—Original—

Cloning of the Elk Common Glycoprotein α -subunit and the FSH and LH β -subunit cDNAs

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Abstract. We report the nucleotide and deduced amino acid sequences of the pituitary glycoprotein hormone common α -subunit, and the FSH and LH β -subunits of the elk (*Cervus elaphus*). These sequences were obtained by RT-PCR of pituitary gland polyadenylated RNA. A partial genomic fragment of the elk follicle stimulating hormone β -subunit was also amplified from genomic DNA and sequenced. These sequences show high similarity to other related domestic species, including sika deer, ovine, bovine, porcine and equine. The presently reported cDNAs were used as probes for Northern analysis of pituitary gland and muscle samples from elk and sheep. Mature mRNA transcripts of approximately 700, 1700 and 550 bases were expressed in the pituitary gland but not in muscle tissue, for the glycoprotein hormone common α -subunit and FSH and LH β -subunits, respectively. These observations are consistent with reports for other related species. The genomic fragment of elk FSH β -subunit revealed an intronic microsatellite of 6 CT repeats, which differs from the 19 CT repeats documented for the sheep genomic sequence. We propose that differences in the CT repeats of the elk FSH β -subunit gene may be used as a marker for elk parentage testing. The elk DNA sequences presently reported represent a unique addition to the growing collection of the pituitary hormone genes for various vertebrates, which will facilitate evolutionary and phylogenetic studies of the gonadotropin genes. In addition, this information and the cloned cDNAs will be useful for studying seasonal expression of the elk pituitary glycoprotein genes.

Key words: Elk, Cervids, FSH, LH, Gene cloning

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Follicle stimulating hormone (FSH) and luteinizing hormone (LH) are structurally related heterodimeric glycoproteins, composed of two subunits, α and β . The α -subunit is common for both hormones, while the β -subunit is hormone-specific [1]. FSH and LH are produced by the gonadotrope cells of the pituitary gland and are essential for ovarian follicular development, maturation of the oocyte, steroidogenesis and ovulation. In males, LH is involved in regulation of

steroidogenesis in the Leydig cells, whereas FSH regulates spermatogenesis by binding to receptors in the Sertoli cells [2]. The gene and protein structures of the pituitary glycoprotein hormones of a variety of vertebrates including, mammals, birds, amphibians and fish have been reported over recent years [3]. This information has been useful for the isolation and analysis of these genes in different species, identification and quantification of their mRNA and production of biologically active recombinant hormones. Analysis of the nucleotide and predicted amino acid sequences of the pituitary glycoproteins also provides further

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insight into the evolution of this family of hormones [4]. We are interested in studying expression of the gonadotropin genes in the North American elk, or wapiti (*Cervus elaphus*). The elk is a temperate seasonal breeder related to the Japanese water deer, or sika deer (*Cervus nippon*). The only information on cervid pituitary gene structures is the recently reported sika deer common α -subunit [5] and FSH β -subunit [6] and our recently reported elk pre-prolactin cDNA [7]. We presently report the cloning and expression of the elk pituitary common glycoprotein α -subunit and the FSH and LH β -subunit cDNAs and their deduced amino acid sequences. Overall, this work is aimed at generating information on the structure of the gonadotropin genes and developing the reagents for studying the molecular basis of the seasonal reproductive events in elk.

Materials and Methods

Reagents

TRI[®] Reagent, general chemicals, antibiotics and reagents for RNA electrophoresis, were purchased from Sigma (St. Louis, MO). Oligotex[®] and QiaexII[®] kits were purchased from Qiagen (Mississauga, ON). The FirstChoice[®] RLM-RACE kit was purchased from Ambion (Austin, TX). Hybond-H[®] nylon membranes were purchased from Amersham Pharmacia Biotech (Piscataway, NJ). [α -³²P]-dCTP was purchased from New England Nuclear (Boston, MA). Agarose, X-gal (5-bromo-4-chloro-3-indolyl-B-D-galactoside), Moloney murine leukemia virus reverse transcriptase (MMLV-RT), custom primers and EcoRI were purchased from GibcoBRL (Burlington, ON). *Taq* DNA polymerase (catalogue #18038-018), 10X PCR buffer, dNTPs and the TOPO TA[®] kit were purchased from Invitrogen (Carlsbad, CA).

