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**Abstract**

Mohair, an important source of fiber, is only obtained from Angora goats. The important characteristics that determine the economic value of mohair are fiber diameter and quantity. In countries where mohair is produced, efforts are made to improve these characteristics. It is stated that hair follicle characteristics and/or genetic regulation mechanisms that form animal fibers directly affect fiber production and quality. In this study, it was aimed to determine the genes affecting mohair development in two varieties of Angora goat and the molecular mechanisms affecting these genes. The biopsy samples were collected during three distinct phases of the hair growth cycle: anagen (active growth, September), catagen (transition, January), and telogen (resting, March). The specific timing and location of the biopsies suggest a potential influence of seasonality or a controlled experimental design. RNA was isolated from these biopsy samples, and differentially expressed genes and the pathways affected by these genes were evaluated using the RNA sequencing method. It has been observed that the expression of *KRTAP8-1, KRTAP16.4,* and *KRTAP21-1* genes was quite high in the group in which catagen and telogen phases were compared in Eskisehir variety females. Interestingly, the *KRTAP21-1* gene was found to be expressed in four different protein isoforms. Interestingly, the analysis revealed a cluster of keratin-associated protein genes (*KRT40, KRT72, KRTAP10*) solely differentially expressed in the male Eskisehir versus Lalahan comparison. This suggests potential sex-specific regulatory mechanisms involving keratinocyte differentiation during the catagen phase, which might be unique to the Eskisehir variety. In the Eskisehir variety, unlike the Lalahan variety, DEGs identified in the anagen-catagen comparison in males were significantly enriched in the Reactome mediated keratinization pathway, and these genes were down regulated. The results showed that *KRT* and *KRTAP* genes are highly functional and have different expression patterns between males and females, in addition to being different between the Eskisehir and Lalahan varieties. This study provides valuable insights into the genetic regulation of mohair development, potentially paving the way for targeted breeding strategies to improve mohair quality and production.

**Keywords**: Mohair, Hair follicle cycling, Angora goat, Transcriptomics

# Introduction

Goats are widely considered to be one of the earliest species to be domesticated. The bezoar goat (*Capra aegagrus*), which inhabits the rugged mountainous regions stretching from the Taurus Mountains in Turkey to Pakistan, is considered to be the wild ancestor of the domestic goat (*Capra hircus*) [1]. It is therefore plausible that the indigenous goat breeds found in present-day Turkey are direct descendants or closely related to the ancestral populations.

Goat breeds are classified into dairy, meat, fiber, and combined productivity breeds. The Angora goat holds significant prominence in Turkey due to its crucial role in mohair production, characterized by its lengthy and glossy coat [2]. Angora goats have been raised in numerous countries for an extended period, including Türkiye, Australia, America, Argentina, New Zealand, France, and South Africa. The origins of this breed can be traced back to Ankara, the capital city of Türkiye. Mohair is a valuable animal product and an important raw material for the textile industry. It absorbs moisture easily, it is durable, shiny, elastic, resistant to harmful sun rays, heat-resistant, highly insulating, and easy to dye [3]. The economic value of fiber is primarily determined by its fineness and quantity, which are considered to be the most crucial factors. The value of mohair in world markets depends primarily on its quality characteristics, including fineness, quantity, length, curl, and strength. Therefore, efforts are being made in mohair-producing countries to enhance these characteristics and improve the overall quality of the product [4].

Before these traits are accepted as selection criteria, genetic parameters need to be estimated. Numerous research studies have consistently shown that the characteristics of hair follicles (HF) and the genetic regulatory mechanisms associated with them have a direct impact on the production and quality of hair fibers [5-16]. Enhancing our knowledge and comprehension of the developmental and biological properties of the fibers generated by hair follicles can offer valuable strategies for obtaining fibers that possess the desired properties [6]. Animal fibers are generated through the hair follicles found in the skin. These fibers possess unique mechanisms that enable their growth, development, and self-renewal. The hair follicles themselves are regarded as miniature organs that arise from the interaction between

neuroectodermal and mesodermal tissues [17]. The embryonic period initiates hair follicle formation, leading to its full growth within the same developmental stage [4]. The initial phase of development involves a series of stages collectively known as morphogenesis, including induction, organogenesis, and cytodifferentiation [18]. Following birth, hair follicles undergo a recurrent process known as the hair follicle cycle. This cycle consists of three specific phases: anagen, catagen, and telogen. Each phase of the hair follicle cycle has a unique pattern of gene activation and suppression. The transition between stages is intricately controlled by transcription factors and enzymes, which are influenced by specific signaling molecules present in the immediate environment. These signaling molecules consist of cytokines, hormones, neurotransmitters, and essential mediator molecules [19].

During the anagen phase, hair follicles undergo a period of rapid growth. Subsequently, in the catagen phase, the hair bulbs undergo contraction, resulting in a gradual thinning as they ascend. Hair follicles in the telogen phase are situated solely above the sebaceous glands and remain in a stationary condition [20]. The cycle initiates as the HF reaches maturity and enters the catagen phase in response to various activating signals. Apoptosis is a distinctive feature of the catagen phase, wherein programmed cell death takes place in the inner root sheath (IRS), outer root sheath (ORS), and hair matrix. This apoptotic process leads to the regression of the lower two-thirds of the hair follicle, ultimately resulting in the development of the epithelial column. Simultaneously, the dermal papilla (DP) ascends, approaching the bulge region in close proximity. During the telogen phase, the main hair shaft experiences a change and transforms into club hair, all the while maintaining the close proximity of the DP to the bulge. The onset of the anagen phase is triggered by the interaction between the bulge and the DP. During this stage, the activation of hair follicle stem cells (HFSC) occurs in the upper segment of the hair follicle. Subsequently, the hair follicle starts to grow downward, giving rise to the bulb and other related structures. As a result, new hair is produced while the old club hair is shed [21]. These phases, the duration of which varies from organism to organism and from follicle to follicle, are the anagen, catagen, and telogen phases, respectively. In goats, the secondary follicles that form cashmere and mohair fibers enter the active anagen phase between June and November and grow for about 185 days. This is followed by the catagen

phase, which lasts about 60 days between December and January. From February to the end of May, the telogen phase extends for approximately 120 days [22].

Hair follicle regulation involves the activation of specific genes known as Keratin genes (*KRTs*) and the presence of Keratin-associated proteins (*KAPs*). Moreover, the growth and maturation of hair follicles are profoundly impacted by a multitude of signaling pathways, including but not limited to MAPK, PI3K-Akt signaling, Ras signaling, and the cell cycle [20]. Nevertheless, the exact features, chronological order, and intersections of these signaling pathways are yet to be fully elucidated.

To produce finer animal fiber, it is crucial to comprehend the regulatory role that genes, signaling pathways, and other processes play in hair follicle growth. Transcriptome sequencing technology was utilized in this study to explore the mRNA expression profile of goat skin tissue. The research was conducted on two Ankara goat varieties, Eskisehir and Lalahan, with both males and females studied in three different growth phases: anagen, catagen, and telogen. The aim of this study was to determine the mRNA expression profile of skin tissues collected from Angora goat varieties using RNA-seq analysis. This is the first study to use the mRNA expression profile of skin tissues to analyze differences between Angora goat varieties in the anagen, catagen, and telogen phases for both males and females.

# Materials and Methods

**Animals and Sampling**

The animal material for this study consisted of Lalahan and Eskisehir varieties of Ankara goats. The Lalahan variety of Ankara goats has been purebred since 1930 and has been under protection since 1997. The Eskisehir variety of Ankara goats carries US Ankara goat blood in its genotype and was brought to the International Center for Livestock Research and Training (ICLRT) in 1997 from the Eskisehir Anatolian Agricultural Enterprise as part of the "Protection of Animal Genetic Resources" project conducted by TAGEM, which is the General Directorate of Agricultural Research and Policies under the Ministry of Agriculture and Forestry. For this study, four female and three male Lalahan variety Angora goats and three female and three male Eskisehir variety Angora goats (located at 39°58'16.71 "N latitude

and 33° 6'33.27 "D coordinates) were used. Angora goats in each group were selected from clinically healthy goats born in 2021, and the same management and nutritional conditions were applied to all animals.

Three skin samples were taken from each goat as biological duplicates at every stage of hair follicle growth (September, January, and March). In total, 72 samples were analyzed in this study, including samples from two Angora goat varieties, two gender, three developmental stages (three biological replicates), and two technical replicates for RNA sequencing.

Skin tissue samples were taken from the right shoulder region, which is the upper one-third of the left scapula, of these goats during the anagen phase (September), catagen phase (January), and telogen phase (March) using a 0.3 mm diameter punch biopsy tool while adhering to sepsis and antisepsis protocols. The shoulder region was shaved and wiped with 70% alcohol prior to the biopsy. For each animal, prilocaine hydrochloride was applied subcutaneously for local anesthesia to reduce animal pain. A 0.3 mm diameter punch biopsy instrument was used to obtain a biopsy sample from the shoulder region, and the biopsy materials were placed in an RNA preservation solution to prevent degradation and stored at -80°C until RNA isolation. Ethics committee permission dated September 15, 2022, and numbered 205 was obtained from the ICLRT Animal Experiments Local Ethics Committee for this study.

