

## Cloning and Characterisation of Orthologous Genes Encoding Growth Hormone in Egyptian Holstein Crossbred and Ossimi Sheep

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**Abstract:** This study aims to investigate the compatibility between growth hormone (GH) cDNA sequence in Egyptian cows and sheep as an introduction to produce bovine GH-Transgenic sheep using sperm mediated gene transfer (SMGT) technique to improve their productivity. Our previous in vivo study indicates a significant increase in the concentration of GH mediator (IGF-1) in blood, and daily growth rate, when Egyptian rams were injected with bovine somatotropin. The GH gene of Egyptian Holstein crossbred (EH\_GH) and Ossimi sheep breed (Os\_GH) has been characterized in this study. The mRNA of pituitary gland has been isolated and then the cDNA was cloned into pTZ57R/T cloning vector. The complete coding domain sequences (CDSs) of EH\_GH (AC: KP221576) AND Os\_GH (Ac: KP221575) were detected and annotated comprehensively. The sequenced GH genes varied slightly along gene length; EH\_GH was 740 bp instead of 690 bp in Os\_GH, but with similar gene structure (5 exons and 4 introns). High homology (98%) in GH protein length and structure was observed; the predicted open reading frames (ORF) length was 217 aa and 216 aa of EH\_GH and Os\_GH, respectively, which included only four different amino acids in the predicted ORFs. A gene synteny was developed of the homologous bovine and ovine GH1 genes Ensemble annotated genome database. The results showed strong conserved structure and function, indicated that to GH gene of Egyptian Holstein crossbred and Ossimi breed are orthologous genes. We assume in this study that the novel genetic information about the bovine and ovine GH in Egyptian breeds can support; further success possibilities of producing the bovine GH-transgenic sheep to improve the growth performance and milk production.

**Keywords:** Growth hormone (GH), Orthologous genes, Egyptian Holstein crossbred, Ossimi breed.

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### 1. INTRODUCTION

Sheep is one of the most important domestic animals reared in Egypt. There are almost 5.5 million head that contributed about 6% of the total red meat that produced in Egypt [1]. The cows' population in Egypt is estimated about 4.53 million heads that produce 2.90 and 0.41 million tons of milk and meat, respectively [2]. In the last three decades, Egyptian farms adopted crosses between the Holstein breed and the Egyptian Baladi breeds to improve their productivity by producing Egyptian Holstein crossbred adapted under the Egyptian environment. Due to the

world food gap in the world, a lot of scientists have been turned on to gene transfer techniques as a quick tool to increase livestock production. This study aims to investigate the compatibility between the growth hormone cDNA sequence in Egyptian cows and sheep as an introduction to produce bovine GH-Transgenic sheep using sperm mediated gene transfer (SMGT) to improve their productivity. The candidate gene is growth hormone that spans approximately 1.8 kilobases and consists of five exons interrupted by four introns [3]. Growth Hormone gene is located at chromosome 19 in *Bos taurus* genome, while at chromosome 11 in *Ovis aries* genome [4] and [5]. The growth hormone is a single-chain polypeptide synthesised and secreted by the pituitary gland under hypothalamic control and regulates the body growth, cell reproduction and regeneration in cattle and sheep [6]. Growth hormone affects the partitioning of nutrients among tissues in sheep and cattle, increasing bone growth and milk production and decreasing fatness [7]. It belongs to the same hormonal family as prolactin (PRL) and placental lactogen (PL), all of them sharing certain biological, immunological and structural features; they are considered to be derived from duplication of a single ancestral gene [8] and [9]. Plasma concentration of GH is increased in cows genetically selected for high milk production [10] and in sheep selected for low levels of fatness [11], indicating that GH is genetically correlated with these traits.

In our previous study, recombinant bovine somatotropin (100 mg rbST/14 days) had been injected in Egyptian Rahmani lambs. The average daily gain has been increased significantly (8.43%,  $P < 0.05$ ) in rbST treated rams comparing to the control. The feed conversion did not differ significantly in rbST treated rams compared to the control that may be gives us an indicator that the treated group utilizes the feed intake in a good way to increase the average daily gain [12]. Based on that aforementioned, our aim was cloning and characterization of growth hormone gene in both Egyptian Holstein crossbred and Ossimi sheep breed. The functional bioinformatic analysis can guide the research accurately to predict for the success possibilities to introduce and express bovine GH gene into transgenic sheep for enhancing their productivity.

## 2. MATERIALS AND METHODS

### 2.1. cDNA Synthesis

Total RNA has been isolated from pituitary gland (50 mg) of Egyptian Holstein crossbred and Ossimi breed using Booze reagent kit (Bioflux<sup>®</sup>). Total RNA was reverse transcribed with Oligo dT<sub>18</sub> using RevertAid First Strand cDNA Synthesis Kit (Fermentas, Canada). The EH\_GH primer was designed using custom primers-OligoPerfect™ designer tool (<https://tools.lifetechnologies.com/content.cfm?pageid=9716&icid=fr-oligo-6?CID=fl-oligoperfect>) based on NCBI database (Ac: M27325.1, 783bp). The EH\_GH primer sequence was EH\_GH-F 5'-GAGCTCCAGGGTCCTGTGGACAGC-3' and EH\_GH-R 5'-CCGCGGTGCGATGCAATTTCC TCAT-3'. While, the Os\_GH primer was published on NCBI (Ac: EU935861.1), Os\_GH-F 5'-GCTCACCAGCTATGATGGCTG-3' and Os\_GH-R 5'-TGGCAACTAGAAGGCGCAGCT-3'.

### 2.2. Gene Cloning

The PCR reaction mixture (10µl) was prepared for each gene as follow; [1µl of 10X buffer containing MgCl<sub>2</sub> (25mM), 1µl of dNTBase mixture (200 µM), 1µl of F-primer, 1µl of R-primer, 0.2 µl of Taq polymerase (1U/µl), 1µl of both EH\_GH/Os\_GH cDNA and water nuclease-free up to 10µl) [13]. The amplification reaction was carried out in a Thermocycler PCT-100 (MJ Research, USA). The PCR conditions of EH\_GH-cDNA was denaturation for 1 min at 94°C, annealing for 2 min at 57°C, and extension for 3 min at 72°C for 29 cycles, and final extension at 72°C for 7 min, while for Os\_GH-cDNA, denaturation for 1 min at 94°C, annealing for 2 min at 63°C, and extension for 3 min at 72°C for 29 cycles. A final extension was 72°C for 7 min. The PCR products were visualized on a 1% agarose gel stained with Ethidium bromide in 1x TAE buffer at 100 v for 60 min with 10 Kb BioLabs ladder, (New England, UK). The image of the gel band was taken using Bio-Rad Gel Doc XR. The detected EH\_GH and Os\_GH amplicons were extracted from the gel using GeneElute™ Gel Extraction Kit (Fermentas, USA), then cloned into pTZ57R/T vector (Fig.1). The vector construction was designed according to the cloning kit procedure (Fermentas, USA) as follow; 3µl of pTZ57R/T vector, 2.5µl of both EH\_GH/Os\_GH cDNA, 6µl of 10x ligase buffer, 1µl T4 DNA ligase and 17.5µl sterile water in total 30 µl reaction mixture, incubated at 22°C/1h for ligation. The ligated product was transformed into DH5α cells (Fermentas, USA). The constructed plasmids (EH\_GH and Os\_GH-PTZ57R/T) were extracted

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using GeneJET plasmid Miniprep kit, and sequenced. The efficiency of transformation was determined using the polymerase chain reaction.