Purification and Northern blot analysis of mRNA

Total RNA was isolated from pituitary glands of three female elk and one female sheep. Total RNA was also isolated from female elk muscle. Tissue samples were collected immediately after death and stored at -80 C until processed. Then samples were homogenized in TRI[®] Reagent with a Polytron[®] tissue homogenizer (Brinkmann, Mississauga, ON). Total RNA was extracted

following the manufacture's protocol and dissolved in a solution containing 0.5% SDS and 20 mM EDTA. Ten μ g of total RNA were size-fractionated by electrophoresis in an agarose/formaldehyde gel, transferred to a nylon membrane by capillary blotting and cross-linked using a UV Stratalinker 1800[®] (Stratagene, La Jolla, CA). The cDNA probes were labelled by primer extension with [α -³²P]-dCTP (50 μ Ci). Northern blot membranes were hybridized and autoradiographed as previously described [8].

Synthesis, cloning and sequencing of the pituitary gonadotropin cDNAs

Polyadenylated mRNA was obtained using the Oligotex[®] kit following the manufacture's protocol. The common α -subunit cDNA was amplified by polymerase chain reaction (PCR), using cDNA synthesised by RNA ligation mediated-rapid amplification of cDNA ends (RLM-RACE). The FSH and LH β -subunit cDNAs were amplified by PCR, using first strand cDNA synthesised with a chimeric oligo (dT) adapter primer and MMLV-RT. The custom primers were designed based on sequence homology to GenBank sequences, shown in Table 1. Genomic PCR was performed on white blood cell DNA extracted from whole blood samples following standard procedures [9].

The PCR mixture included 2 μ l of RT-cDNA, 5 μ l of the 10X PCR buffer, 4 μ l of 2.5 mM dNTP, 2.5 μ l of 50 mM MgCl₂, 50 pmol of reverse and forward primers, and 1.5 units of *Taq* DNA polymerase. The cycling condition were (1) 94 C for 3 min, (2) 94 C for 1 min, (3) 65 C for 1 min, (4) 72 C for 1.2 min, (5) 30 cycles of steps 2, 4, and (6) a terminal extension step at 72 C for 7 min. The amplified products were separated on a 1% agarose gel. The resulting band was excised from the gel and purified using the Qiaex II[®] kit, following the manufacturer's protocol and cloned into the TOPO TA[®] vector. Both strands of the isolated fragment were sequenced with vector-specific universal forward and reverse primers using a fluorescent automated sequencer (National Research Council, Plant Biotechnology Institute, Saskatoon, SK). Cloned cDNA were analysed and compared with sequences available in GenBank using the Clustal W alignment and ExPASy translation public domain computer software (<http://clustalw.genome.jp> and <http://ca.expasy.org>).

Results

Cloning of the pituitary glycoprotein common α -subunit cDNA

A 24 oligonucleotide forward primer was designed containing the putative common glycoprotein α -subunit start codon (Table 1) based on the reported ovine cDNA nucleotide sequence [10]. Using this primer, and the 3' RACE reverse primer provided by the manufacturer, a DNA fragment of 691 bp was amplified (Fig. 1). Sequencing of clones from three independent PCR revealed high similarity, between 85 and 95%, to the glycoprotein common α -subunit cDNA of other domestic species (Table 2). Comparison analysis suggests that 363 bp of this fragment represent the entire coding region, and 309 bp represent the 3' untranslated region (UTR) including the polyadenylated tail of the mRNA. A deduced 120 amino acid peptide chain would be encoded by this nucleotide sequence (Fig. 1). The deduced amino acid sequence similarity between elk and a sub-set of related species is shown in Table 3.

Cloning of the pituitary FSH β -subunit cDNA

A 22 oligonucleotide forward primer, ending 5 bases upstream of the putative start codon, named forward FSH β -subunit (Table 1), was designed based on the reported ovine FSH β -subunit cDNA [11]. A 21 oligonucleotide reverse primer, named reverse FSH β -subunit primer 1, was designed to amplify within the putative coding region of the