**Total RNA extraction**

The extraction of RNA from the skin tissue samples was carried out utilizing the Qiagen RNeasy® Fibrous Tissue Mini Kit (Qiagen, Germany) in accordance with the provided kit protocol. The assessment of RNA quality and quantity was conducted using Thermo NanoDrop 2000 spectrophotometry, measuring the absorbance at A260 / A280 nm wavelengths. RNA purity was determined by ensuring that the absorbance ratio at A260/A280 nm was equal to or greater than 2.0. To assess the integrity and quality of the RNA sample, the Qubit RNA IQ Assay was performed on a Qubit 4.0 fluorometer (Life Technologies, CA, USA) using the Qubit RNA BR Assay Kit (500 Assays, Invitrogen-Thermo Fischer Scientific, Cat. No: Q10211). The RNA Integrity Number (RIN) was evaluated using the Bioanalyzer 2100 system (Agilent Technologies, CA, USA) with the RNA Nano 6000 Assay Kit. For the purpose of RNA sequencing, only RNA samples meeting the criteria of having a

RIN value above 8.0 and an OD260/OD280 ratio higher than 1.9 were utilized. The total RNA was appropriately stored in a freezer maintained at a temperature of -80 °C.

**Library preparation and RNA sequencing**

The cDNA library was constructed using the Illumina Stranded Total RNA Prep Ligation With Ribo-Zero Plus kit, adhering to the guidelines provided by the manufacturer. The Illumina Ribo-Zero Plus rRNA Depletion Kit was used to deplete rRNA from the samples and to obtain mRNA before RNA sequencing. The Illumina stranded total RNA kit was used to generate libraries from ribosomal RNA-depleted samples. The constructed cDNA library was sequenced using the Illumina Nova Seq 6000 platform, and paired-end reads of 150 base pairs (bp) were generated during the sequencing procedure.

**Quality control and Data analysis**

Within the scope of the study, expression counts of goat (*Capra hircus*) samples were calculated, and comparisons were performed between the groups. The quality of the raw data was evaluated using the FastQC software. The raw data in FASTQ format underwent primary quality control as the first step in the processing pipeline. In this stage, raw reads including more than three poly-N sequences or adaptor sequences, as well as those containing a base percentage with a quality value, were filtered away in order to produce clean reads. All further analyses were performed using only the high-quality, clean data from the previous processing step. Then, the Trimmomatic tool (version 0.39) was used (i) to trim the reads with a phred score less than 20 and (ii) to filter out the reads shorter than 20 bases long. Moreover, Kallisto v0.44.0 was utilized to estimate transcript abundance by pseudo aligning the reads [23].

**Gene Level Quantification and Transcriptome Assembly**

The expression values at the gene level were obtained using the Kallisto tool (version 0.46.1) and the tximport R package. *Capra hircus* cDNA, ncRNA, and gtf files were obtained from the genome database (https://www.ncbi.nlm.nih.gov/datasets/genome/?taxon=9925) [24]. cDNA and non-coding RNA (ncRNA) were combined and indexed using the Kallisto tool. Transcript-level raw counts were obtained using the Kallisto quant function. The computation of gene-level raw counts was carried out using the tximport tool. The alignment of clean reads from each sample to the goat reference genome, ARS1.2 (GCA_001704415.2, https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_001704415.2/) [25],

was performed using the HISAT2 software. StringTie software was employed for the tasks of transcriptome assembly, annotation, and expression calculations [26].

**Analysis of Differentially Expressed Genes (DEGs)**

The Detection of differentially expressed genes (DEGs) was performed by the DESeq R package [27]. DESeq first normalizes raw counts and uses the normalized values for statistical analysis. Prior to normalization with DESeq, genes with a count of zero were removed from the calculation in all the groups being compared. PCA (Principal Component Analysis) was performed using all samples to test for the presence of outliers. The data used for PCA analysis was the DESeq normalized gene counts. According to this result, no outlier sample was detected, and differential expression analysis was conducted using all samples. The adjusted p-values (Benjamini-Hochberg corrected) and fold changes for each gene in the dataset were computed using the DeSeq package.

DEGs were detected by applying a false discovery rate (FDR) threshold of $\leq 0.001$ and an absolute log2Ratio value of $\geq 1$ (representing a two-fold change) as the thresholds for significance. In cases where the fold change exceeded 1 and the FDR was less than 0.05, the observed change was classified as significant upregulation. Conversely, if the fold change was less than 1 and the FDR was less than 0.05, the change was designated as significant downregulation.

In the scope of the differential expression analysis, comparisons were performed for a total of 3 main groups, which include “Gender”, “Variety”, and “Inter Variety” groups. Differentially expressed genes were identified in the gender group by comparing male and female individuals within each variety at the anagen, catagen, and telogen phases. Therefore, a total of six comparisons were made within this group. In the Variety group, differences in gene expression patterns between anagen and catagen, anagen and telogen, and catagen and telogen phases were analyzed for each variety and gender. Thus, a total of 12 comparisons were performed within this group. Finally, in the inter-variety group, each phase was compared between varieties separately for male and female individuals, and as a result DEGs in each phase were identified between varieties separately in male and female individuals. This group was also subject to a total of six comparisons.

To assess the influence of gender on differentially expressed genes associated with hair growth, six distinct groups were defined for each variety: (1) Eskisehir Anagen Female versus Eskisehir Anagen Male; (2) Eskisehir Catagen Female versus Eskisehir Catagen Male; (3) Eskisehir Telogen Female versus Eskisehir Telogen Male; (4) Lalahan Anagen Female versus Lalahan Anagen Male; (5) Lalahan Catagen Female versus Lalahan Catagen Male; (6) Lalahan Telogen Female versus Lalahan Telogen Male.

In the variety group, to identify DEGs between phases in each variety and each hair growth, phases were compared separately in males and females. A total of 12 comparisons were performed within this group. For the purpose of comparing experimental groups, the following contrast groups were established: (1) Eskisehir Female Anagen versus Eskisehir Female Catagen; (2) Eskisehir Female Anagen versus Eskisehir Female Telogen; (3) Eskisehir Female Catagen versus Eskisehir Female Telogen; (4) Eskisehir Male Anagen versus Eskisehir Male Catagen; (5) Eskisehir Male Anagen versus Eskisehir Male Telogen; (6) Eskisehir Male Catagen versus Eskisehir Male Telogen; (7) Lalahan Female Anagen versus Lalahan Female Catagen; (8) Lalahan Female Anagen versus Lalahan Female Telogen; (9) Lalahan Female Catagen versus Lalahan Female Telogen; (10) Lalahan Male Anagen versus Lalahan Male Catagen; (11) Lalahan Male Anagen versus Lalahan Male Telogen; (12) Lalahan Male Catagen versus Lalahan Male Telogen.

**Functional Enrichment and Protein–Protein Interaction Analyses of DEGs**

The genes that were expressed differentially were subject to enrichment analyses through the use of the g: Profiler tool for investigating their roles in the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genome (KEGG) pathways [28] and KOBAS [29] online analysis database. The KOBAS analysis has been conducted by configuring the query parameters to align with the chosen organism, *Capra hircus*. Subsequent analysis focused on GO terms and KEGG pathways, with significance determined by an adjusted P value below 0.05. Functional annotations for differentially expressed transcripts were performed using the GO database, which assigned them to one of three categories: biological processes (BPs), molecular function (MF), or cellular component (CC). The candidate genes were analyzed using the STRING database [30] to generate a functional protein-protein interaction (PPI) network, which was visualized with the Cytoscape program [31].

bioDBnet is a tool that allows users to perform ortholog conversions, converting identifiers from one species to those of another species. The conservation of gene identities across species has been analyzed using bioDBnet [32].

**Relative Gene Expression Analysis by qPCR**

To confirm the differential gene expression determined by RNA sequencing, Quantitative Real-Time PCR (qPCR) validation was performed with five randomly selected genes (*KRTAP8.1, FOS, FGF5, FOSL1,* and *FOSB*) that exhibited differential expression across the three developmental stages. *GAPDH* was utilized as the endogenous control in this experiment. The qPCR reaction was conducted using a total volume of 10ul, comprising of 2ul of cDNA, 5ul of SYBR green real-time master mix, and 0.5ul of each primer. Relative gene expression levels were determined using the 2-ΔΔCT method [33]. At three distinct developmental stages, total RNA extraction was performed on all experimental goat skin samples. The QuantiTect Reverse Transcription Kit (Qiagen, Cat ID: 205311) was used for synthesizing cDNA from the RNA in accordance with the manufacturer's instructions. The primer sequences are provided in Table S1. The quantitative polymerase chain reaction (qPCR) was conducted on the Bio-Rad CFX96 Real-Time PCR System using the Bio-Rad Universal SYBR® Green Supermix. The accuracy of the SYBR green PCR signal was verified through an analysis of the melting curve, ensuring its specificity.

# Results

**Overview of high-throughput sequencing**

From 36 samples, a cumulative RNA-Seq dataset of 211.94 GB was generated, with Q20 percentages exceeding 99.69% and Q30 percentages surpassing 99.17% for each sample. The GC content ranged from 42% to 50%. Supplementary File 1 provides a comprehensive overview of quality control and alignment statistics, affirming the high quality and uniformity of the sequencing data across all sample sets. The quality control outcomes ascertain that the sequencing results are robust and reliable, establishing a solid foundation for subsequent data processing.

**Analysis of the DEGs**

Following the mapping of clean reads to the goat reference genome, comparisons were made between each growth cycle. Differences in gene expression patterns between anagen and catagen, anagen and telogen, and catagen and telogen phases were compared between varieties and between genders to identify DEGs of hair follicle development and cycle at different stages.