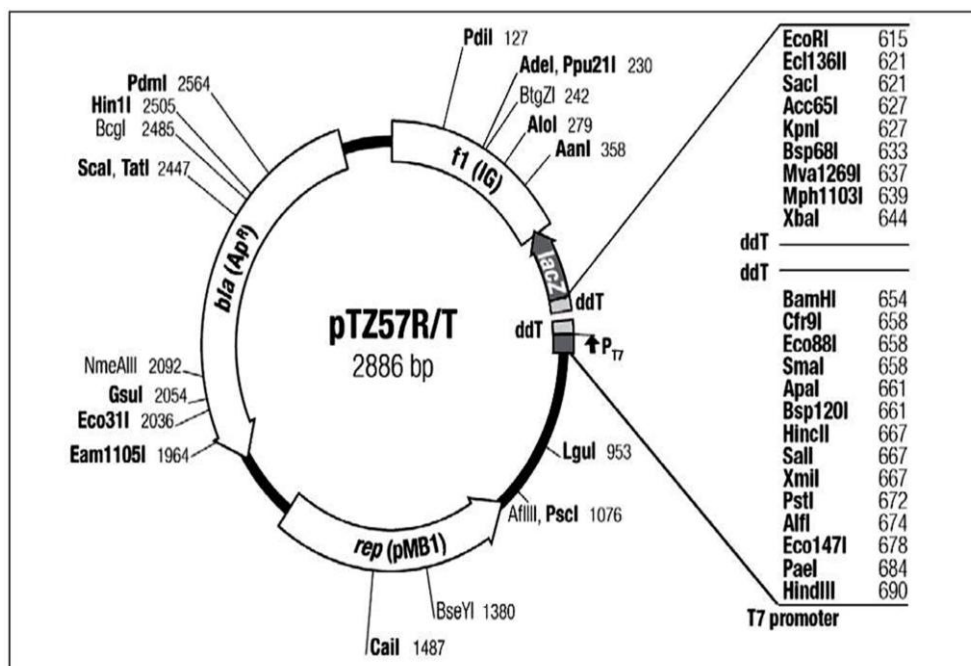


Fig1. Map of the pTZ57R/T cloning vector.

### 2.3. Bioinformatics Analysis

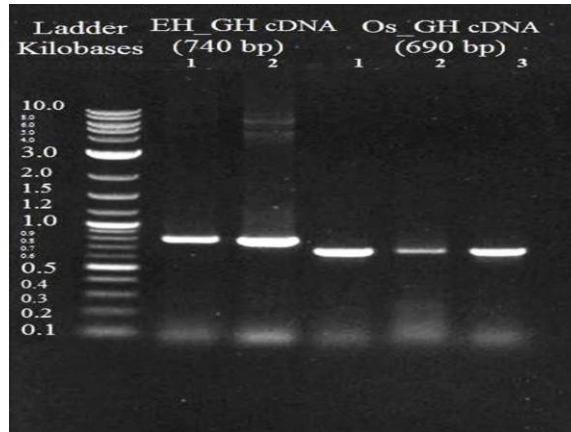
The consensus sequence of cloned EH\_GH and Os\_GH cDNA were created using CodonCode Aligner V4.2.7 software. The SNPs among EH\_GH and Os\_GH cDNA sequence have been done using the pairwise alignment via EMBOSS Needle tool ([http://www.ebi.ac.uk/Tools/psa/emboss\\_needle/](http://www.ebi.ac.uk/Tools/psa/emboss_needle/)). The transcription promoter has been predicted using Neural Network Promoter Prediction via [http://www.fruitfly.org/seq\\_tools/promoter.html](http://www.fruitfly.org/seq_tools/promoter.html) [14]. The open reading frames (ORF) of EH\_GH and Os\_GH cDNA sequences were predicted using <http://web.expasy.org/translate/> [15]. Predicted protein codons relative to the nucleotide sequence were done using translated tool via <http://www.fr33.net/translator.php>. The predicted cleavage site in signal peptide sequence was done using SignalP 4.1 Server tool <http://www.cbs.dtu.dk/services/SignalP/> [16]. The conserved domain of EH\_GH and Os\_GH cDNA sequences were detected using NCBI conserved domains tool via <http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi> [17]. The motif of EH\_GH and Os\_GH cDNA was predicted using search motif library tool at <http://www.genome.jp/tools/motif/>. The cysteine state and disulfide bonds in the EH\_GH and Os\_GH protein sequences were carried out using cysteine and disulfide bond prediction tool at <http://clavius.bc.edu/~clotelab/DiANNA/> [18]. Pairwise alignment was done between EH\_GH and Os\_GH protein sequences using EMBOSS Needle tool ([http://www.ebi.ac.uk/Tools/psa/emboss\\_needle/](http://www.ebi.ac.uk/Tools/psa/emboss_needle/)). Also, the multiple alignments of EH\_GH and Os\_GH protein sequences vs. NCBI protein database have been carried out using Bioedit 7.2.5 software [19]. Phylogenetic tree of EH\_GH and Os\_GH protein sequences was conducted using MEGA version 6 [20]. The synteny location of GH in ovine chromosome (Chr11) vs. bovine chromosome (Chr19) has been achieved using annotation and prediction tool available at <http://www.ensembl.org>.