reported ovine cDNA (Table 1). With this set of primers, a fragment of 398 bp was amplified. Sequencing analyses revealed high similarity to FSH β -subunit cDNA of sheep and other domestic species. We considered this to be a truncated cDNA, since the sequence ended 36 bp before the putative stop codon of the sheep FSH β -subunit cDNA. Therefore, to gain additional information on the last part of the elk FSH β -subunit gene-coding region, a second set of primers were designed based on sheep genomic sequences [12]. A 23 oligonucleotide forward primer was designed based on intronic sequences located upstream of the second exon, and a 25 oligonucleotide reverse genomic primer was designed based on sequences located in the 3' UTR of the gene, named genomic forward FSH β -subunit and genomic reverse FSH β -subunit, respectively (Table 1). Using this second set of primers we amplified a DNA fragment of 397 bp that included genomic sequences, containing 6 intronic CT microsatellite repeats and the last 237 bp of the coding region. Based on this information, a 26 oligonucleotide reverse primer was designed, named FSH β -subunit reverse primer 2 (Table 1), which was used with the forward FSH β -subunit primer. A DNA fragment of 414 bp was amplified (Fig. 3), which demonstrated high similarity, between 88 and 93%, to the FSH β -subunit cDNA of other domestic species (Table 2). This fragment included 390 bp, representing the entire coding region, excluding the 5' and the 3' UTR of the FSH β -subunit gene. Four PCR fragments were

Table 1. Oligonucleotide primers used for amplification of the pituitary glycoprotein cDNAs

Primer	Sequence
Forward α -subunit	5' AGGCAGAGGACGAAGAGCCATGGA 3'
Reverse 3'RACE	5' CGCGGATCCGAATTAATACGACTCACTATAGG 3'
Forward FSH β -subunit	5' AGCATCCACAGTTACCAAGTGC 3'
Reverse FSH β -subunit primer 1	5' CTGAAGGAGCAGTAGCTGGGC 3'
Genomic Forward FSH β -subunit	5' AGCAGTATTCAATCCCTGTCTCA 3'
Genomic Reverse FSH β -subunit	5' CATGTACACACAGACAGCTTGGATG 3'
Forward FSH β -subunit	5' AGCATCCACAGTTACCAAGTGC 3'
Reverse FSH β -subunit primer 2	5' TTACTCTCTGACGTCGCTGAAGGAGC 3'
Forward LH β -subunit	5' GGTACCAGGCACCAAGGATGGAG 3'
Reverse LH β -subunit	5' GGTACCAGGCACCAAGGATGGAG 3'

The forward α -subunit primer was designed based on the NCBI X16977.1, ovine gonadotropin α -subunit cDNA sequence. The forward and reverse FSH β -subunit primer 1 were designed based on the NCBI X15493.1, ovine FSH β -subunit cDNA sequence. The genomic forward and reverse FSH β -subunit primers were designed based on the NCBI S64745, ovine FSH β genomic sequence. The reverse FSH β -subunit primer 2 was designed based on the presently reported elk FSH β genomic sequence. The forward and reverse LH β -subunit primers were designed based on the NCBI S64695.1, ovine LH β -subunit cDNA sequence.

-19	AGGCAGAGGACGAAGAGCC																ATG	GAT	TAC	TGC	AGA	AAA	TAT	GCA	GCT	GTC	ATT	CTG	36
-24																	M	D	Y	C	R	K	Y	A	A	V	I	L	-13
37	GCC	GTA	TTG	TCT	CTG	TTT	CTG	CAG	ATT	CTC	CAT	TCC	TTT	CCT	GAT	GGA	GAG	87											
-12	A	V	L	S	L	F	L	Q	I	L	H	S	F	P	D	G	E	5											
	-1 /+1																												
88	TTT	ATG	ATG	CAG	GGT	TGT	CCT	GAA	TGC	AAG	CTA	AAG	GAA	AAC	AAA	TAC	TTC	138											
6	F	M	M	Q	G	C	P	E	C	K	L	K	E	N	K	Y	F	22											
139	TCC	AAG	CCA	GAC	GCT	CCA	ATC	TAT	CAG	TGC	ATG	GGC	TGC	TGC	TTC	TCC	AGG	189											
23	S	K	P	D	A	P	I	Y	Q	C	M	G	C	C	F	S	R	39											
190	GCA	TAC	CCC	ACT	CCA	GCG	AGG	TCT	AAG	AAG	ACA	ATG	TTG	GTC	CCC	AAG	AAC	240											
40	A	Y	P	T	P	A	R	S	K	K	T	M	L	V	P	K	N	56											
241	ATC	ACC	TCG	GAA	GCC	ACA	TGC	TGT	GTG	GCC	AAA	GCG	TTT	ACC	AAG	GCC	ACA	291											
57	I	T	S	E	A	T	C	C	V	A	K	A	F	T	K	A	T	73											
292	GTG	ATG	GGA	AAC	GTC	AGA	GTG	GAG	AAC	CAC	ACC	GAC	TGC	CAC	TGC	AGT	ACT	342											
74	V	M	G	N	V	R	V	E	N	H	T	D	C	H	C	S	T	90											
343	TGT	TAT	TAT	CAC	AAA	TCT	TAG	AGGGTTT	GCAATGGGCCGTGTTGGT	GATGGCTGATTTGC	393																		
91	C	Y	Y	H	K	S	Stop											96											
394	CCAAAAGGAAAGCTAATTTGTCCAGTGTCTACGGCTTTGTGAGATAAAACCCTCCTTCTCCTTGCCA																	460											
461	TACCGCTTTTAACCTGCTTTGAGAATGTACTGCAGCTTCATTGCTTTTCTCCTTGCCCTACAATATA																	527											
528	ATCAGCAGCCTCGATCTTTTTCATTGGAATGAAATATGGCATTAGCATGACCATAAAAAGCTGGTT																	594											
595	CCACTGGAAATAAAGTCTTTTAAATCATCAAAAAAAAAAAAAACCTATAGTGAGTCGTATTAATTCGGA																	661											
662	TCCGCGAAGGG																	672											