**DEGs analysis results for gender:**

Through the comparison of gene expression profiles in female and male goats within the same variety and hair growth phase, we identified genes exhibiting differential expression between males and females. In the anagen phase, a total of 164 and 355 DEGs were detected between male and female individuals of the Eskisehir and Lalahan varieties, respectively. Furthermore, a notably higher count of DEGs was observed during the anagen phase in the Lalahan Angora goat in comparison to the Eskisehir Angora goat. This suggests that there may be some important differences in the way that these two varieties of goats regulate hair growth. The volcano plot generated for this group is shown in Figure 1.

In the catagen phase, a total of 73 and 87 DEGs were detected in the comparison between male and female individuals of the Eskisehir and Lalahan varieties, respectively. Additionally, 24 DEGs were detected in the telogen phase between male and female individuals of both the Eskisehir and Lalahan Angora goats (Figure 2 and Supplementary File 2). This observation suggests that when comparing male and female individuals in both Eskisehir and Lalahan Angora goats, the differentiation in genes expressed during the anagen phase is more prominent than during the catagen and telogen phases. This information could be used to improve the quality of Angora goat mohair and could be important for understanding the genetic basis of hair growth.

**DEGs analysis results for variety group:**

By conducting this comparison, we were able to identify genes that exhibited differential expression among the three different stages within the same gender and variety. Between the anagen and catagen phases in the female Eskisehir variety of Angora goats, a total of 111 DEGs were identified. Among these genes, 30 were upregulated, while 81 were downregulated. On the other hand, a total of 151 DEGs were detected between the anagen and catagen phases in the male Eskisehir variety of Angora goat, of which 100 genes were upregulated and 51 genes were downregulated. Moreover, a total of 18 DEGs

were detected between the anagen and catagen phases in the female Lalahan variety of Angora goats, of which 11 were upregulated and 7 were downregulated. Furthermore, a total of 76 DEGs were detected between anagen and telogen phases in the female Eskisehir variety of Angora goat, of which 13 were upregulated and 63 were downregulated. In this group, *KRTAP7-1* and *KRTAP8-1* were identified as the first two DEGs (padj= 6,09E-15 and 7,04E-14, respectively; and FC:0,2745; 0,2168, respectively. Supplementary File 3). The volcano plot generated for this group is shown in Figure 3.

For the female Lalahan variety of Angora goat, a total of 23 DEGs were detected between the anagen and telogen phases, of which 12 were upregulated and 11 were downregulated. In this group, *KRTAP7-1* and *PTGS2* were identified as the first two DEGs (padj= 9,83E-09 and 2,08E-06, respectively; and FC: 0,127; 5,115, respectively). The comparison results obtained for the other groups are shown in Figure 4. Gene expression patterns in the male groups appeared to be significantly different from those in the female groups at each hair growth phase.

In the inter-variety group, a separate comparison was performed between varieties for each phase, considering male and female individuals. As a result, differentially expressed genes were identified in each phase, separately for male and female individuals, leading to a total of six comparisons within this group. There were no statistically significant DEGs observed in the telogen phase between the female Eskisehir and Lalahan Angora goat varieties. A total of 4 genes (*TNNT1, B2M, KRTAP 16.2,* and *ENSCHIG00000011606*) were identified as statistically significantly downregulated with differential expression in the anagen phase between the female Eskisehir and Lalahan Angora goat varieties (Figure 5).

In the catagen phase, 63 DEGs were found between the female Eskisehir and Lalahan Angora goat varieties, with 7 upregulated (*LOC108635997: Keratin, type II cytoskeletal 6A-like; LOC102174594: Keratin-associated protein 9-9-like; Keratin-associated protein 4-11; Keratin-associated protein; LOC102171368: Keratin-associated protein 4-9-like; ARHGEF19;* and *LOC108633884*. Supplementary File 4) and 56 downregulated genes (Figure 6A). On the other hand, there were no statistically significant DEGs observed in the anagen phase between the male Eskisehir and Lalahan Angora goat varieties. A total of five DEGs (*TRPM1, KLHL4, ENSCHIG00000011606, ENSCHIG00000018556*, and *HAL*) were

found between the male Eskisehir and Lalahan Angora goat varieties in the telogen phase. In the catagen phase, a total of 177 DE genes were found between the male Eskisehir and Lalahan Angora goat varieties. Of these, 47 were upregulated, and 130 were downregulated (Figure 6B).

To identify differentially expressed genes specific to each goat variety in each phase of hair growth, we compared the DE genes for each variety and sex in each phase. Figure 7 shows the Venn diagram of the DEGs identified in this comparison group. These findings indicate that the DEGs identified are phase and variety-specific, demonstrating sex-specific patterns as well.

The findings revealed that among the female Eskisehir Angora goat varieties, the Differentially Expressed (DE) genes exhibited the greatest variation between the anagen and catagen phases, with a comparatively smaller differentiation observed between the catagen and telogen phases. On the other hand, in the female Lalahan Angora goat varieties, the DEGs in all groups were smaller than those in the Eskisehir Angora goat variety. Our findings suggest that there are significant physiological differences between Eskisehir and Lalahan Angora goats at different stages of hair follicle cycling.

**Enrichment of DEGs**

In order to gain a comprehensive understanding of the biological processes and pathways related to the development of mohair hair follicles, we carried out GO and KEGG analyses. The top enriched GO terms (p-value < 0.05) were identified by comparing DE across groups. We conducted gene ontology and KEGG pathway analyses on each of the 24 groups, and the obtained results are shown in Supplementary Figure S1-Figure S15 and Supplementary File 5.

In the gender group, the comparison of data between the Eskisehir Telogen Female and Male groups revealed that DEGs were significantly enriched in the keratin filament (GO:0045095) and intermediate filament (GO:0005882) categories, according to the GO CC analysis. *ENSCHIG0000000025280* (*LOC106503217: keratin-associated protein 4-9-like*), *ENSCHIG0000000020416* (*LOC108638288: keratin-associated protein 4*), *ENSCHIG0000000009215*, *ENSCHIG00000002703, ENSCHIG00000007577* (*LOC108638300: Keratin, high-sulfur matrix protein, IIIA3-like*) genes that were upregulated were significantly enriched in the keratin filament and intermediate filament categories (Supplementary Figure S3 and Supplementary File 5).

The development and cycling of goat hair follicles have been extensively investigated in previous studies. These investigations have shed light on the involvement of multiple key pathways, including tumor necrosis factor (*TNF*), fibroblast growth factor (*FGF*) [5], *MAPK* signaling, *WNT* pathway [33], bone morphogenetic protein (*BMP*) family [34], transforming growth factor (*TGF*) family [35], Sonic hedgehog (*SHH*) [36], and *NOTCH* signal transduction pathways [37].

Consistent with the preceding investigations, our results suggest a notable enrichment of the TNF signaling pathway (chx04668) within the comparison group of Lalahan Catagen Female vs. Male (Supplementary Figure S5 and Supplementary File 5). *ENSCHIG00000007846* (*JUNB: JunB proto-oncogene, AP-1 transcription factor subunit*), *ENSCHIG00000014217* (*LOC102182395: growth-regulated protein homolog gamma*), *ENSCHIG00000025384* (*FOS: Fos proto-oncogene, AP-1 transcription factor subunit*), *ENSCHIG00000021461* (*TNFAIP3: TNF alpha induced protein 3*), and *ENSCHIG00000011290* (*SOCS3: suppressor of cytokine signaling 3*) genes were significantly enriched in the TNF signaling pathway.

In the variety group, for the Eskisehir Female Anagen vs. Catagen comparison, DE genes for GO BP were significantly enriched in muscle system processes (GO:0003012). For the cellular component, DEGs were enriched mainly in myofibrils (GO:0030016) and intermediate filaments (GO:0005882). Then, for molecular function, most of the DEGs were enriched in actin binding and actin filament binding. The KEGG pathway analysis showed that DEGs in comparison between Eskisehir Female Anagen and Catagen were significantly enriched in the Calcium signaling pathway (chx04020) (Supplementary Figure S6 and Supplementary File 5). Moreover, out of the 111 genes that were identified through the comparison of Eskisehir Female Anagen vs. Catagen, 94 were found to be orthologous to Homo sapiens. We conducted a Reactome pathway analysis using these 94 genes. The Reactome pathway analysis revealed that the DEGs identified in this group were significantly enriched in the keratinization (*R-HSA-680556; KRTAP11-1, KRTAP13-3, KRTAP10-9, KRTAP10-6, KRTAP3-3, KRTAP4-5, KRT33A, KRTAP3-1*) pathway (Figure 8).

In the Lalahan variety of Angora goat, 716 DEGs were identified between anagen and catagen phases in males, out of which 654 were found to be orthologous with humans. In contrast to the results of

the Eskisehir Female Anagen and Catagen comparison, the Reactome pathway analysis revealed that the DEGs in the Lalahan Male Anagen vs. Catagen comparison group were significantly involved in the "*Keratinization*" (R-HSA-6805567), "*Muscle Contraction*" (R-HSA-397014; 27 genes), and "*Formation of the Cornified Envelope*" (R-HSA-6809371; 21 genes) pathways (Figure 9). On the other hand, DE genes for GO BP were significantly enriched in "muscle structure development" (GO:0061061, 57 genes) and "*actin filament-based processes*" (GO:0030029). For the cellular component, DEGs were enriched mainly in "*keratin filament*" (GO:00045095) (Table 1) and intermediate filament (GO:0005882) (Supplementary Figure S11 and Supplementary File 5).