## 3. RESULTS AND DISCUSSION

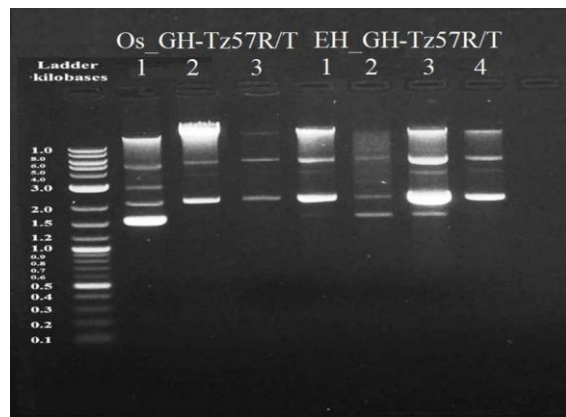
### 3.1. Gene Cloning of GH

The cDNA and translated protein sequences of EH\_GH and Os\_GH had been published at Genebank-NCBI database with accession number KP221576 and KP221575, respectively. The electrophoretic band of EH\_GH was 740 bp and was 690 bp for Os\_GH (Fig. 2). In Fig. 3, five uncut plasmid forms were showed on one sample of EH\_GH-TZ57R/T, whereas the Os\_GH-TZ57R/T had three to four forms. About five forms of uncut plasmid on the electrophoretic gel based on its form and molecular weight are; 1) Nicked open-circular 2) Relaxed circular 3)

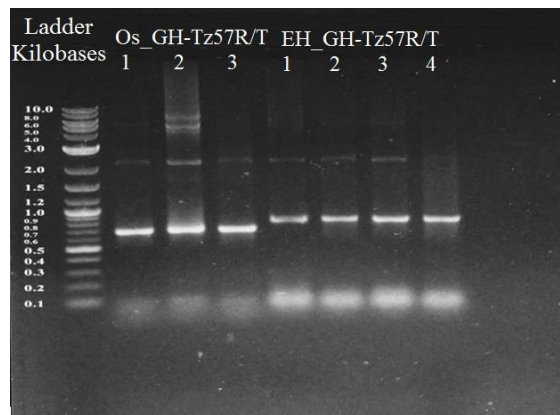
Linear, 4) Supercoiled, 5) Supercoiled denature [21]. The electrophoresis results of EH\_GH-TZ57R/T and Os\_GH-TZ57R/T are shown in Fig. 4.



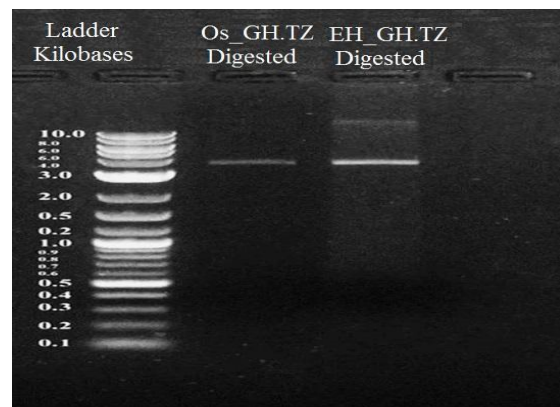
**Fig2.** Gel electrophoresis of cDNA band of EH\_GH and Os\_GH.



**Fig3.** Photograph of Holstein and Ossimi GH-TZ57R/T uncut plasmid electrophoresis.



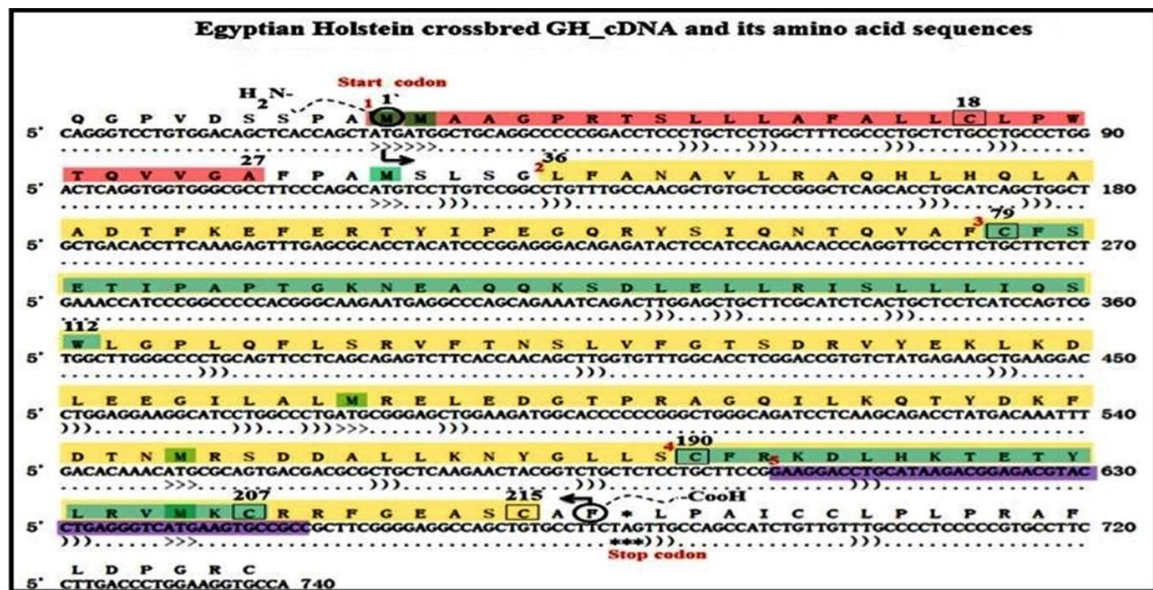
**Fig4.** Photograph of EH\_GH-TZ57R/T and Os\_GH-TZ57R/T PCR plasmid electrophoresis.



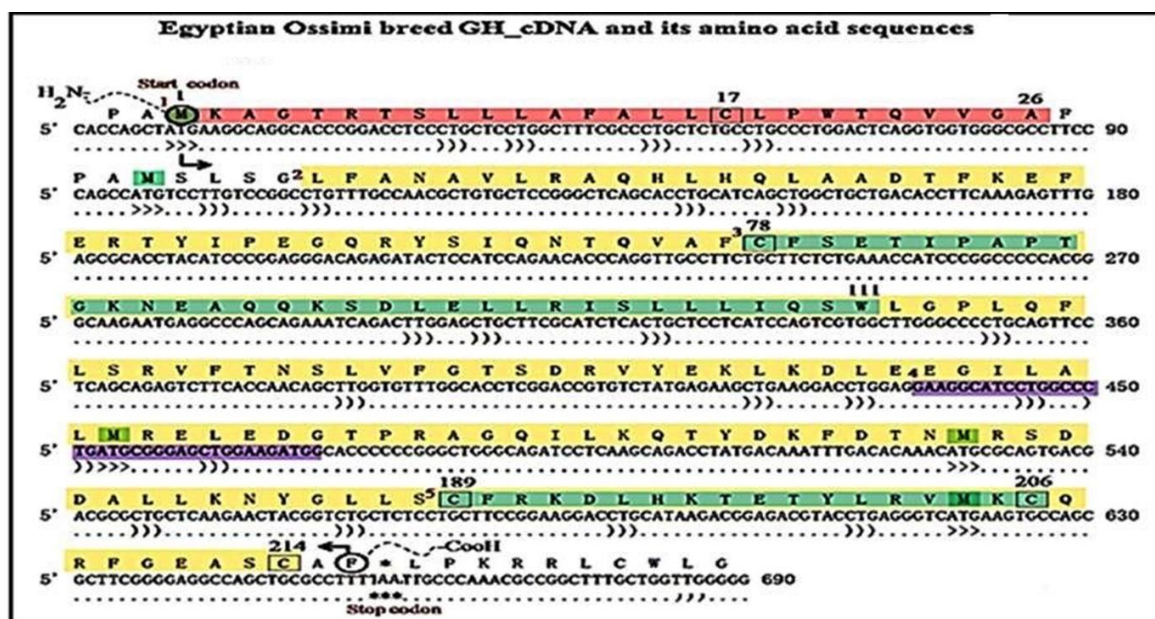
**Fig5.** Photograph of EH\_GH-TZ57R/T and Os\_GH-TZ57R/T cut plasmid.

