissues. Three micrograms of poly(A)$^+$RNA from each tissue was used for electrophoresis as described in Materials and Methods. A significant signal showing the presence of follistatin mRNA was detected in the uteroplacental tissues, follicles and copora lutea, and the size of the transcript in these tissues was 2.7 kb. CBR, cerebrum; CBL, cerebellum; PIT, pituitary; LU, lung; AD, adrenal; UP, uteroplacental tissue; T, testis (adult); FT, fetal testis; FL, follicle; CL, corpus luteum; and FO, fetal ovary.

investigating cellular localization of follistatin mRNA in the equine follicle indicated that follistatin mRNA was expressed exclusively in granulosa cells, with no expression in either theca and stromal cells (Fig. 2). Such an exclusive expression of follistatin mRNA in granulosa cells of mature follicles was previously described in the rat [16] and our result indicates that this is also the case in equine species.

Based on the result of *in situ* hybridization analysis, we have performed cDNA cloning to obtain the full length cDNA of equine follistatin by screening the equine follicle cDNA library [26]. Around 1.2×10^5 plaques were screened, and one positive clone was isolated. The sequence of isolated cDNA, which was 2,315 bp in length, revealed that this encodes the complete open reading frame (ORF) of equine follistatin. The restriction enzyme map and sequencing strategies for the equine follistatin cDNA (EQ-FS-1) obtained are shown in Fig. 3. The EQ-FS-1 ORF contained 1032 bp encoding 344 amino acid residues, and 153 bp and 1130 bp of 5'- and 3'-flanking regions, respectively. Two potential poly(A) signal sequences (AATAAA) were found at 1067 bp and 1086 bp downstream of the termination codon. The sequences of cDNA and its deduced amino acids are shown in Fig. 4. Esch *et al.* [2] reported the presence of two forms of precursor proteins of follistatin, consisting of 344 and 317 amino acid residues. These different forms are due to alternative splicing of follistatin mRNA, one of which encodes a follistatin precurser with 317 amino acids but without 27 amino acid residues encoded by exon 6 of the follistatin gene [2]. Although in the present study we obtained one equine follistatin cDNA encoding 344 amino acid residues, an mRNA species encoding 317 amino acid residues in equine species is still possible.

A comparison of the deduced amino acid sequence of eqine follistatin with those of six other mammals is shown in Fig. 5. In the deduced amino acid sequence of equine follistatin, the signal peptide of 29 amino acids (Fig. 5, Met^{-29} to Ala^{-1}) and its cleavage site (Fig. 5, Ala^{-1}-Gly^{1}) were found as are seen in the sequences of follistatin of the other species [1, 3, 16–18, 20]. The potential N-glycosylation sites ($Asn^{95, 259}$), the highly acidic region (Glu^{292}-Asp^{304}), the heparin-binding basic region (Lys^{75}-Lys^{86}) and the