In the Eskisehir Female Anagen vs. Telogen comparison, the DEGs involved in gene ontology biological processes were significantly enriched in "*actin-myosin filament sliding*" (GO:0033275) and "*myofibril assembly*" (GO:0030239). For the cellular component, the DEGs were mainly enriched in "*myofibril*" (GO:0030016; 18 genes) and "*intermediate filament*" (*GO:0005882; KRT33A, KRTAP1-1, KRTAP1-3, KRTAP1-4, KRTAP1-5, KRTAP11-1, KRTAP15-1, KRTAP7-1, KRTAP8-1*). A detailed cnetplot visualization of these cellular component processes is presented in Figure 10.

The Lalahan Female Anagen vs. Catagen, Anagen vs. Telogen, Catagen vs. Telogen, and Eskisehir Female Catagen vs. Telogen groups were excluded from GO and pathway analysis due to the limited number of genes in these groups.

In the inter-variety group, for the Catagen Female Eskisehir vs Lalahan comparison, DEGs for GO CC were significantly enriched in "*myofibril*"(GO:0030016, 22 genes) and "*keratin filament*" (GO:0045095). For molecular function, most of the DEGs were found to be enriched in the "*process of acting binding*" (GO:0003779, 14 genes) (Supplementary Figure S14 and Supplementary File 5). For the Catagen Male Eskisehir vs. Lalahan comparison, DE genes for GO BP were significantly enriched in "*muscle structure development*" (GO:0061061, 18 genes), For the cellular component, DEGs were significantly enriched in the "*keratin filament*" (GO:0045095, 19 genes). The KEGG pathway analysis showed that DEGs in the comparison between Catagen Male Eskisehir and Lalahan were significantly enriched in the Metabolic pathway (chx01100) (Supplementary Figure S15 and Supplementary File 5). Moreover, to identify DEGs specific to each gender in the catagen phase of hair growth for each goat

variety, we compared the DEGs for each variety and sex in this phase (Figure 11). The results showed that *KRT40, KRT72, KRTAP10, KRTAP9, KRTAP2, KRTAP3*, and *KRTAP8* were only differentially expressed in the Male catagen Eskisehir vs. Lalahan group, while KRT6 was only differentially expressed in the Female catagen Eskisehir vs. Lalahan group. This information can be used in breeding selection.

**Validation of RNA-Seq data by qPCR**

To confirm the results of the transcriptomic analysis, we randomly selected five genes with differential expression and performed a qPCR analysis. The genes chosen for analysis —*FGF5, FOS, KRTAP8, FOSL1,* and *FOSB*—exhibited a consistent expression pattern in both the qPCR results and RNA-Seq data, thereby confirming the reliability of our transcriptome information (Supplementary Figure S16).

# Discussion

Within a dynamic environment, the hair follicle, a functional miniature organ, undergoes development that is influenced by various molecular signals. The hair cycle, which consists of three distinct phases, controls the growth, regression, and resting periods of hair follicles. This cycle serves as an important model for investigating the regulation, activation, and quiescence of stem cells, as well as the processes of cell proliferation, differentiation, and apoptosis within adult regenerative epithelial tissue.

Insufficient knowledge exists regarding the alterations in gene expression that occur during the transition from anagen to catagen and telogen in the Angora goat and its specific varieties. To advance our understanding of the molecular mechanisms underlying this transformation, it would be beneficial to conduct global gene expression measurements and perform differential gene expression profiling of distinct hair follicles. As of our current knowledge, the application of RNA-Seq technology for analyzing hair follicles in Angora goats and their specific varieties has not been reported to this date.

In this study, according to the comparison results of males and females in each phase and variety, a higher number of DEGs were found in the anagen phase than in the catagen and telogen phases, while the lowest number of DEGs were found in the telogen phase. In contrast to our results, Su et al. observed

406 distinctive gene expression patterns in Inner Mongolian Cashmere goat skin samples across various stages
407 of the hair growth cycle [38]. Notably, they identified 51 DEGs between the anagen and catagen stages,
408 443 DEGs between the catagen and telogen stages, and 779 DEGs between the telogen and anagen stages.
409 These findings provide insights into the molecular mechanisms that lie under the hair growth cycle in this
410 specific breed of goats. The authors emphasized that the most substantial number of DEGs was observed
411 between telogen and anagen, whereas the smallest number of DEGs was identified between anagen and
412 catagen. The contradictory results of these studies confirm that hair follicle development in goats may
413 vary according to breeds. However, our results are consistent with the process of the hair growth cycle. A
414 hair shaft is produced by the follicle from tip to root during the growth period, or anagen. The hair follicle
415 experiences epithelial proliferation and differentiation during the anagen phase. During this stage, the
416 inner root sheath undergoes keratinization, a process that imparts structural stability and direction to the
417 growing hair shaft. In contrast, the catagen phase represents a transitional stage where the follicle
418 prepares for the telogen phase, which is a period of dormancy where no new hair is produced. The
419 transition from the anagen phase to the catagen phase is of utmost importance in the hair cycle. In this
420 phase, the matrix cells, which are responsible for hair growth, undergo a restricted number of cell
421 divisions before initiating the process of differentiation. As the availability of matrix cells decreases, the
422 process of differentiating the hair shaft (HS) and inner root sheath (IRS) slows down, leading the follicle
423 into a destructive phase known as catagen. Subsequently, the hair follicles enter a resting phase called
424 telogen. The activation of one or two quiescent stem cells, located at the base of the telogen follicle near
425 the dermal papilla (DP), is accountable for the transition from telogen to anagen. This transition leads to
426 the formation of a new hair shaft. The cells initiate a rapid proliferation process, resulting in the rapid
427 multiplication of transit-amplifying daughter cells that will eventually give rise to the formation of the
428 new hair follicle. This newly formed follicle takes shape adjacent to the existing pocket that houses the
429 club hair, which will eventually be shed. As a consequence, a bulge is formed, comprising a fresh layer of
430 stem cells that play a crucial role in replenishing the stem cell reservoir. Interestingly, the emerging hair
431 emerges from the same upper opening as the previous hair. The transition from the telogen to anagen
432 phase bears similarity to the activation of embryonic skin stem cells, wherein they are stimulated to

generate a new hair follicle from scratch. Most of signaling molecules that regulate hair follicle morphogenesis belong to Wnt pathway, fibroblast growth factor (FGF) family, tumor necrosis factor (TNF) family, bone morphogenetic protein (BMP) family, Sonic hedgehog (Shh) transduction pathway, transforming growth factor (TGF) family and Notch transduction pathway. Some of them show activator and some inhibitory effects. However, the exact nature, timing, and intersections of these signaling pathways remain unclear. [39].

The expression levels of most genes in the skin were correlated with the activity of the hair follicle, suggesting that the two are closely linked. Therefore, these results suggest that there were probably gradual changes in the skin between male and female Angora goats as the hair follicle moved from the growth phase to the resting phase.

Similarly, in the variety group, the gene expression patterns in the male groups appeared to be significantly different from those in the female groups when comparing each hair growth phase. This result confirms the effect of androgens and estrogens on the hair growth process. It's been known for over 70 years that estrogens play a role in skin physiology and the control of hair growth. Previous research has established a connection between sex steroid hormones and the regulation of different aspects of skin structure and function. These hormones play a role in controlling processes such as hair growth, pigmentation, vascularity, water retention, and elasticity. The current understanding of sexual hair growth acknowledges the involvement of both adrenal androgens and ovarian hormones in human females. It is no longer limited to just adrenal androgens. Estrogens have the ability to directly stimulate hair growth at the hair follicle level in the skin, either independently or in conjunction with androgens. Furthermore, estrogens serve as effective regulators of hair follicle growth and cycling [40-41]. Androgens are responsible for anagen shortening or premature catagen entry [41]. The relationship between sex and hair growth in goats, as well as the underlying molecular mechanisms, are worthy of further investigation.

Moreover, Figure 7 visually depicts the complexity of differentially expressed genes identified in this study through a Venn diagram. These findings highlight the phase and variety-specific nature of DEGs, with an additional layer of sex-specific patterns. Notably, female Eskisehir Angora goats displayed the most variation in DEGs between the anagen and catagen phases, suggesting a crucial transition in

gene expression during this active hair growth stage. Interestingly, the differentiation between catagen and telogen phases was less pronounced. Conversely, female Lalahan Angora goats exhibited a consistently lower number of DEGs across all phases compared to Eskisehir goats. This observation suggests significant physiological differences between these two varieties, potentially influencing hair follicle cycling at the molecular level. Further investigation into the specific genes and pathways involved in these breed-specific variations could provide valuable insights into the regulation of hair growth in Angora goats. Further delving into sex-specific differences, Figure 11 explores DEGs specific to the catagen phase for each goat variety. Interestingly, the analysis revealed a cluster of keratin-associated protein genes (*KRT40, KRT72, KRTAP10*, etc.) solely differentially expressed in the male Eskisehir versus Lalahan comparison. This suggests potential sex-specific regulatory mechanisms involving keratinocyte differentiation during the catagen phase, which might be unique to the Eskisehir variety. Conversely, only *KRT6* emerged as a differentially expressed gene specific to the female Eskisehir versus Lalahan comparison. These findings highlight the intricate interplay between sex, breed, and specific hair growth phases. Additionally, the identification of these DEGs holds significant value for breeding programs. By incorporating this information into selection strategies, breeders can potentially target specific traits related to hair quality and growth patterns in Angora goats.