Fig. 1. cDNA nucleotide and deduced amino acid sequences of the elk common glycoprotein α -subunit. The nucleotides of the 5' UTR are designated with negative numbers and the first nucleotide of the ATG translational start codon as +1. The forward custom primer was designed from nucleotide positions -19 to +5, overlapping the first two codons from the translational start site. The primers sequences are shown in bold. The polyadenylation signal is underlined. The end of the signal peptide and the beginning of the mature protein amino acid sequence is designated as -1/+1 boundary. The proposed leader sequence is 24 amino acids in length. The free α -subunit is proposed to be O-glycosylated at Thr⁴³, which is bolded and underlined. The α -subunit in the heterodimeric glycoprotein is putatively N-glycosylated at Asn⁵⁶ and Asn⁸², which are bolded and underlined. Proposed proper subunit folding involves 10 cysteines, shown in bold and located at positions 11, 14, 32, 35, 36, 63, 64, 86, 88 and 91 that form 5 disulfide bonds.

sequenced, one truncated fragment from one animal, two genomic fragments from a second animal and one full-length fragment from a third animal. The sequence differences between the different elk are diagrammed in Fig. 3. A deduced 129 amino acid peptide chain would be encoded by this nucleotide sequence (Fig. 4). The deduced amino acid sequence similarity between elk and a sub-set of related species is shown in Table 3.

Cloning of the pituitary LH β -subunit cDNA

A 23 oligonucleotide forward primer was designed based on the reported ovine cDNA,

containing the putative LH β -subunit start codon [13]. A 23 oligonucleotide reverse primer was designed to sequences located downstream of the putative stop codon, within the 3' UTR (Table 1). By using these primers a fragment of 524 bp was amplified, which revealed high similarity, between 79 and 97%, to the LH β -subunit cDNA of other domestic species (Table 2). The elk LH β -subunit sequence was confirmed by sequence analysis of three independent PCR fragments. This sequence includes the entire coding region, with an open reading frame of 366 bp, which encodes a deduced peptide chain of 121 amino acids (Fig. 5). The

Table 2. Nucleotide similarities between the elk glycoprotein cDNAs and cDNAs from a subset of related species were examined over the entire coding region

	Elk gonadotropin α -subunit cDNA (363 bases)	Elk FSH β -subunit cDNA (390 bases)	Elk LH β -subunit cDNA (426 bases)
Sika deer	99% (362/363) [5]	98% (385/390) [6]	N/A
Ovine	95% (346/363) [10]	93% (362/390) [11]	97% (415/426) [13]
Bovine	95% (346/363) [14]	92% (357/390) [23]	97% (415/426) [20]
Porcine	92% (335/363) [15]	87% (338/390) [24]	90% (384/426) [21]
Equine	85% (307/363) [16]	88% (344/390) [25]	79% (336/426) [22]

Similarities were calculated as a percent of identical nucleotides over the entire coding region.