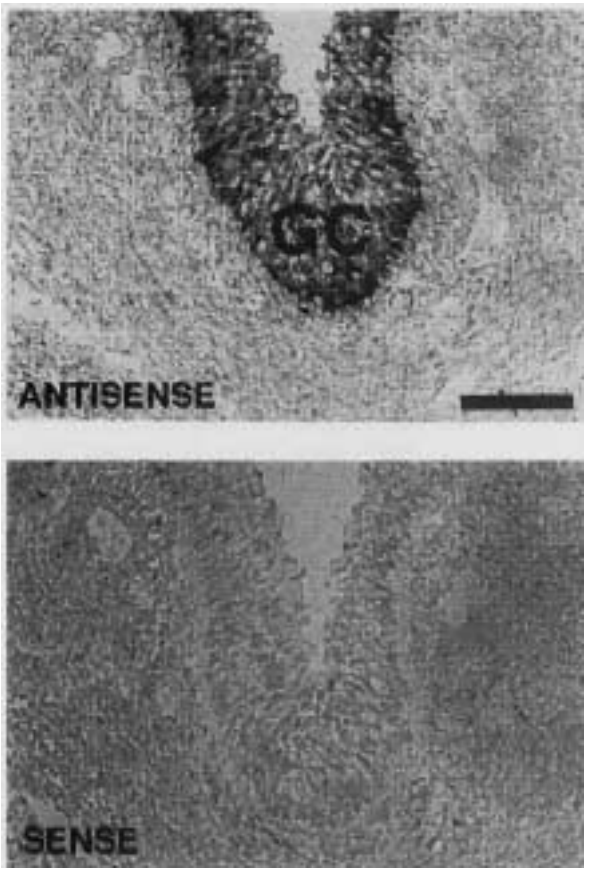

Fig. 2. *In situ* hybridization of follistatin mRNA in the equine follicle. Digoxigenin-labeled antisense cRNA probe specific for equine follistatin mRNA and the control probe (sense probe) were used. Only granulosa cells were positive for follistatin mRNA. GC, granulosa cells. Magnification, × 300.

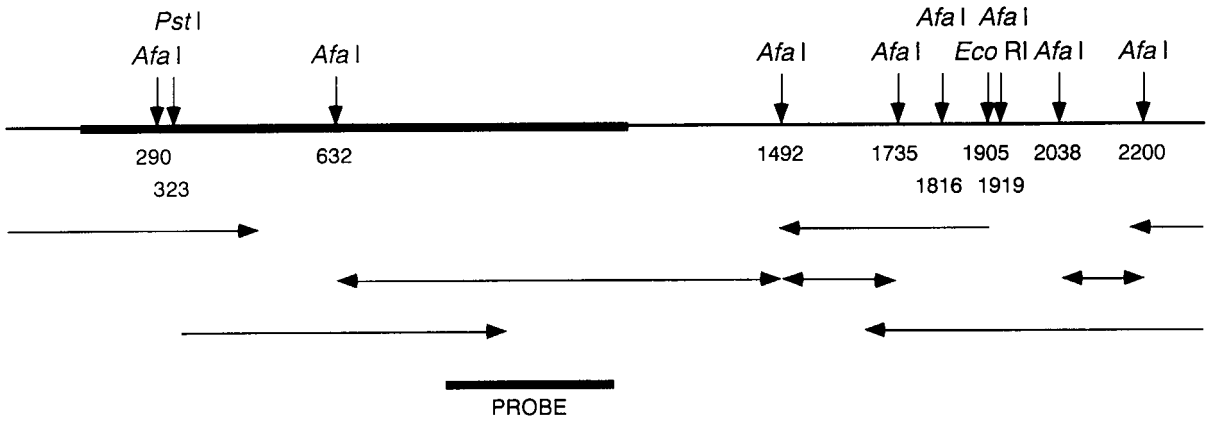

Fig. 3. Structure and sequencing strategies for equine follistatin cDNA (EQ-FS-1) clone. The dark box indicates the open reading frame. The restriction sites and the position corresponding to the probe used are shown.