In the female, when we compared the catagen and telogen phases, we observed that the expression of genes for keratins and keratin-associated proteins (*KRTAP8-1*, *KRTAP16.4*, and *KRTAP21-1*) was quite higher in Eskisehir Angora goats. Interestingly, *KRT21-1* was found to be expressed as four different protein isoforms: *ENSCHIG00000010356*, *ENSCHIG00000009372*, *ENSCHIG00000015280*, and *ENSCHIG00000011204*. The UniProt accession numbers for these genes are A0A452E3E7 (93 aa), A0A452E013 (90 aa), A0A452EQT5 (87 aa), and A0A452E733 (87 aa), respectively. This may indicate that alternative isoforms of *KRT21-1* may be functional in the transition from catagen to telogen. Further studies are necessary to confirm the roles of the *KRT21* gene in hair follicle development.

As the hair cycle transitions from anagen to catagen, apoptosis of epithelial cells in the hair strand begins. These apoptotic cells are then phagocytosed by macrophages and neighboring epithelial cells. Throughout this process, keratin remains the major protein in the hair. Consistent with this information,

for the Eskisehir Female Anagen vs. Catagen comparison, we found that DEGs identified in this group were significantly enriched in the keratinization pathway (*R-HSA-680556; KRTAP11-1, KRTAP13-3, KRTAP10-9, KRTAP10-6, KRTAP3-3, KRTAP4-5, KRT33A, KRTAP3-1*) for Reactome. We also found that DEGs in this group were significantly enriched in the "*Calcium signaling pathway*" for KEGG analysis. A total of 7 DEGs (*ATP2A1, TNNC2, FGF21, HRC, CAMK2A, RYR1, CACNA1S*) were involved in the calcium signaling pathway chx04020 (Supplementary Figure 17), which may have a potential indirect role in *MAPK* signaling and apoptosis pathways. The *MAPK* signaling pathway and the cell cycle pathway, among others, also play an important role in hair follicle development [20]. The enrichment of DEGs in the *MAPK* signal pathway suggests that these genes may have a role in cell proliferation and differentiation, which are essential processes in hair follicle growth and development. This is consistent with previous findings that the *MAPK* signal pathway is an important regulator of hair follicle growth and development in goats [38,42]. In the anagen-catagen comparison, in contrast to Eskisehir variety, we found that DEGs identified in Lalahan male group were significantly enriched in the keratinization pathway (*R-HSA-6805567; KRTAP15-1, KRTAP11-1, KRT75, KRT40, KRT28, KRT32, DSG4, KRT36, KRTAP13-3, KRT73, KRT72, KRT26, KRT85, KRT39, KRTAP24-1, KRTAP8-1, KRT33A, KRT71, RPTN, KRT74, KRTAP3-1, DSC2, KRT25, KRT84, KRTAP9-1, IVL, KRT27, KRT35*) for Reactome and all these genes were found as downregulated. The downregulation of *KRT* and *KRTPs* suggests a significant decrease in keratinization from the anagen to catagen transition, consistent with the molecular process of hair growth. The transition from the anagen to the catagen of the hair follicle may indicate that gradual changes are taking place within the skin. Additionally, the internal molecular microstate of the skin undergoes a significant change when the hair follicle enters a new cycle of growth from the resting phase. Furthermore, the findings of this study are consistent with those of [43], who suggested that *KRT84* and *KRT25* can be considered as candidate genes for hair follicle morphogenesis. Additionally, according to the GO CC results, 30 DEGs were enriched mainly in keratin filament. As a result, we determined that *KRT* and *KRTAPs* were highly functional and had quite different expression patterns between female and male goats, as well as between Eskisehir and Lalahan Angora goats.

The results of comparing varieties for each phase, considering male and female individuals, show that DEGs were only determined in the catagen phase in both female and male goats. For the Catagen Female Eskisehir vs. Lalahan comparison, DEGs for GO CC were significantly enriched in "*keratin filament*" (*GO:0045095, ENSCHIG00000025429: LOC108635997 keratin, type II cytoskeletal 6A-like, ENSCHIG00000020255: LOC102174594 keratin-associated protein 9-9-like, ENSCHIG00000023156, ENSCHIG00000015597, ENSCHIG00000017056: LOC102171368 keratin-associated protein 4-9-like*).For the Catagen Male Eskisehir vs Lalahan comparison, DE genes for GO CC, DEGs were enriched significantly in keratin filament (*GO:0045095, 19 genes: ENSCHIG00000010601, ENSCHIG00000006780, ENSCHIG00000021086, ENSCHIG00000006463:LOC108638298: keratin, high-sulfur matrix protein, IIIA3-like; ENSCHIG00000000533:LOC108636561: keratin-associated protein 10-11-like; ENSCHIG00000010344:LOC108636554: keratin-associated protein 10-8-like; ENSCHIG00000023156, ENSCHIG00000007612:LOC108636430: keratin-associated protein 10-12-like; ENSCHIG00000008117:LOC108636431: keratin-associated protein 12-2-like; ENSCHIG00000001659:KRTAP3-1; ENSCHIG00000011548:LOC108636548: keratin-associated protein 10-1; ENSCHIG00000004564:LOC102170546: keratin-associated protein 3-3; ENSCHIG00000015017:LOC108636550: keratin-associated protein 10-8-like; ENSCHIG00000024813:LOC102172766: keratin-associated protein 10-11-like; ENSCHIG00000026079, ENSCHIG00000011563:LOC100861174: keratin associated protein 12.1; ENSCHIG00000026525:LOC102177517: keratin, type II cytoskeletal 72; ENSCHIG00000026301:LOC102184693: keratin, type II cuticular Hb5; ENSCHIG00000026924:LOC108638297: keratin, high-sulfur matrix protein, B2C*). The KEGG pathway analysis showed that DEGs in the comparison between catagen male Eskisehir and Lalahan goats were significantly enriched in the metabolic pathway, while they were significantly enriched in the calcium signaling pathway in female goats. During the catagen phase, hair growth ceases and several morphological changes occur, such as dermal papilla condensation and movement upwards. Additionally, cytological changes may also occur, including apoptosis of epithelial cells and the outer root sheath. Catagen, the intermediate phase between anagen and telogen, plays a crucial role in the hair growth cycle.

During this phase, the lower portion of the hair follicle regresses entirely, including the bulb and outer root sheath (ORS). This intricate process is characterized by apoptosis, or programmed cell death, of the epithelial cells within these layers [39,44,45]. The findings presented in this study indicate that distinct molecular pathways governing regulation are implicated in the hair follicle cycle of Eskisehir and Lalahan Angora goats. These pathways exhibit variations not only among different varieties but also between males and females.

Fibroblast Growth Factor 5 (*FGF5*) belongs to the Fibroblast Growth Factor gene family and is primarily expressed during the anagen phase of the hair cycle. Its main function is to regulate the transition from the growth phase (anagen) to the resting phase (telogen) [39,46]. Research has indicated that the absence of the *FGF5* gene leads to a prolonged anagen phase during hair growth. In a study conducted by Su et al. it was observed that *FGF5* and *FGFR1* had an impact on the growth cycle of hair follicles in Inner Mongolian Cashmere goats [38]. Furthermore, Zhang et al. utilized RNA-seq to analyze genome-wide expression and investigate differentially expressed genes associated with the cycling of hair follicles in cashmere and milk goat breeds across various seasons [42]. The researchers discovered that the *FGF5* gene exhibited its lowest expression levels during December, which corresponds to the catagen phase, while its highest expression levels were observed in September, during the anagen phase, in milk goats. This finding supports the notion that the transition from anagen to catagen occurs between the fall and winter seasons. Additionally, the expression patterns of *FGF2, FGF8,* and *FGF22* mirrored those of *FGF5*, with their highest expression levels occurring in September (anagen) and lowest in December (catagen). Furthermore, a significant number of *FGF* genes demonstrated their highest expression levels in March (telogen), while some genes exhibited high expression levels in both March and June. The researchers claim that the discrepancy in the quantity of DEGs observed in September and December in cashmere goats, in contrast to the higher number of DEGs found in milk goats, provides compelling evidence that cashmere goats do not experience the anagen to catagen transition during the transition from autumn to winter. The evidence for this conclusion is reinforced by the increased level of *FGF5* expression observed in December, which closely resembles that of September. Additionally, the

expression of telogen *FGF18* was significantly elevated in September, further supporting the aforementioned conclusion.

Moreover, the distinctively high levels of *FGF8* and *FGF22* expression were observed exclusively in the identical group of September milk goats, and this elevated expression pattern persisted in both September and December cashmere goats. Moreover, *TP53*, an inhibitor of anagen, demonstrated the most significant level of expression during the month of September. Additionally, the authors highlighted that the expression profiles of *FGF5* in milk goats suggest that the hair follicles undergo both catagen and telogen phases during winter, while anagen begins in March and concludes in autumn [42]. Our findings indicate that the *FGF5* gene exhibited a significant differential expression exclusively in male Lalahan Angora goats during the transition from telogen to anagen. This observation aligns with the notion that the *FGF5* gene plays a crucial role in this specific stage of hair growth in male Lalahan Angora goats.