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2338 AGCAGTATTCAATCCCTGTCTCATTTTGATTACGCTACACAGAACTTCCAGACTACTGTAACATCAT 2405
2406 CTCTCTCTCTCTGTGTCTCCTAA ACC ACT CAG GAC TTG GTG TAC AGG GAC CCA GCG 2461
39 T T Q D L V Y R D P A 49
2462 AGG CCC AAC ATC CAG AAG ACA TGT GCC TTC AAG GAG CTG GTC TAC GAG ACG 2512
50 R P N I Q K T C A F K E L V Y E T 66
2513 GTG AGA GTG CCC GGC TGC GCT CAC CGC GCA GAC TCC CTG CAC ACG TAC CCG 2563
67 V R V P G C A H R A D S L H T Y P 83
2564 GTG GCC ACC GCG TGT CAC TGC GGC AAG TGT GAC AGC GGC AGC ACC GAC TGC 2614
84 V A T A C H C G K C D S G S T D C 100
2615 ACC GTG CGG GGC CTG GGG CCC AGC TAC TGC TCC TTC AGC GAC GTC AGA GAG 2665
101 T V R G L G P S Y C S F S D V R E 117
2666 TAA GGGACGGCGGCGCTCTGAGCTGCCACCCCTCACAGGCCAGGCATCCAAGCTGTCTGTGTGTA 2713
Stop
2732 CATG 2735

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Fig. 2. Genomic nucleotide and deduced amino acid sequences of elk FSH β -subunit. The genomic nucleotide numbering is based on the NCBI S64745 ovine FSH- β genomic sequence. The forward custom primer was designed from nucleotide positions 2338 to 2361 and the reverse custom primer was designed from nucleotide positions 2710 to 2735.

deduced amino acid sequence similarity between elk and a sub-set of related species is shown in Table 3.

Northern blot analysis

Expression of the elk gonadotropin genes in elk and sheep tissues is shown in the Northern blot autoradiograms of Fig. 6. The common α -subunit, FSH β -subunit and LH β -subunit genes are

expressed in the elk pituitary gland as mature transcripts of approximately 700, 1700 and 600 bases, respectively. Transcripts of these genes migrated to the same position as the corresponding mRNA expressed in the sheep pituitary gland. Expression of the gonadotropin genes was not observed in elk muscle tissue. GAPDH expression was observed in all samples.

Table 3. Pituitary gonadotropin deduced amino acid sequences similarities between elk and a subset of related species

	Elk α -subunit 120 a.a.	Elk FSH β -subunit 129 a.a.	Elk LH β -subunit 141 a.a.
Sika deer	99% (119/120) [5]	96% (125/129) [6]	—
Ovine	95% (115/120) [10]	91% (118/129) [11]	89% (126/141) [13]
Bovine	95% (116/120) [14]	90% (116/129) [23]	88% (125/141) [20]
orcine	93% (112/120) [15]	89% (115/129) [27]	85% (111/141) [21]
Equine	78% (94/120) [26]	87% (113/129) [25]	78% (109/141) [22]

Similarities are expressed as percent of identical amino acids over the total for the specie shown in brackets.

partial cDNA	TGCTGGAGAGCAATCTGCTGCAGAAGCTGCGAGCTGACCAACATC CCC ATCACGGTGGAG TGCTGGAGAGCAATCTGCTGCAGAAGCTGCGAGCTGACCAACATC ACC ATCACGGTGGAG *****
partial cDNA genomic	TACACCCGG GACTTGGTGTACAGGGACCCAGCGAGGCCCAACATCCAGAAGACATGTGCCT TACACCCGG GACTTGGTGTACAGGGACCCAGCGAGGCCCAACATCCAGAAGACATGTGCCT ACCACTCAG GACTTGGTGTACAGGGACCCAGCGAGGCCCAACATCCAGAAGACATGTGCCT *** *
partial cDNA genomic	TCAAG GGG CTGGTCTACGAGACGGTGAGAGTGCCCGGCTGCGCTCACC GC GCAGACTCCC TCAAG GAG CTGGTCTACGAGACGGTGAGAGTGCCCGGCTGCGCTCACC GC GCAGACTCCC TCAAG GAG CTGGTCTACGAGACGGTGAGAGTGCCCGGCTGCGCTCACC GC GCAGACTCCC *****
partial cDNA genomic	TGCACACGTACCCGGTGGCC ACT GCGTGTCACTGCGGCAAGTGTGACAGCGGCAGCACCG TGCACACGTACCCGGTGGCC ACC GCGTGTCACTGCGGCAAGTGTGACAGCGGCAGCACCG TGCACACGTACCCGGTGGCC ACC GCGTGTCACTGCGGCAAGTGTGACAGCGGCAGCACCG *****

Fig. 3. Alignment of elk FSH β -subunit fragments amplified from 3 different animals. The stars represent the sequence, which is the same between the fragments. The codons where differences were observed are bolded. The first difference occurs at the codon encoding the amino acid at position 15, where the partial fragment would encode proline and the full-length cDNA would encode threonine. The codons encoding the amino acids at positions 39, 40 and 41 show differences among the DNA fragments sequenced. The partial and full-length fragments would encode tyrosine at position 39, whereas the genomic fragment would encode threonine. At position 40, the nucleotide difference between the partial and full-length cDNA fragments compared to the genomic sequence does not result in an amino acid change. At position 41, the nucleotide difference would result in the genomic fragment encoding the amino acid glutamine, whereas the partial and full-length sequences would encode arginine. The full-length and genomic sequences would encode glutamic acid at amino acid position 61, whereas the partial fragment would encode glycine. The nucleotide difference observed between the samples encoding amino acid 86, do not cause an amino acid change.