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   1 TCGCGCGGGCGGCCGGCGGCGAGCGCGGGGCTGCGCCGGGATCGCTGCGCCCTCAGCCGC
  61 TCGCCTCTGCGACGCGCGCCGCTCGCCAGAGCCACCCGCCGCCGCGCCCGCTCCCCGCGC
 121 CGCCGCGCTCCTCGCCCCGCGCCTGCCCCCAGGATGGTCCGTCCCAGGCACCAGCCCGGC
                                    MetValArgProArgHisGlnProGly    -21
 181 GGGCTTTGCCTCCTGCTGCTGCTGCTCTGCCAGTTCATGGAGGACCGCAGCGCCCAGGCT
     GlyLeuCysLeuLeuLeuLeuLeuLeuCysGlnPheMetGluAspArgSerAlaGlnAla    -1
 241 GGGAATTGCTGGCTCCGCCAAGCGAAGAACGGCCGCTGCCAGGTCCTGTACAAGACAGAA
     GlyAsnCysTrpLeuArgGlnAlaLysAsnGlyArgCysGlnValLeuTyrLysThrGlu    20
 301 CTGAGCAAGGAGGAGTGCTGCAGCACCGGCCGCCTGAGCACCTCGTGGACCGAGGAGGAC
     LeuSerLysGluGluCysCysSerThrGlyArgLeuSerThrSerTrpThrGluGluAsp    40
 361 GTCAATGATAACACGCTCTTCAAGTGGATGATTTTCAACGGGGGCGCCCCCAACTGCATC
     ValAsnAspAsnThrLeuPheLysTrpMetIlePheAsnGlyGlyAlaProAsnCysIle    60
 421 CCCTGTAAAGAAACGTGCGACAACGTGGACTGTGGACCCGGGAAAAAATGCCGAATGAAC
     ProCysLysGluThrCysAspAsnValAspCysGlyProGlyLysLysCysArgMetAsn    80
 481 AAGAAGAATAAGCCCCGCTGCGTCTGTGCCCCAGATTGTTCCAATATCACCTGGAAAGGC
     LysLysAsnLysProArgCysValCysAlaProAspCysSerAsnIleThrTrpLysGly   100
 541 CCAGTCTGTGGGCTGGACGGGAAAACCTACCGCAACGAATGTGCGCTCCTCAAGGCCAGA
     ProValCysGlyLeuAspGlyLysThrTyrArgAsnGluCysAlaLeuLeuLysAlaArg   120
 601 TGTAAAGAGCAGCCAGAACTGGAGGTCCAGTACCAGGGCAAATGTAAAAAGACCTGCCGG
     CysLysGluGlnProGluLeuGluValGlnTyrGlnGlyLysCysLysLysThrCysArg   140
 661 GATGTTAACTGTCCAGGCAGCTCCACGTGCGTGGTGGACCAGACAAATAATGCCTACTGT
     AspValAsnCysProGlySerSerThrCysValValAspGlnThrAsnAsnAlaTyrCys   160
 721 GTGACATGTAACCGGATTTGCCCAGAGCCCACCTCCTCTGAACAGTATCTCTGTGGGAAT
     ValThrCysAsnArgIleCysProGluProThrSerSerGluGlnTyrLeuCysGlyAsn   180
 781 GATGGAGTGACCTACTCCAGTGCCTGTCACCTGAGAAAGGCTACCTGCCTGCTGGGCAGA
     AspGlyValThrTyrSerSerAlaCysHisLeuArgLysAlaThrCysLeuLeuGlyArg   200
 841 TCGATTGGATTAGCCTATGAGGGAAAGTGTATCAAAGCAAAGTCCTGTGAAGATATCCAG
     SerIleGlyLeuAlaTyrGluGlyLysCysIleLysAlaLysSerCysGluAspIleGln   220
 901 TGCACTGGTGGAAAAAAATGTTTATGGGATTTCAAGGTTGGCAGAGGCCGGTGCTCCCTC
     CysThrGlyGlyLysLysCysLeuTrpAspPheLysValGlyArgGlyArgCysSerLeu   240
 961 TGTGATGAGCTGTGCCCTGACAGTAAGTCTGAGGAGCCTGTCTGTGCCAGTGACAATGCC
     CysAspGluLeuCysProAspSerLysSerGluGluProValCysAlaSerAspAsnAla   260
1021 ACTTACGCCAGCGAGTGTGCCATGAAGGAAGCTGCCTGCTCCTCAGGTGTGCTGCTGGAA
     ThrTyrAlaSerGluCysAlaMetLysGluAlaAlaCysSerSerGlyValLeuLeuGlu   280
1081 GTAAAGCATTCCGGATCTTGCAACTCCATTTCGGAAGACACCGAGGAAGAGGAGGAAGAT
     ValLysHisSerGlySerCysAsnSerIleSerGluAspThrGluGluGluGluGluAsp   300
1141 GAAGACCAGGACTACAGCTTTCCTATATCTTCCATTCTAGAGTGGTAAACCCTCCGCCCA
     GluAspGlnAspTyrSerPheProIleSerSerIleLeuGluTrp***               315
1201 TGTTCAGTGTTGATATAGCCTTTGGGCAGAAAAAAAAAAAAAAAACAGGAAAGAAAAGAAA
1261 AAATATATTGTCCATACTGTAAATAAGTGTATGCTTATTTATTTGGGGGGAAAAGTATAC
1321 ATAAAGGACCTTTGTCCTAAAGCTGTCTCCCAGGCCACCTTGTTACTCATTGGACCCGGA
1381 GAGGCGGTCATTGTGAGGTCTACTGGATGAGGCCCATAGTTGAGAATTGTAGACATTTAT
1441 TTATACTGTGTCATGTTTTATAATTTATACATAAAATGTCTGGTTGACTGTACACCTTGT
1501 TTTTGAAGAAATTTATTCGTGAAAGGAAGAGCAGTTGTTATTTATTGTGAGGTCTCTTGC
1561 TTGTAAAGTAAAAGCTTTTTTTTTCCTTGTAAACCATTTAAGTCCATTCCTTACTATTGA
1621 CTCACTCATCTGTCTCCCTTCATTTCACTGTTGTTAGACTCTTTTCCACTTTAACAAACT
1681 TGCATGTCAGTTTCTGTCATGTTTATTTATTGGATTCTCTGCTGCCTGTTCTGTACATAC
1741 ATGATCCCTCGGGTTTTGTTTACAAGGAATCTTGACTGACCAAAAGGCATTATAACTCTG
1801 ACTTAAATAGAAGGTACAGAGAATACATCTTGGGGGAAACTCCTATTTCAGTTCTCATGT
1861 CGTGACAAAGACTTCTGTGAGAGAGGGGGTGATAGCAATGTTAGAATTCTAGTAACGTAC
1921 CTTCAAGATGTTACAGATCCAAAGAGATTGAGTGATATGGGTGTCGGGAGACTGAAGGGA
1981 GCAGAATGCTACACTTTCAGCCTTTCATTAGGTGTTTCCTTTAAGCTAGTCTGCTGTACC
2041 TTTTAAATCAGTTCTTTTTCAACCAGTGTGTCACTAAAAGTTATATCAAAGCTTTATCAG
2101 TTCAAGTTTCTTGCTTTTCATAATACTTTTTTCTGATGCAATTTTATATTTTCAAACATG
2161 GCAAGTTAAATATAAATTCATTTAAATATATAGTTTTGTACTTTTCTACCATGTAAATGT
2221 GCAATGTATATAAAAGTTATAATGTGTATTTGTAAATAAATGATGAGTGAAAAAATAAAA
2281 AAATTTTAAAAAGCCAAAAAAAAAAAAAAAAAAAA
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Fig. 4. Sequences of nucleotides and deduced amino acids of equine follistatin cDNA. The deduced amino acid sequence is shown under the nucleotide sequence. Nucleotides and amino acids are numbered on the left and right of the lines, respectively. Amino acids are numbered from the signal peptide cleavage site (Ala^{-1}-Gly^{1}, see text). The potential N-glycosylation sites ($Asn^{95, 259}$), highly acidic region (Glu^{292}-Asp^{304}), heparin binding basic region (Lys^{75}-Lys^{86}) and two poly(A) signal sequences are double-underlined, italicized, dotted-underlined and underlined, respectively.