### 3.2. Growth Hormone cDNA SNPs

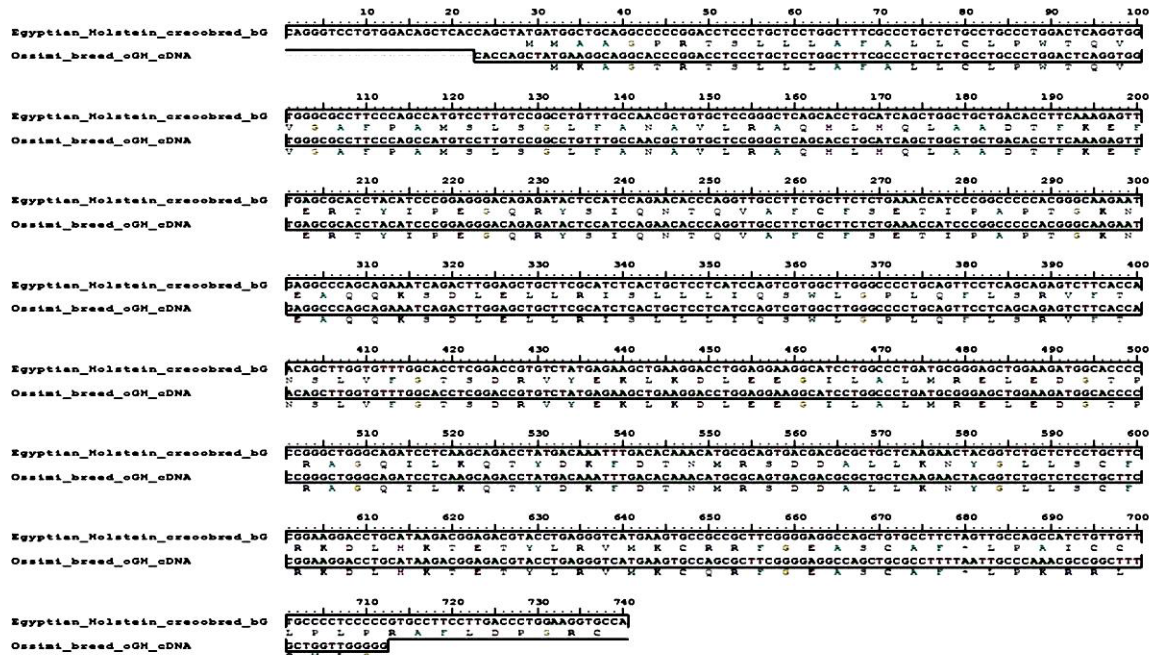
The pairwise alignment (Fig. 8) has been shown that there are ten nonsynonymous alleles substitutions (small letter) which yield a new codon that encodes a different amino acids in EH\_GH cDNA sequence vs. Os\_GH cDNA sequence (Table 1); gct for Alanine (Ala) at 34,35 and 36, ccc for Proline (Pro) at 43, 44 and 45, Cgc for Arginine (Arg) at 650 and 651, gcc for Alanine (Ala) at 688, 689 and 690, atC for Isoleucine (Ile) at 691 and 692, t Gt for cysteine (Cys) at 694 and 696, tgT for Cysteine (Cys) at 697 and 698, Ttg for Leucine (Leu) at 701 and 702, ccc for Proline (Pro) at 703, 704 and 705 and ccc for Proline (Pro) at 709, 710 and 711 in EH\_GH cDNA sequence vs. aag for Lysine (Lys) at 12, 13 and 14, aCC for Threonine (Thr) at 21, Cag for Glutamine (Glu) at 628 and 629, aaa for Lysine (Lys) at 666, 667 and 668, cgC for Arginine (Arg) at 669 and 670, cGg for Arginine (Arg) at 772 and 774, ctT for leucine (Leu) at 675 and 676, Tgc for Cysteine (Cys) at 780, tgg for Tryptophan (Trp) at 681, 682 and 683 and ggg for Glycine (Gly) at 687, 688 and 689 in Os\_GH cDNA sequence.



**Fig6.** The growth hormone cDNA sequence and the encoded amino acid residues in Egyptian Holstein crossbred. <sup>1</sup>Signal protein sequence (Red, 1...27 aa), <sup>2</sup>Conserved domain of predicted residues sequence (Yellow: 36...215 aa), <sup>3</sup>First motif Somatotropin\_1 (Green: 79...112, 192 related sequences and 22 related structures). <sup>4</sup>Second motif Somatotropin\_2 (Green: 190...207, 194 related sequences and 22 related structures). <sup>5</sup>Predicted promoter (Purple, start 603 bp....end 653 bp and score 0.85). The conserved Cysteine residues are illustrated by boxes.



**Fig7.** The growth hormone cDNA sequence, predicted amino acids sequences and protein features in Ossimi breed. <sup>1</sup>Signal protein sequence (Red, 1...26 aa), <sup>2</sup>Conserved domin of predicted residues sequence (Yellow: 35...214 aa), <sup>3</sup>First motif Somatotropin\_1 (Green: 78...111, 192 related sequences and 22 related structures). <sup>4</sup>Second motif Somatotropin\_2 (Green: 189...206, 194 related sequences and 22 related structures). <sup>5</sup>Predicted promoter (Purple, start 581bp... end 631bp and score 0.85). The conserved Cysteine residues are illustrated by boxes.