Discussion

We cloned and sequenced the elk pituitary gonadotropin α - and β -subunit cDNAs, and assessed expression of these genes by Northern blot

analysis. The amplified elk α -subunit cDNA is 95% similar, but 62 bases shorter, than the sheep α -subunit cDNA reported by Bello *et al.* (1989). This discrepancy is due to the design of the forward α -subunit primer, aimed to a sequence located close

-27	AGCATCCACAGTTACCAAGTGC															CCAGG	ATG	AAG	TCC	ATC	CAG	TTC	TGC	TTC	CTT	TTC	30
-12																	M	K	S	I	Q	F	C	F	L	F	-3
31	TGT	TGC	TGG	AGA	GCA	ATC	TGC	TGC	AGA	AGC	TGC	GAG	CTG	ACC	AAC	ATC	ACC	81									
-2	C	C	W	R	A	I	C	C	R	S	C	E	L	T	N	I	T	15									
	-1 /+1																										
82	ATC	ACG	GTG	GAG	AAA	GAG	GAA	TGC	AGC	TTC	TGC	ATA	AGC	ATC	AAC	ACC	ACG	132									
16	I	T	V	E	K	E	E	C	S	F	C	I	S	I	N	T	T	32									
133	TGG	TGT	GCG	GGC	TAC	TGC	TAC	ACC	CGG	GAC	TTG	GTG	TAC	AGG	GAC	CCA	GCG	183									
33	W	C	A	G	Y	C	Y	T	R	D	L	V	Y	R	D	P	A	49									
184	AGG	CCC	AAC	ATC	CAG	AAG	ACA	TGT	GCC	TTC	AAG	GAG	CTG	GTC	TAC	GAG	ACG	234									
50	R	P	N	I	Q	K	T	C	A	F	K	E	L	V	Y	E	T	66									
235	GTG	AGA	GTG	CCC	GGC	TGC	GCT	CAC	CGC	GCA	GAC	TCC	CTG	CAC	ACG	TAC	CCG	285									
67	V	R	V	P	G	C	A	H	R	A	D	S	L	H	T	Y	P	83									
286	GTG	GCC	ACC	GCG	TGT	CAC	TGC	GGC	AAG	TGT	GAC	AGC	GGC	AGC	ACC	GAC	TGC	336									
84	V	A	T	A	C	H	C	G	K	C	D	S	G	S	T	D	C	100									
337	ACC	GTG	CGG	GGC	CTG	GGG	CCC	AGC	TAC	TGC	TCC	TTC	AGC	GAC	GTC	AGA	GAG	387									
101	T	V	R	G	L	G	P	S	Y	C	S	F	S	D	V	R	E	117									

Fig. 4. cDNA nucleotide and deduced amino acid sequences of elk FSH β -subunit. The nucleotides of the 5' UTR are designated with negative numbers and the first nucleotide of the ATG translational start codon as +1. The forward custom primer was designed from nucleotide positions -27 to -6. The reverse custom primer was designed from nucleotide positions 362 to 387. Both primer sequences are shown in bold. The end of the signal peptide and the beginning of the mature protein amino acid sequence is designated at the -1/+1 boundary. The proposed leader sequence is 12 amino acids in length. The FSH β -subunit is proposed to be N-glycosylated at Asn¹³ and Asn³⁰, shown in bold and underlined. Proposed proper folding involves 12 half cysteines shown in bold and located at positions 9, 23, 26, 34, 38, 57, 72, 88, 90, 93, 100 and 110 that form 6 disulfide bonds.

to the start codon. Thus, our elk α -subunit cDNA clone has an incomplete 5' UTR, compared to the ovine α -subunit cDNA. During the course of this study, the glycoprotein α -subunit [5] and FSH β -subunit [6] cDNA nucleotide sequences of the sika deer were reported. The presently reported elk cDNA shares 99% nucleotide similarity to the glycoprotein α -subunit cDNA of the sika deer. Only one nucleotide difference was observed at position 11, where an adenine was reported for sika deer whereas in the elk we report a guanine. Using the cloned elk glycoprotein α -subunit cDNA as a probe, a mature transcript size of approximately 700 bases was identified, which is consistent with the sheep glycoprotein α -subunit mRNA reported by Bello *et al.* (1989). Thus, we consider that this cDNA corresponds to the elk common pituitary glycoprotein α -subunit.