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                -20        -10        -1         10         20
EQUINE      MVRPRHQPGG LCLLLLLLCQ FMEDRSAQA GNCWLRQAKN GRCQVLYKTEL
BOVINE      MARPRHQPGG LCLLLLLLCQ FMEDRSAQA GNCWLRQAKN GRCQVLYKTEL
OVINE       .....PGG VCLLLLLLCQ FMEDRSAQA GNCWLRQAKN GRCQVLYKTEL
PORCINE     MVRPKHQPGG LCLLLLLLCQ FMEDRSAQA GNCWLRQAKN GRCQVLYKTEL
HUMAN       MVRARHQPGG LCLLLLLLCQ FMEDRSAQA GNCWLRQAKN GRCQVLYKTEL
MOUSE       MVCARHQPGG LCLLLLLLCQ FMEDRSAQA GNCWLRQAKN GRCQVLYKTEL
RAT         MVCARHQPGG LCLLLLLLCQ FMEDRSAQA GNCWLRQAKN GRCQVLYKTEL

                30         40         50         60         70
EQUINE      LSKEECCSTG RLSTSWTEED VNDNTLFKWM IFNGGAPNCI PCKETCDNVD
BOVINE      LSKEECCSTG RLSTSWTEED VNDNTLFKWM IFNGGAPNCI PCKETCENVD
OVINE       LSKEECCSTG RLSTSWTEED VNDNTLFKWM IFNGGAPNCI PCKETCENVD
PORCINE     LSKEECCSTG RLSTSWTEED VNDNTLFKWM IFNGGAPNCI PCKETCENVD
HUMAN       LSKEECCSTG RLSTSWTEED VNDNTLFKWM IFNGGAPNCI PCKETCENVD
MOUSE       LSKEECCSTG RLSTSWTEED VNDNTLFKWM IFNGGAPNCI PCKETCENVD
RAT         LSKEECCSTG RLSTSWTEED VNDNTLFKWM IFNGGAPNCI PCKETCENVD