**Fig8.** The pair wise alignment of EH and Os\_GH cDNA sequences.

On the other hand, there are five synonymous allele's substitutions (small letter) which yield a new codon that encodes the same amino acids TGt for Cysteine (Cys) at 672, TTc for Phenylalanine (Phe) at 678, TAg Stop codon at 681, CCa for Proline (Pro) at 687 and cTc Leucine (Leu) at 706 and 708, in EH\_GH cDNA sequence vs. TGc for Cysteine (Cys) at 650, TTt for Phenylalanine (Phe) at 656, TAa Stop codon at 659, CCc for Proline (Pro) at 665 and tTg Leucine (Leu) at 684 and 686 in Os\_GH cDNA sequence. That single nucleotide polymorphism can be used to develop SNPs markers for each breed.

### 3.3. The Prediction of Growth Hormone Promoter

The promoter is the most important regulatory region that regulates the very first step of gene expression [22]. The promoter sequence of EH\_GH cDNA was AAGGACCTGCATAAGACGG AGACGTACCTGAGGGTCATGAAGTGCCGCC (50bp), contains start codon (ATG) and spanning between 603bp and 653bp with prediction score 0.85 (Fig. 6). Whereas the promoter of Os\_GH was GAAGGCATCCTGGCCCTGATGCGGGAGCTGGAAGATGG (38bp), contains start codon (ATG) and spanned between 581bp to 631bp with 0.85 prediction score (Fig. 7). The identity score between both promoters was 57.89 considering length differences.

### 3.4. Protein Structure

#### 3.4.1. Signal Peptide Sequence

The signal peptide of EH\_GH sequence ranged from 1 to 27 residues and from 1 to 26 residues in Os\_GH cDNA (Fig. 6 and 7, respectively). The predicted cleavage site was located between 26 and 27 amino acids in EH\_GH protein and between 25 and 26 amino acids in Os\_GH protein (Fig. 9). The signal peptide has been defined as a sequence generally found at the N-terminal end of most secreted and cell-surface protein precursors [23]. The signal sequence that bound at the amino terminus of the emerging protein "flag", which transport the mechanism of the cell to prompt them as to where the emerging protein should go. The flags recognize by signal recognition particle (SRP) locate freely in the cytoplasm or attached to the ribosome, the SRP interacts with the signal sequence and leads to the transient arrest of translation. That may indicate that the isolated GH cDNA of both breed could be expressed in transfected cell [24].

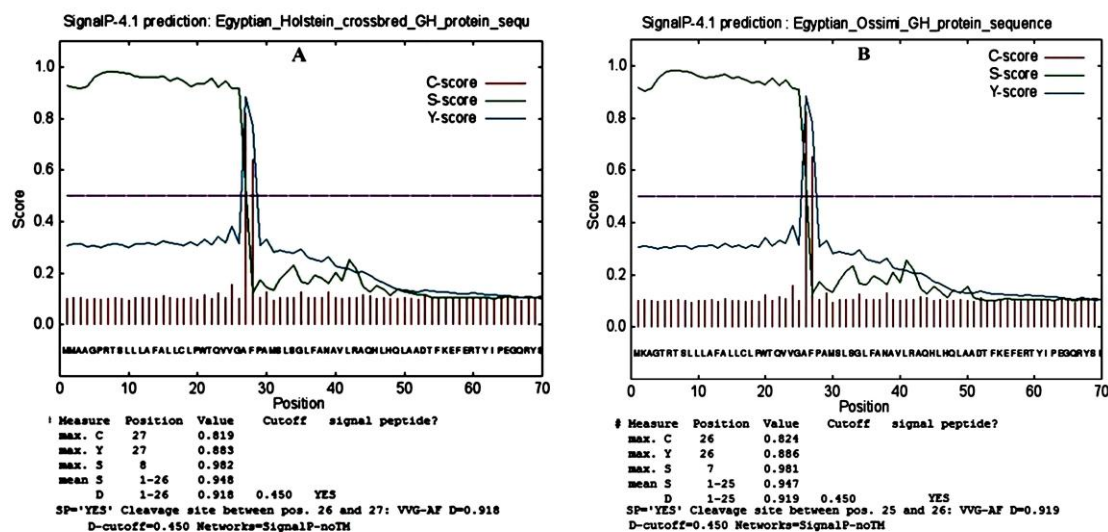
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**Table1.** The modified nucleotides (SNPs) and encoded amino acids in EH\_GH cDNA vs. Os\_GH cDNA sequences.

EH_GH cDNA sequence			Os_GH cDNA sequence		
Nucleotides	Location	Residue	Nucleotides	Location	Residue
*gct	34, 35 and 36	Alanine	aag	12, 13 and 14	Lysine
ccc	43, 44 and 45	Proline	aCC	21	Threonine
Cgc	650 and 651	Arginine	Cag	628 and 629	Glutamine
TGt	672	**Cysteine	TGc	650	**Cysteine
TTc	678	**Phenylalanine	TTt	656	**Phenylalanine
TAg	681	**Stop	TAa	659	**Stop
CCa	687	**Proline	CCc	665	**Proline
gcc	688, 689 and 690	Alanine	aaa	666, 667 and 668	Lysine
atC	691 and 692	Isoleucine	cgC	669 and 670	Arginine
tGt	694 and 696	Cysteine	cGg	672 and 674	Arginine
tgT	697 and 698	Cysteine	ctT	675 and 676	Leucine
Ttg	701 and 702	Leucine	tgc	679 and 680	Cysteine
ccc	703, 7024 and 705	Proline	tgg	681, 682 and 683	Tryptophan
cTc	706 and 708	**Leucine	tTg	684 and 686	**Leucine
ccc	709, 710 and 711	Proline	ggg	687, 688 and 689	Glycine
C.....C	1 to 22	Q..... P	No complement nucleotide		
G.....A	713 to 740	R..... C	No complement nucleotide		

\* Lower case letters have been indicated for SNPs, \*\* The same amino acids with different encoding sequence

### 3.4.2. Conserved Domain and Motifs



**Fig9.** The predicted cleavage site of signal peptide in Egyptian Holstein crossbred (A) and Ossimi breed (B). C-score: Signal peptide cleavage sites. S-score: Signal peptide positions within signal peptides from positions in the mature part of the proteins and from proteins without signal peptides. Y-score: Better cleavage site prediction than the raw C-score alone. Mean S: The average S-score of the possible reticulum the SRP detaches and this leads to the continuation of protein translation, and eventually to the signal peptide (from position 1 to the position immediately before the maximal Y-score). D-score: Discriminate signal peptides from non-signal peptides. For non-secretory proteins all the scores represented in the SignalP output should ideally be very low (close to the negative target value of 0.1) [15].