# Conclusion

Mohair fiber is a valuable animal fiber source. Despite the significance of mohair, existing literature has predominantly focused on cashmere fiber, with limited studies dedicated to mohair. This study is the first to evaluate the impact of genes on hair follicle development in Lalahan and Eskisehir variety Angora goats using RNA sequencing methods. Consistent with the hypothesis, the study revealed notable differences between the two varieties in terms of hair follicle development. Specific genes were identified that play distinct roles in the hair growth cycles of Lalahan and Eskisehir Angora goats, contributing to our understanding of the molecular mechanisms underlying mohair production. This study has certain limitations, including the sample size and the scope of gene expression analysis. Future studies with larger sample sizes and broader genomic analyses are necessary to validate and expand upon these findings. The discoveries made in this study highlight the genetic differences influencing hair follicle development in Angora goats, which can inform breeding programs aimed at improving mohair quality. Understanding these genetic factors can lead to enhanced selection strategies for desirable fiber traits. Further investigations are needed to explore the functional roles of the identified genes and their

interactions in hair follicle development. Additionally, studies examining environmental and management factors in conjunction with genetic data will provide a more comprehensive understanding of mohair production. This study is expected to serve as a valuable resource for future research endeavors in the field of animal fiber genetics, paving the way for advancements in mohair quality and production.

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731 **Table 1.** DEGs involved in the process of keratin filament (GO:00045095) in the comparison
732 group of Lalahan Male Anagen vs. Catagen.

| ENSEMBLE GENE ID | GENE SYMBOL | GENE NAME | FC |
|---|---|---|---|
| ENSCHIG00000026301 | LOC102184693 | Keratin, type II cuticular Hb5 | 0,227 |
| ENSCHIG00000015383 | KRT25 | Keratin 25 | 0,299 |
| ENSCHIG00000024894 | | | 0,276 |
| ENSCHIG00000001659 | KRTAP3-1 | Keratin associated protein 3-1 | 0,180 |
| ENSCHIG00000018684 | LOC102183766 | Keratin, type II cuticular Hb1 | 0,245 |
| ENSCHIG00000010288 | KRTAP24-1 | Keratin associated protein 24-1 | 0,283 |
| ENSCHIG00000026525 | LOC102177517 | Keratin, type II cytoskeletal 72 | 0,235 |
| ENSCHIG00000004564 | LOC102170546 | KRTAP3-3 | 0,228 |
| ENSCHIG00000021223 | KRT74 | Keratin 74 | 0,330 |
| ENSCHIG00000008117 | LOC108636431 | Keratin associated protein 12-2 | 0,186 |
| ENSCHIG00000009215 | | | 5,002 |
| ENSCHIG00000023701 | KRT36 | Keratin 36 | 0,314 |
| ENSCHIG00000022765 | LOC102176522 | Keratin 73 | 0,279 |
| ENSCHIG00000011563 | LOC100861174 | Keratin associated protein 12-1 | 0,221 |
| ENSCHIG00000014439 | | | 0,284 |
| ENSCHIG00000022057 | LOC102185436 | Keratin, type II microfibrillar, component 7C | 0,242 |
| ENSCHIG00000018033 | LOC102183211 | Keratin, type II cuticular Hb1-like | 0,214 |
| ENSCHIG00000026178 | KRT85 | Keratin 85 | 0,258 |
| ENSCHIG00000015120 | LOC102177231 | Keratin 71 | 0,315 |
| ENSCHIG00000007612 | LOC108636430 | Keratin associated protein 10-12 | 0,190 |
| ENSCHIG00000008757 | LOC102177561 | Keratin, high-sulfur matrix protein, IIIA3-like | 0,195 |
| ENSCHIG00000014772 | KRT75 | Keratin 75 | 0,333 |
| ENSCHIG00000024813 | LOC102172766 | Keratin associated protein 10-11-like | 0,227 |
| ENSCHIG00000021086 | | | 0,325 |
| ENSCHIG00000026924 | LOC108638297 | Keratin, high-sulfur matrix protein, B2C | 0,270 |
| ENSCHIG00000010601 | | | 0,247 |
| ENSCHIG00000024531 | KRT84 | Keratin 84 | 0,343 |
| ENSCHIG00000022685 | LOC102178483 | Keratin, high-sulfur matrix protein, B2D | 0,306 |
| ENSCHIG00000023678 | LOC108638291 | Keratin, high-sulfur matrix protein, B2D-like | 0,306 |
| ENSCHIG00000006780 | | | 0,287 |

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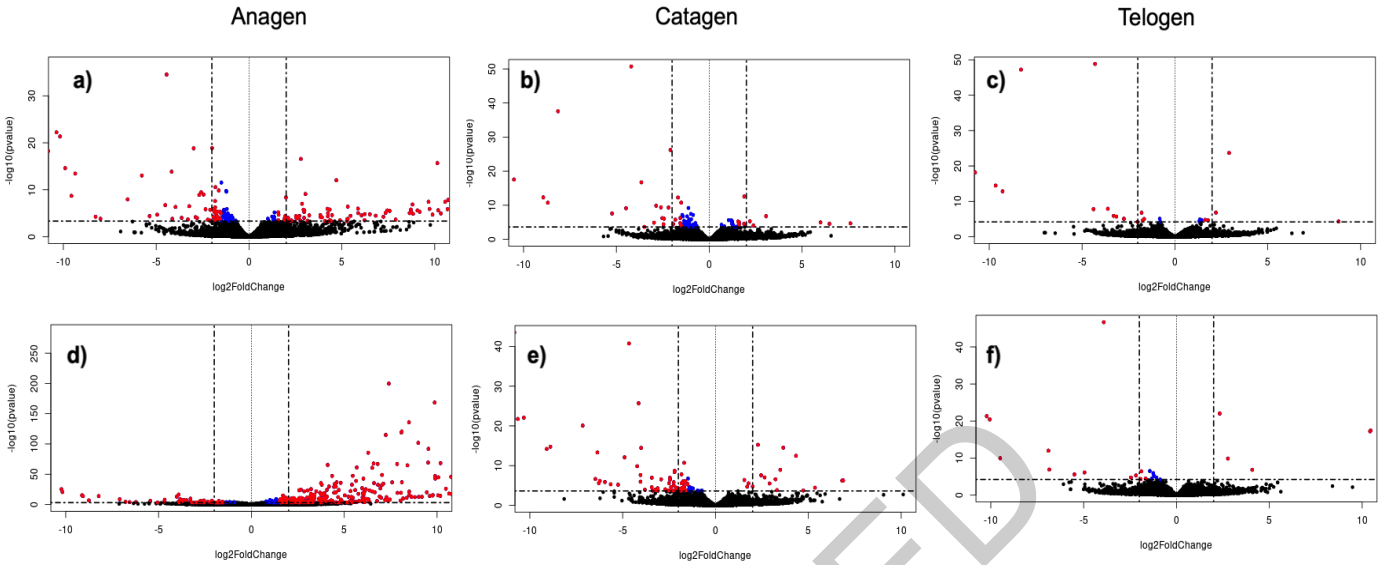
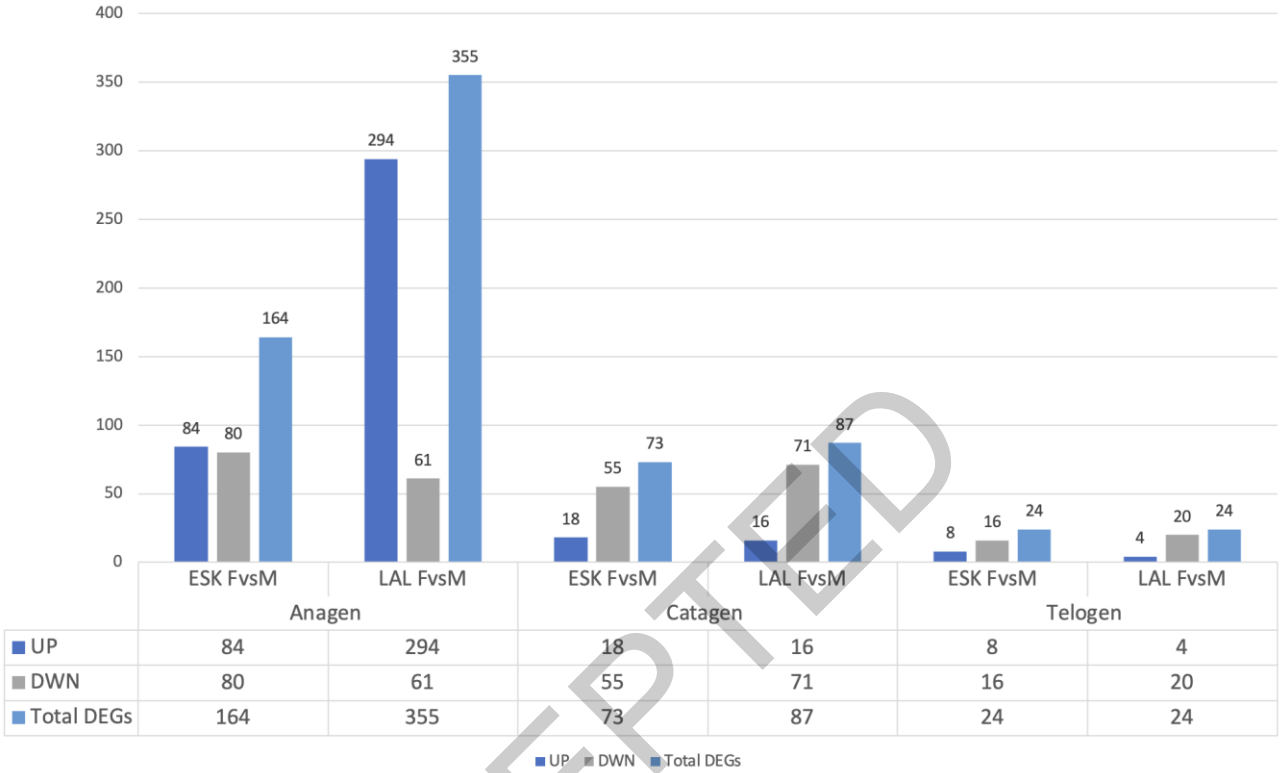