                80         90         100        110        120
EQUINE      CGPGKKCRMN KKNKPRCVCA PDCSNITWKG PVCGLDGKTY RNECALLKAR
BOVINE      CGPGKKCRMN KKNKPRCVCA PDCSNITWKG PVCGLDGKTY RNECALLKAR
OVINE       CGPGKKCRMN KKNKPRCVCA PDCSNITWKG PVCGLDGKTY RNECALLKAR
PORCINE     CGPGKKCRMN KKNKPRCVCA PDCSNITWKG PVCGLDGKTY RNECALLKAR
HUMAN       CGPGKKCRMN KKNKPRCVCA PDCSNITWKG PVCGLDGKTY RNECALLKAR
MOUSE       CGPGKKCRMN KKNKPRCVCA PDCSNITWKG PVCGLDGKTY RNECALLKAR
RAT         CGPGKKCRMN KKNKPRCVCA PDCSNITWKG PVCGLDGKTY RNECALLKAR

                130        140        150        160        170
EQUINE      CKEQPELEVQ YQGKCKKTCR DVNCPGSSTC VVDQTNNAYC VTCNRICPEP
BOVINE      CKEQPELEVQ YQGKCKKTCR DVFCPGSSTC VVDQTNNAYC VTCNRICPEP
OVINE       CKEQPELEVQ YQGKCKKTCR DVFCPGSSTC VVDQTNNAYC VTCNRICPEP
PORCINE     CKEQPELEVQ YQGKCKKTCR DVFCPGSSTC VVDQTNNAYC VTCNRICPEP
HUMAN       CKEQPELEVQ YQGRCKKTCR DVFCPGSSTC VVDQTNNAYC VTCNRICPEP
MOUSE       CKEQPELEVQ YQGKCKKTCR DVFCPGSSTC VVDQTNNAYC VTCNRICPEP
RAT         CKEQPELEVQ YQGKCKKTCR DVFCPGSSTC VVDQTNNAYC VTCNRICPEP

                180        190        200        210        220
EQUINE      TSSEQYLCGN DGVTYSSACH LRKATCLLGR SIGLAYEGKC IKAKSCEDIQ
BOVINE      TSSEQYLCGN DGVTYPSACH LRKATCLLGR SIGLAYEGKC IKAKSCDDIQ
OVINE       TSSEQYLCGN DGVTYPSACH LRKATCLLGR SIGLAYEGKC IKAKSCEDIQ
PORCINE     TSSEQYLCGN DGVTYSSACH LRKATCLLGR SIGLAYEGKC IKAKSCEDIQ
HUMAN       ASSEQYLCGN DGVTYSSACH LRKATCLLGR SIGLAYEGKC IKAKSCEDIQ
MOUSE       SSSEQYLCGN DGVTYSSACH LRKATCLLGR SIGLAYEGKC I-TKSCEDIQ
RAT         SSSEQSLCGN DGVTYSSACH LRKATCLLGR SIGLAYEGKC IKAKSCEDIQ

                230        240        250        260        270
EQUINE      CTGGKKCLWD FKVGRGRCSL CDELCPDSKS EEPVCASDNA TYASECAMKE
BOVINE      CTGGKKCLWD FKVGRGRCSL CGELCPESKS EEPVCASDNA TYASECAMKE
OVINE       CTGGKKCLWD FKVGRGRCSL CGELCPESKS EEPVCASDNA TYASECAMKE
PORCINE     CTGGKKCLWD FKVGRGRCSL CDELCPESKS EEPVCASDNA TYASECAMKE
HUMAN       CTGGKKCLWD FKVGRGRCSL CDELCPDSKS DEPVCASDNA TYASECAMKE
MOUSE       CGGGKKCLWD SKVGRGRCSL CDELCPDSKS DEPVCASDNA TYASECAMKE
RAT         CGGGKKCLWD FKVGRGRCSL CDELCPDSKS DEPVCASDNA TYASECAMKE