Highly conserved domain of predicted growth hormone protein has been detected in EH\_GH and Os\_GH (Fig. 10). Detecting the significant conserved protein domains is often required for basic cellular function, stability or reproduction. Conservation of protein structures is indicated by the presence of functionally equivalent, though not necessarily identical, amino acid residues and structures between analogous parts of proteins [25]. The predicted conserved domain hit somatotropin like hormone and specific family with growth hormone like superfamily; Interval 35 to 214 aa, 1.40e-104 E-value for Os\_GH protein and Interval 36 to 215 aa, 5.30e-106 E-value for EH\_GH protein. Protein motifs are signatures of protein families and can often be used as tools

for the prediction of protein function [26]. We detected two common motifs in both EH\_GH and Os\_GH cDNA but with different spanning (Fig. 6 and 7); Somatotropin\_1 (CFSETIPAPTCKNEAQQKSDLELLRISLLLIQSW) from 79 to 112 aa for EH\_GH cDNA and from 78 to 111 aa for Os\_GH cDNA. Somatotropin\_2 (CFRKDLHKETETYL RVM KC) from 190 to 207 aa for EH\_GH cDNA and from 189 to 206 aa for Os\_GH cDNA which reflects same function and characteristics. A comprehensive summary of EH\_GH and Os\_GH annotation has been covered in Table (2).

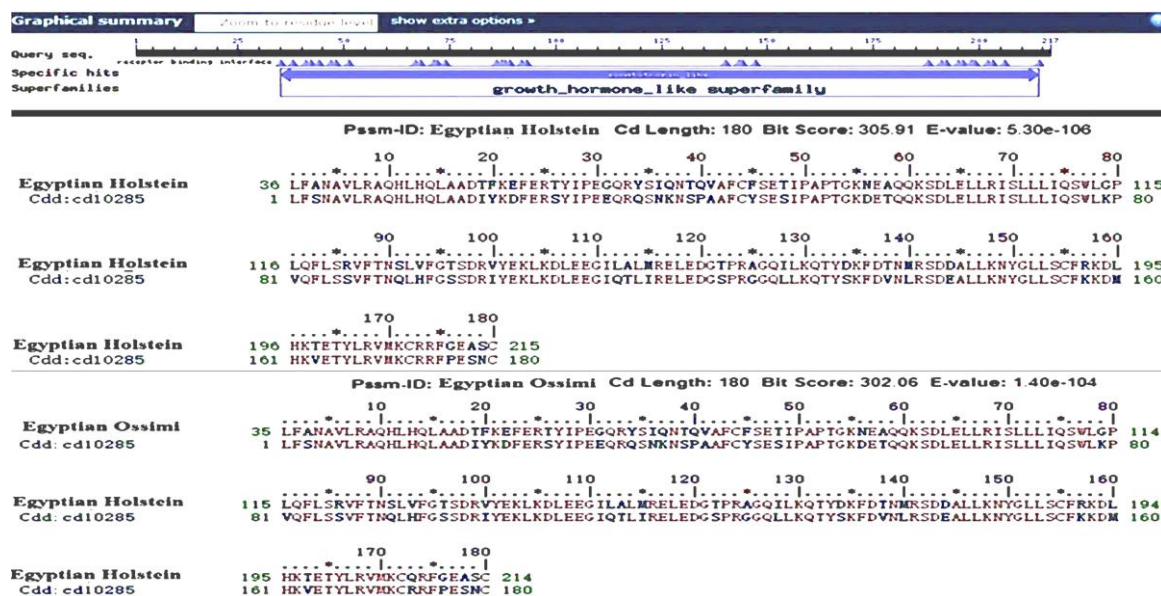


Fig10. Conserved domain of EH\_GH and Os\_GH protein sequences

Table2. Comprehensive summary of EH\_GH and Os\_GH sequences annotation.

Parameters	EH_GH cDNA	Os_GH cDNA
Chromosome location	19	11
Exon number	5	5
Exon protein position	1..31..150..175..205	1..30..149..175..204
Intron number	4	4
Length of mRNA	740 bp	690 bp
Predicted promoter	Start 603, End 653, Score 0.85	Start 581, End 631, Score 0.85
<b>Motifs</b>		
Somatotropin_1	From 79 to 112	From 78 to 111
Somatotropin_2	From 190 to 207	From 189 to 206
Molecular weight	24558 Da	24460 Da
Number of residues	217	216
Disulfide bonds	79 - 190	78 - 189
	207 - 215	206 - 214
Cysteine position	18	17
	79	78
	190	189
	207	206
	215	214
<b>Physical characteristics of growth hormone protein</b>		
Length of protein	217 aa	216 aa
Chain peptide	27-217 (190 aa)	26-216 (190 aa)
Open reading frame	1-217	1-216
Signal peptide	1-27	1-26
Conserved domain	36-215	35-214

### 3.4.3. Cysteine Bridge and Disulfide Bounds

Five conserved cysteine (Cys) are founded in both EH\_GH and Os\_GH protein sequence at the following positions; (Cys<sup>18</sup>, Cys<sup>79</sup>, Cys<sup>190</sup>, Cys<sup>207</sup> and Cys<sup>215</sup>) and (Cys<sup>17</sup>, Cys<sup>78</sup>, Cys<sup>189</sup>, Cys<sup>206</sup> and Cys<sup>204</sup>), respectively (Fig 6 and 7). Two sulfide bounds are predicted between 79 and 190 aa



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(99%) and between 207 and 215 aa (98%) in EH\_GH protein and between at 78 and 189 aa (99%) and between 206 and 214 aa (93%) in Os\_GH protein. The disulfide bonds play an important role in the folding and stability of some proteins, usually proteins secreted to the extracellular medium [27]. Four cysteine residues from five have been participated in the formation of two disulfide bonds between the thiol groups of cysteine residues by the process of oxidative folding. The disulfide bond stabilizes the folded form of a protein in several ways; 1) It holds two portions of the protein together, biasing the protein towards the folded topology. 2) The disulfide bond may form the nucleus of a hydrophobic core of the folded protein [28]. One possible role of this fifth Cysteine residue might be the formation of oligomeric complexes, which might affects the appropriate re-folding of growth hormone protein [29]. In the intracellular environment, cysteines can still play a key structural role. Their sulphhydryl side chain is excellent for binding to metals, such as zinc, meaning that cysteines are very common in metal binding motifs such as zinc fingers [30].