**Figure 1.** Volcano plots of DE genes in female and male goats of the same variety and in the same phase of hair growth. **a)** Eskisehir Anagen Female versus Eskisehir Anagen Male; **b)** Eskisehir Catagen Female versus Eskisehir Catagen Male; **c)** Eskisehir Telogen Female versus Eskisehir Telogen Male; **d)** Lalahan Anagen Female versus Lalahan Anagen Male; **e)** Lalahan Catagen Female versus Lalahan Catagen Male; **f)** Lalahan Telogen Female versus Lalahan Telogen Male.




| | Anagen | | Catagen | | Telogen | |
|---|---|---|---|---|---|---|
| | ESK FvsM | LAL FvsM | ESK FvsM | LAL FvsM | ESK FvsM | LAL FvsM |
| UP | 84 | 294 | 18 | 16 | 8 | 4 |
| DWN | 80 | 61 | 55 | 71 | 16 | 20 |
| Total DEGs | 164 | 355 | 73 | 87 | 24 | 24 |

**Figure 2.** Determined of differentially expressed genes in female and male goats of the same variety and in the same phase of hair growth.

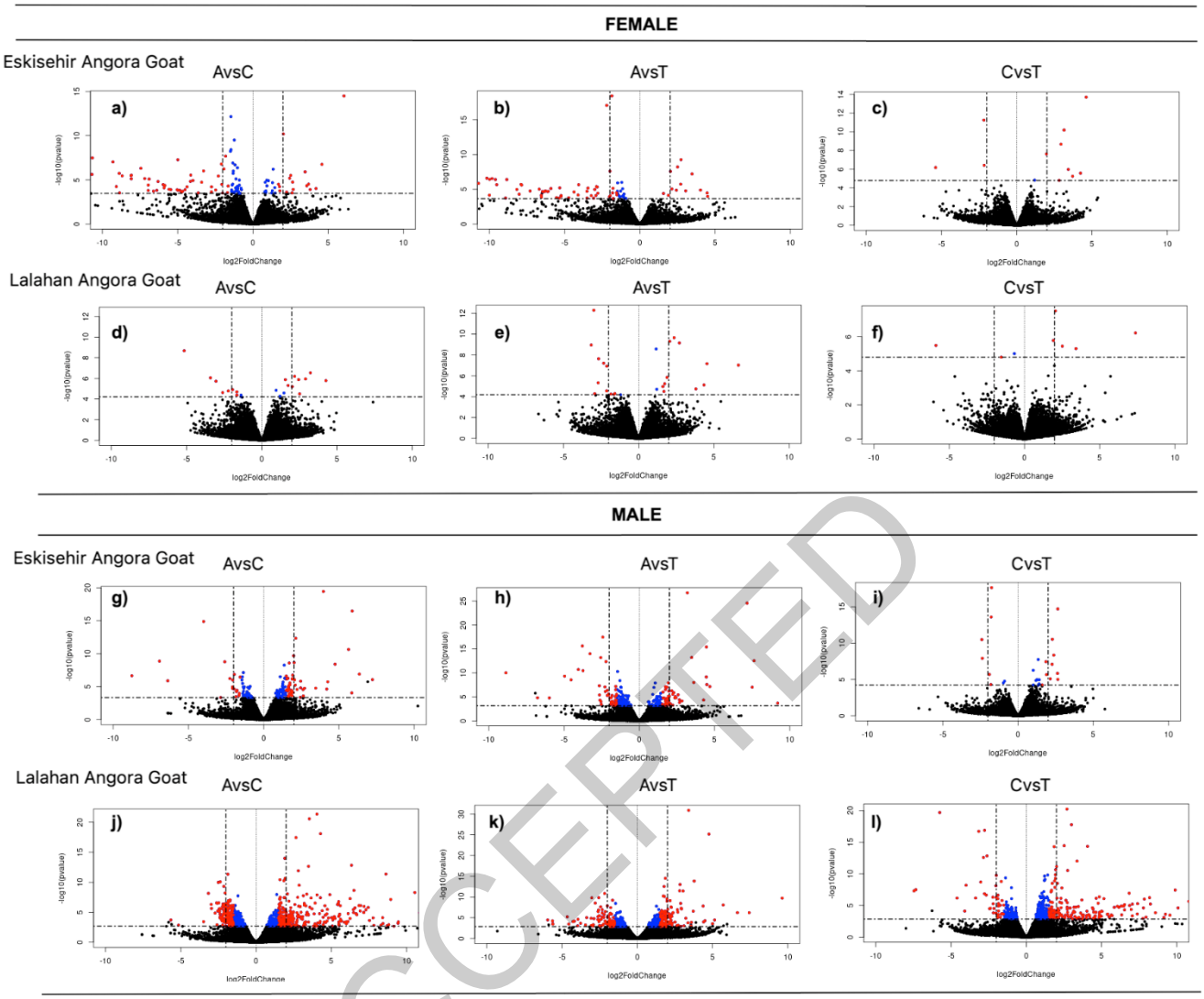
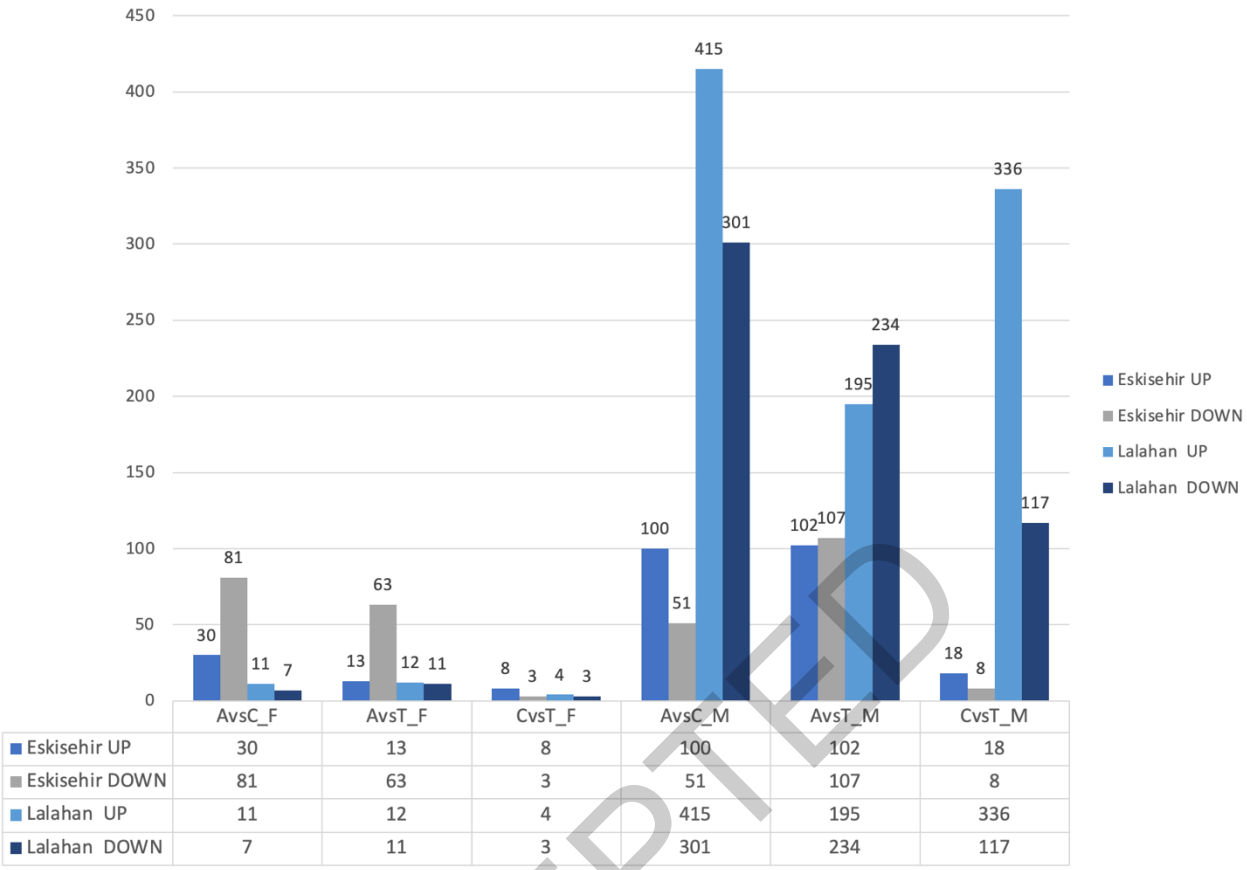