                280        290        300        310        320
EQUINE      AACSSGVLLE VKHSGSCNSI SEDTEEEEED EDQDYSFPIS SILEW*....
BOVINE      AACSSGVLLE VKHSGSCNSI SEDTEDEEED EDQDYSFPIS SILEW*....
OVINE       AACSSGVLLE VKHSGSCNSI SEDTEDEEED EDQDYSFPIS SILEW*....
PORCINE     AACSSGVLLE VKHSGSCNSI SEDTEEEEED EDQDYSFPIS SILEW*....
HUMAN       AACSSGVLLE VKHSGSCNSI SEDTEEEEED EDQDYSFPIS SILEW*....
MOUSE       AACSSGVLLE VKHSGSCNSI SEETEEEEEE EDQDYSFPIS SILEW*....
RAT         AACSSGVLLE VKHSGSCNSI SEETEEEEEE EDQDYSFPIS STLEW*....
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Fig. 5. Comparison of deduced amino acid sequences of equine follistatin with those of seven mammalian species. The sequences shown are those for bovine [3], ovine [20], porcine [18], human [17], mouse [1] and rat [16].

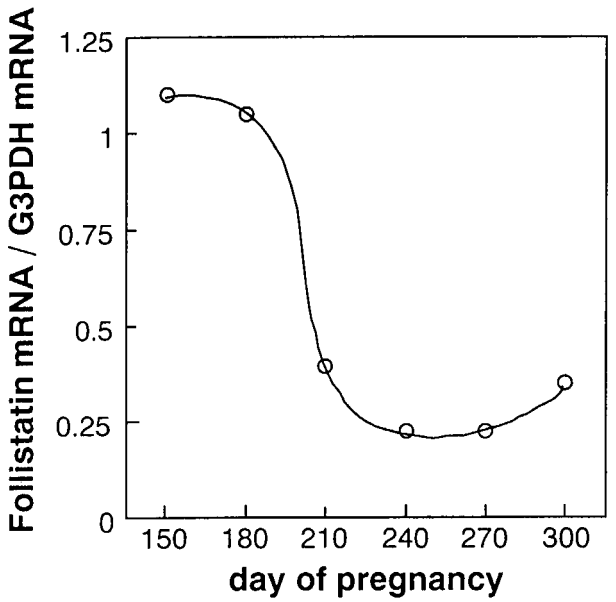

Fig. 6. Changes in the expression level of follistatin mRNA in uteroplacental tissues of the mare during the mid to late stages of pregnancy determined by Northern blot analysis (see Results and Discussion).

positions of 36 Cys residues involved in intra-molecular disulfide bonds were also conserved.

We have previously shown that inhibin-activin βA-subunit mRNA is strongly expressed in the uteroplacental tissue of the pregnant mare whereas no expression of α-subunit mRNA was detected [22]. The cellular localization of βA-subunit mRNA in the equine uteroplacental tissues was quite different from both the rat [6, 13] and human [6, 11–12] — expression in the equine uteroplacental tissue was identified in the endometrial glands but not in the trophoblasts [22]. The absence of α-subunit mRNA in the uteroplacental tissue of the mare suggests that activin rather than inhibin is produced predominantly in this tissue and that the activin produced may have roles in maintaining pregnancy. Michel *et al.* [8], who examined the distribution of follistatin mRNA in various mammalian tissues, found that most tissues expressing follistatin mRNA overlaps with the tissues producing activin [7]. This suggested that follistatin plays roles as a local modulator of activin action in these tissues and led us to examine the expression pattern of follistatin mRNA in the uteroplacental tissue of the mare and compare it with that of βA-subunit mRNA. As shown in Fig. 6, the level of expression of follistatin mRNA was relatively high on days 150 and 180 of pregnancy (second trimester) and thereafter declined to a lower level on days 210 to 300 (third trimester). Although no statistical analysis was performed due to limited sample availability, it is worth noting that the changing pattern in the expression level of follistatin mRNA was quite similar to that of βA-subunit mRNA, as reported previously [22], suggesting that follistatin acts by modulating activin action in the uteroplacental tissues of the mare. A preliminary investigation employing *in situ* hybridization failed to localize the expression of follistatin mRNA in the uteroplacental tissue of the mare. This may be related to the low sensitivity of this detection method.

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