### 3.4.4. Pair wise alignment of Open Reading Frame

The pairwise alignment of EH\_GH protein vs. Os\_GH protein (Fig. 11) has been indicated that the protein sequences in both breeds are highly identical residues (98%) except in four positions (Table 3). The EH\_GH protein sequence was started with two start codon (MM), while the Os\_GH protein sequence was started with unusual single start codon (M). The modified residues of EH\_GH protein sequence were Ala<sup>3</sup>, Pro<sup>6</sup> (signal peptide) and Arg<sup>208</sup> vs. Lys<sup>2</sup>, Thr<sup>5</sup> (signal peptide) and Gln<sup>207</sup> in Os\_GH protein sequence. The differences in residues substitution in signal peptide did not affect translocation efficiency and cleavage site position and this conclusion is confirmed based on the results of highly values of C, Y, S and D in signalP chart (Fig. 9). But the difference in the N-terminus Methionine can be higher growth hormone transcription in EH\_GH, and lower expression in Os\_GH, refer to the active and inactive forms of GH [31].

Egyptian_Hols	1	MMAAGPRTSLLLAFALLCLPWTQVVGAFPAMSLSGLFANAVLRAQHLHQL	50
		. .	
Ossimi_breed_	1	-MKAGTRTSLLLAFALLCLPWTQVVGAFPAMSLSGLFANAVLRAQHLHQL	49
Egyptian_Hols	51	AADTFKEFERTYIPEGQRYSIQNTQVAFCFSETIPAPTGKNEAQQKSDLE	100
Ossimi_breed_	50	AADTFKEFERTYIPEGQRYSIQNTQVAFCFSETIPAPTGKNEAQQKSDLE	99
Egyptian_Hols	101	LLRISLLLIQSWLGPLQFLSRVFTNSLVFGTSDRVYEKLDLEEGILALM	150
Ossimi_breed_	100	LLRISLLLIQSWLGPLQFLSRVFTNSLVFGTSDRVYEKLDLEEGILALM	149
Egyptian_Hols	151	RELEDGTPRAGQILKQTYDKFDTNMRSDDALLKNYGLLSCFRKDLHKTET	200
Ossimi_breed_	150	RELEDGTPRAGQILKQTYDKFDTNMRSDDALLKNYGLLSCFRKDLHKTET	199
Egyptian_Hols	201	YLRVMKCRRFGEASCAF	217
		:	
Ossimi_breed_	200	YLRVMKCQRFGEASCAF	216

**Fig11.** The pairwise alignment between EH\_GH and Os\_GH protein sequences

The Ala and Lys can substitute each other because both belong to the same amino acid group (small) as well as Arg vs. Gln both are polar residues. Although, the Pro is a small residue and Thr is a polar residue, in addition to a common role of Pro and Thr in facilitating the intracellular signal transduction but they can substitute without negative effect on protein function [15], [32] and [33]. It may worth to look into the unique residue in Os\_GH (Lys) in exon2, which was associated with a mutation (AAG) vs. (Ala, GCT), which is a common codon among all the aligned proteins, and see if the mutation affect the receptor efficiency especially with the missing initial Met in Ossimi. In respect to the important role of Lys in structure and locate on the outside of the protein, while Ala rarely involved in protein function directly but can play a role in substrate recognition or specificity [33]. There is about 97.5% homology in the coding regions

between bovine GH and ovine GH genes [34]. The GH homology can supports and explain the assumption the expression possibility of bovine growth hormone in injected sheep breed as mentioned previously in rams [12], [35], [36], [37] and [38].

### 3.4.5. Multiple Alignment

Gene families arisen by gene duplication and natural selection [39]. The multiple alignments are an essential study to understand the evolution among the bovine and ovine growth hormone precursors. The multiple alignment of EH\_GH and Os\_GH against other GH complete CDS (Genebank- NCBI database), and the main published GH1 of bovine and ovine (Ensemble genomes database) are shown in Fig. 12. There are three pakistani ovine breeds (Latti, Lohi and Afghani) and four bovine breeds (Indian Zebo, Iranian cattle, Pakistani Sahiwal cattle and

**Table3.** Substituted residues of growth hormone protein of Egyptian Holstein crossbred and Ossimi breed vs. investigated NCBI breeds.

Studied breeds	Modified residues				Investigated breeds
	Residue	Position	Residue	Position	
Egyptian Ossimi breed	Glycine	34	Serine	35	Afghani breed, Pakistan
	Leucine	106	Proline	107	Dhanni cattle, Pakistan
	Glutamine	109	Arginine	110	
	Phenylalanine	122	Leucine	123	Lezhi Black breed Capra hircus, China
	Glutamic acid	152	Glycine	153	Tibetan ovine breed, China
	Glycine	155	Valine	156	All tested breeds except Afghani ovine breed
	Tyrosine	167	Histamine	168	Latti ovine breed, Pakistan
	Glutamine	207	Arginine	208	All tested breeds database
Egyptian Holstein crossbred	Leucine	107	Proline	107	Dhanni cattle, Pakistan
	Glutamine	110	Arginine	110	
	Glycine	156	Valine,	156	Kamori cattle, Pakistan
	Leucine	189	Phenylalanine	189	Irani cattle
Egyptian Holstein crossbred	Methionine	1	-	1	Egyptian Ossimi breed
	Alanine	3	Lysine	2	
	Proline	6	Threonine	5	
	Arginine	208	Glutamine	207	

Chinese cattle) have partial sequences (four exons) while in Zebu cattle contains five exons but the first exon is very short contains only four residues. In most mammals, GH sequence is strongly conserved, but differences in the biological and receptor-binding properties are due to the species-specificity of receptor-binding [40]. Interestingly, the results of multiple alignment have been illustrated that there is a distinct mutation in growth hormone protein sequence where the conserved Gly<sup>156</sup> has been substituted with Val<sup>156</sup> in following breeds; Ovis aries ref synteny, Pakistani Kamori cattle, Pakistani Latti ovine, Chines Tibetan ovine, Pakistani lohi ovine, Chines Kazakh ovine, Swiss Saanen goat, Portugal Serra da Estrela ovine, Chines Lezhi Black goat, Chines Tibet goat and Chines ovine compared to the other breeds (Table 3). Normally, if the conserved Glycine has been changed to any other amino acid, this change could have a drastic impact on protein function [33]. Therefore, it could be concluded that it is possible to substitute conserved Gly and get a functional growth hormone protein, this mutation may be considered as a specific genetic marker related to specific breed in specific region especially in China and Pakistan based on the investigated breeds. The Ossimi breed contains distinct modified residues; Lys<sup>2</sup> and Thr<sup>5</sup> vs. Ala<sup>3</sup> and Pro<sup>6</sup> in all investigated breeds, respectively (Table 3). The substitution of Pro<sup>6</sup> to Thr<sup>6</sup> and Ala<sup>3</sup> to Lys<sup>2</sup> are located out open reading frame therefore did not affect the protein function [32], [33] and [41]. In the present study, the Gly<sup>35</sup> residue was presented in all investigated breeds vs. Ser<sup>35</sup> residue in Afghani sheep breed. Because the both Gly and Ser are tiny and small residues and the Gly is not in the conserved domain sequence; therefore the substitution is functional [32]. The substitution of Phe<sup>123, 217</sup> and Leu<sup>188</sup> in all tested breeds with Leu<sup>123, 217</sup> and Phe<sup>188</sup> in Lezhi Black goat, Dhanni x Friesian cattle crossbred and Iranian cattle breed, respectively are acceptable and may use this substitution as a distinct genetic marker in Lezhi Black goats and in Iranian cattle breed. In the present study, the Glu<sup>154</sup> residue is presence in all breeds vs. Gly<sup>154</sup> in Pakistani Afghani ovine breed. This mutation may causes reduction in growth hormone function in this breed resulting in the differences between them in physio-

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chemical properties. The Tyr<sup>168</sup> residue is present in all tested breeds vs. His<sup>168</sup> residue in Pakistani Latti ovine breed. Because the both histidine and tyrosine are polar amino acids (neutral), therefore Tyr can substitute with His residue [32].