If the signal peptide of the elk α -subunit is of the same length as reported for ovine [10], the elk α -subunit cDNA would encode a mature protein of 96 amino acids. The nucleotide difference observed

at position 11, translated into a difference in the fourth deduced amino acid. We report a cysteine for the elk, whereas a tyrosine was reported for sika deer, ovine, bovine, porcine and equine [5, 10, 14–16]. In addition, the forward primer we designed to amplify the elk α -subunit cDNA biased the -24 and -23 deduced amino acids. Therefore, most likely the resulting deduced amino acids at -24 and -23 in the elk α -subunit gene would be methionine and aspartic acid, which correspond to the amino acids reported for the other related species [5, 10, 14–16]. The hydrophobic residues in the signal peptide, including valine, alanine, leucine, and isoleucine, remain the same in the elk as in sika deer, ovine, bovine and porcine. The amino acid difference observed is a conserved change since tyrosine and cysteine are both uncharged polar residues. Therefore, the difference observed may not affect the biological activity of the hormone, since the signal peptide is cleaved from the nascent peptide.

The elk FSH β -subunit cDNA reported in this study is 98% similar, but 1175 bp shorter than the

-17	GGTACCAGGCACCAAGGATGGAG															ATG	CTC	CAG	GGG	CTG	CTG	CTG	TGG	CTG	CTG	CTG	39		
-20																M	E	M	L	Q	G	L	L	L	W	L	L	L	-8
40	GGC	GTG	GCC	GGG	GTG	TGG	GCT	TCC	AGG	GGG	CCA	CTG	CGG	CCG	CTG	TGC	CAG	90											
-7	G	V	A	G	V	W	A	S	R	G	P	L	R	P	L	C	Q	10											
																			-1	/+1									
91	CCC	ATC	AAC	GCC	ACC	CTG	GCG	GCT	GAG	AAG	GAG	GCC	TGC	CCC	GTC	TGC	ATC	141											
11	P	I	N	A	T	L	A	A	E	K	E	A	C	P	V	C	I	27											
142	ACT	TTC	ACC	ACC	AGC	ATC	TGC	GCC	GGC	TAC	TGC	CCC	AGC	ATG	AAG	CGG	GTG	192											
28	T	F	T	T	S	I	C	A	G	Y	C	P	S	M	K	R	V	44											
193	CTG	CCT	GCC	ATC	CTG	CCG	CCC	ATG	CCC	CAG	CGG	GTG	TGC	ACC	TAC	CAC	GAG	243											
45	L	P	A	I	L	P	P	M	P	Q	R	V	C	T	Y	H	E	61											
243	CTG	CGC	TTC	GCC	TCC	GTT	CGG	CTC	CCC	GGC	TGC	CCA	CCT	GGC	GTG	GAC	CCA	294											
62	L	R	F	A	S	V	R	L	P	G	C	P	P	G	V	D	P	78											
295	ATG	GTC	TCC	TTC	CCC	GTG	GCC	CTC	AGC	TGT	CAC	TGC	GGG	CCC	TGC	CGC	CTC	345											
79	M	V	S	F	P	V	A	L	S	C	H	C	G	P	C	R	L	95											
346	AGC	AGC	ACT	GAC	TGT	GGG	GGT	CCT	AGA	ACC	CAA	CCC	TTG	GCC	TGT	GAC	CAC	396											
96	S	S	T	D	C	G	G	P	R	T	Q	P	L	A	C	D	H	112											
397	CCC	CCG	CTC	CCA	GAC	ATC	CTC	TTC	CTC	TAA	GGATGCCCACTTCAACCTCCCATGCC							453											
113	P	P	L	P	D	I	L	F	L	Stop								121											
454	TCCTAACTCCCGGAGCCAGCAGACGCTCCTCCCTCCCTTCCCAATAAAGACTTCT																			509									

Fig. 5. cDNA nucleotide and deduced amino acid sequences of elk LH β -subunit. The nucleotides of the 5' UTR are designated with negative numbers and the first nucleotide of the ATG translational start codon as +1. The forward custom primer was designed from nucleotide positions -17 to +6, overlapping the first two codons from the translational start site. The reverse custom primer was designed from nucleotide positions 485 to 509. Both primer sequences are shown in bold. The end of the signal peptide and the beginning of the mature protein amino acid sequence is designated as the -1/+1 boundary. The proposed leader sequence is 20 amino acids in length. The LH β -subunit is presumed to be N-glycosylated at Asn¹³, shown in bold and underlined. Proposed proper folding involves 12 cysteines shown in bold and located at positions, 9, 23, 26, 34, 38, 57, 72, 88, 90, 93, 100 and 110 that form 6 disulfide bonds.

ovine FSH β -subunit cDNA reported by Mountford *et al.* (1989). This discrepancy is attributed to the forward FSH β -subunit primer, which hybridised at a site close to the start codon, and to the reverse FSH β -subunit primer 2, designed from the genomic elk FSH β -subunit clone, which hybridized to the last 9 codons of the gene. Thus, this cDNA does not include 29 bp of the 5' UTR, nor the 1144 bp of the 3' UTR compared to the ovine FSH β -subunit cDNA. The reported cDNA shares 98% nucleotide similarity to the FSH β -subunit cDNA of the sika deer. Using the cloned elk FSH β -subunit as a probe, a mature transcript size of approximately 1700 bases was identified, which is consistent with the reported size of the bovine FSH β -subunit mRNA [17]. Therefore, we conclude that the reported cDNA corresponds to the elk FSH β -

subunit.

The strategy used to clone the elk FSH β -subunit cDNA involved sequencing of a genomic fragment, from which we gained additional information on the structure of the gene. The elk FSH β -subunit genomic fragment presently reported contains 6 CT microsatellite repeats in the intronic sequence amplified. This differs from the genomic sheep FSH β -subunit sequence [12], which contains 19 CT microsatellite repeats at the same location. If adequate variability in the number of these CT repeats were shown, this microsatellite could represent a marker for DNA fingerprinting in elk parentage testing [18].

Five nucleotide differences within the coding region of the FSH β -subunit were observed between elk and sika deer. The nucleotide

Fig. 6. Autoradiographs of Northern blot membranes hybridized with elk α -subunit, FSH β -subunit, LH β -subunit and GAPDH cDNA probes. Lanes 1, 2, 3 and 4 were loaded with total RNA from sheep pituitary gland, elk muscle, Chinese hamster ovary cells and elk pituitary gland, respectively.

sequence of the FSH β -subunit cDNA was deduced from information obtained from three different animals, from which we amplified a truncated cDNA, a genomic fragment and a full-length cDNA. We observed discrepancies in the nucleotide sequences between these fragments, diagrammed in Fig. 3. The discrepancies reported could be due to amplification errors [19] or to genetic polymorphisms between different animals. The full-length cDNA would encode a peptide chain of 129 amino acids, of which 117 correspond to the mature protein.

The LH β -subunit cDNA we amplified is 96% similar, but 7 bp shorter than the ovine LH β -subunit cDNA reported by d'Angelo-Bernard *et al.* (1990). This discrepancy is due to the forward and reverse LH β -subunit primers, which did not target the complete 3' or 5' UTR sequences. Using the cloned elk LH β -subunit cDNA as a probe, a mature transcript size of approximately 550 bases was

identified, which is consistent with the report for ovine [13]. Thus, we consider that this cDNA corresponds to the elk LH β -subunit. This cDNA would encode a peptide chain of 121 amino acids, of which 101 correspond to the mature protein, assuming that the signal peptide of elk would be the same length as reported for ovine [13]. The signal peptide is proposed to be the first 20 amino acids, which are cleaved to yield the mature protein. The forward LH β -subunit primer used compromises the methionine and glutamic acid amino acids at positions -20 and -19. These amino acids were reported in the same positions in ovine, bovine, porcine and equine LH β -subunit [13, 20, 22]. Therefore, we speculate that despite the introduced bias of the forward LH β -subunit primer, the elk LH β -subunit gene may encode for methionine and glutamic acid at positions -20 and -19, respectively.

In summary, we cloned the elk glycoprotein α -subunit and FSH and LH β -subunit cDNAs. Sequence and Northern blot analyses demonstrates high similarities between the gonadotropin hormones genes and their transcripts between elk, sika deer and sheep. The elk DNA sequences presently reported represent an addition to the growing collection of pituitary hormone genes for various vertebrates, which will facilitate evolutionary and phylogenetic studies of the gonadotropin genes.

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