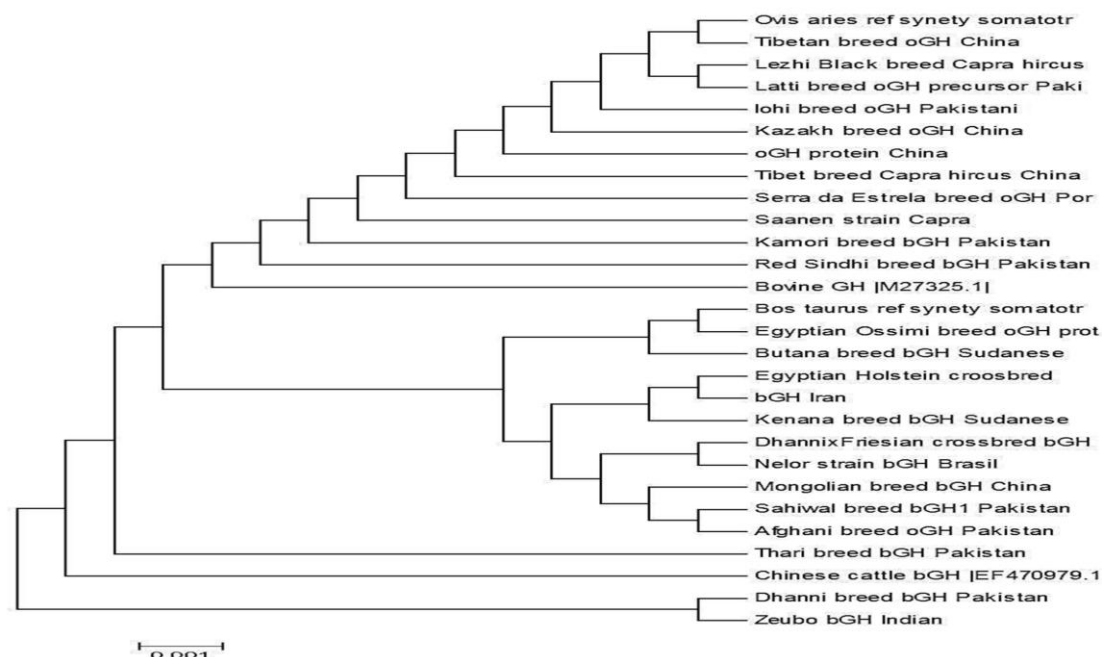


**Fig12.** Multiple alignments of the encoded proteins of Egyptian Holstein crossbred and Ossimi breed vs. tested NCBI database breeds.

### 3.4.6. Phylogeny Tree and Synteny

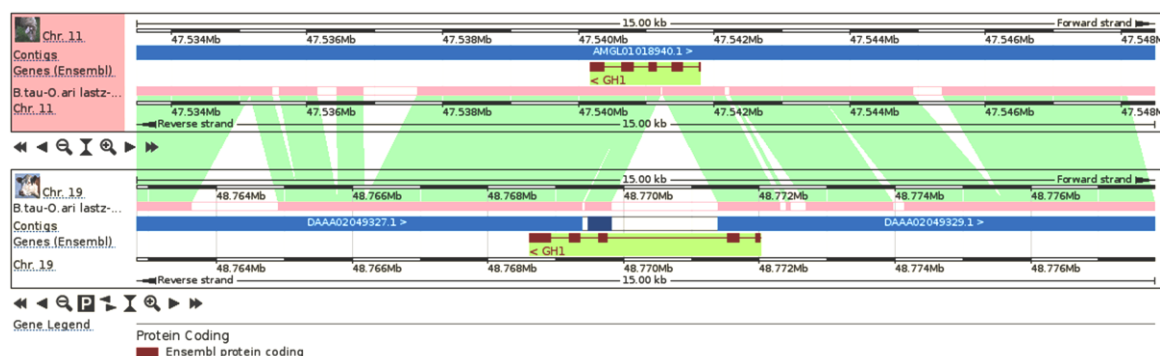
The phylogenetic tree of EH\_GH and Os\_GH protein sequences vs. investigated breeds has been shown in Fig. 13. The predicted synteny location between Egyptian Holstein crossbred (Chr 19) and Ossimi breed (Chr 11) is shown in Fig. 14. Comparisons of mammalian gene maps show

large regions of conserved synteny where the same genes map to equivalent regions in different species [4], [5], [42], [43] and [44].



**Fig13.** Phylogenetic tree of Egyptian Holstein crossbred and Ossimi breed growth hormone protein sequences vs. tested NCBI breeds.

From phylogenetic tree and synteny results have been illustrated that EH\_GH gene at chromosome 19 is an orthologous gene to the Os\_GH gene at chromosome 11, as expected from their close evolutionary relationship [45]. The results support our assumptions and our previous work [12], and can induce a clue for further studies to produce transgenic sheep carry an expressed copy of bovine growth hormone gene using Sperm Mediated Gene Transfer (SMGT) technique as mentioned previously in different mammalian systems [46], [47] and [48] in mice; [49] in rabbits; [50] in pigs; [51] and [52] in Goat; [53] and [54] in sheep and [55] in cattle.



**Fig14.** The predicted synteny location of growth hormone protein sequences between Egyptian Holstein crossbred (Chr 19) and Ossimi breed (Chr 11).

#### 4. CONCLUSION

It could be concluded that GH sequence of Egyptian Holstein crossbred cattle and Ossimi sheep breed are orthologous genes (98%). We assume in this study that the novel genetic information about the bovine and ovine GH such Egyptian breeds can support further success possibilities of producing transgenic sheep carrying bovine GH, using sperm mediated gene transfer (SMGT) technique to enhance their performance.

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