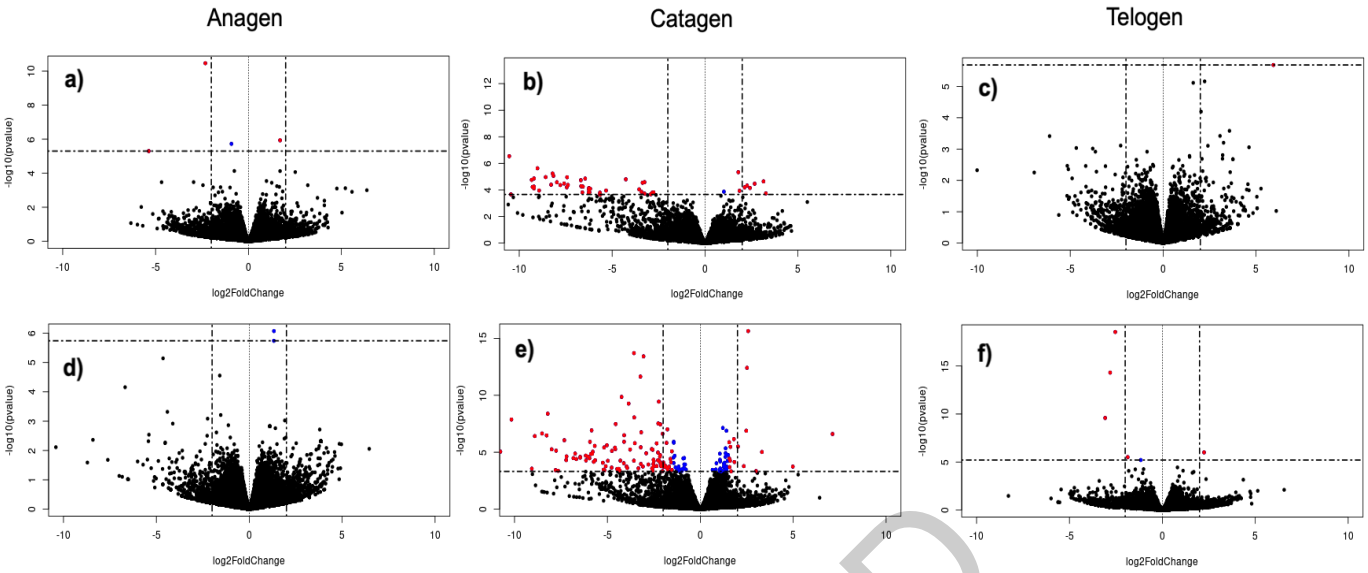



**Figure 3.** Volcano plots of DE genes between the three different stages of the same gender within the same variety. **a)** Eskisehir Female Anagen versus Eskisehir Female Catagen; **b)** Eskisehir Female Anagen versus Eskisehir Female Telogen; **c)** Eskisehir Female Catagen versus Eskisehir Female Telogen; **d)** Lalahan Female Anagen versus Lalahan Female Catagen; **e)** Lalahan Female Anagen versus Lalahan Female Telogen; **f)** Lalahan Female Catagen versus Lalahan Female Telogen; **g)** Eskisehir Male Anagen versus Eskisehir Male Catagen; **h)** Eskisehir Male Anagen versus Eskisehir Male Telogen; **i)** Eskisehir Male Catagen versus Eskisehir Male Telogen; **j)** Lalahan Male Anagen versus Lalahan Male Catagen; **k)** Lalahan Male Anagen versus Lalahan Male Telogen; **l)** Lalahan Male Catagen versus Lalahan Male Telogen.




| | AvsC_F | AvsT_F | CvsT_F | AvsC_M | AvsT_M | CvsT_M |
|---|---|---|---|---|---|---|
| Eskisehir UP | 30 | 13 | 8 | 100 | 102 | 18 |
| Eskisehir DOWN | 81 | 63 | 3 | 51 | 107 | 8 |
| Lalahan UP | 11 | 12 | 4 | 415 | 195 | 336 |
| Lalahan DOWN | 7 | 11 | 3 | 301 | 234 | 117 |

**Figure 4.** Differentially expressed genes between the three different stages of the same gender within the same variety.

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782 **Figure 5.** Volcano plots of DE genes between Eskisehir and Lalahan Angora goat varieties in the
783 female and male group. **a)** Anagen Female Eskisehir versus Anagen Female Lalahan; **b)** Catagen
784 Female Eskisehir versus Catagen Female Lalahan; **c)** Telogen Female Eskisehir versus Telogen
785 Female Lalahan; **d)** Anagen Male Eskisehir versus Anagen Male Lalahan; **e)** Catagen Male
786 Eskisehir versus Male Female Lalahan; **f)** Telogen Male Eskisehir versus Telogen Male Lalahan
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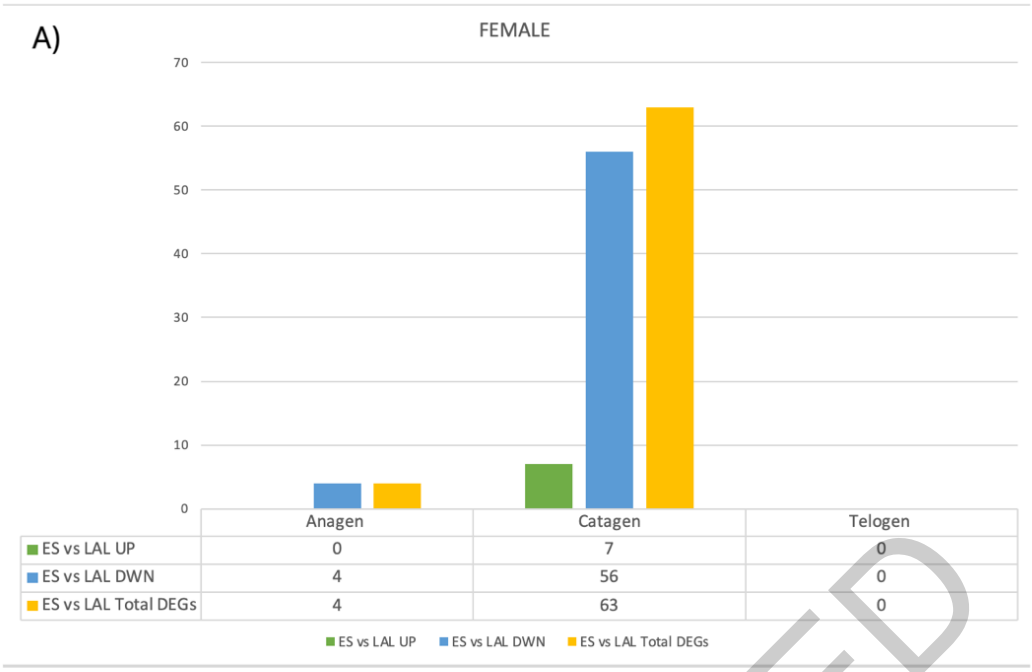


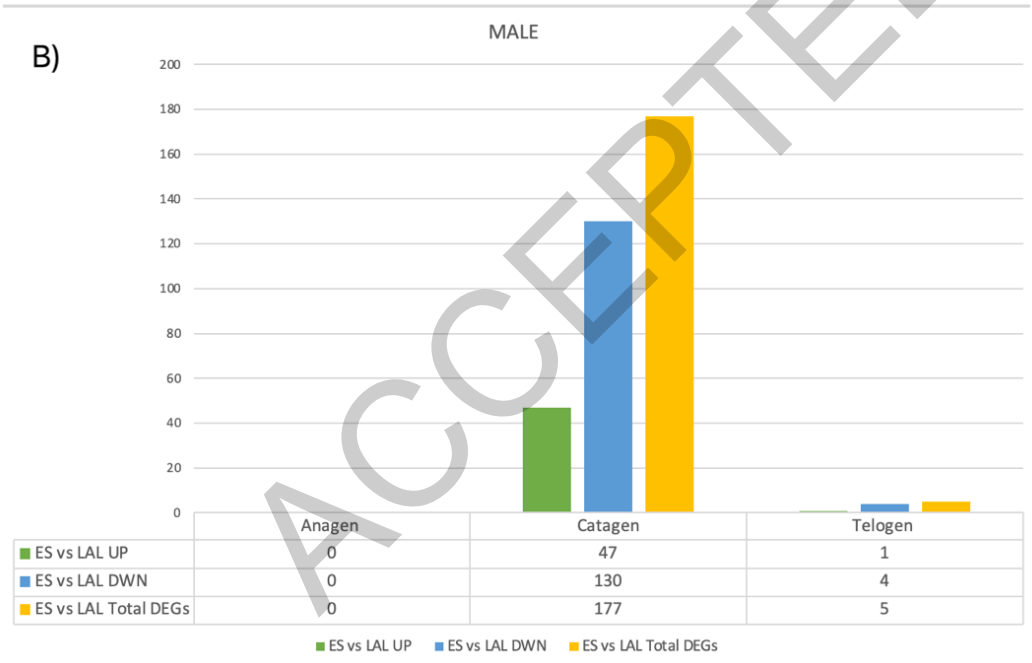


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828 **Figure 6.** Differentially expressed genes between Eskisehir and Lalahan Angora goat varieties in
829 the (A) female and (B) male group.

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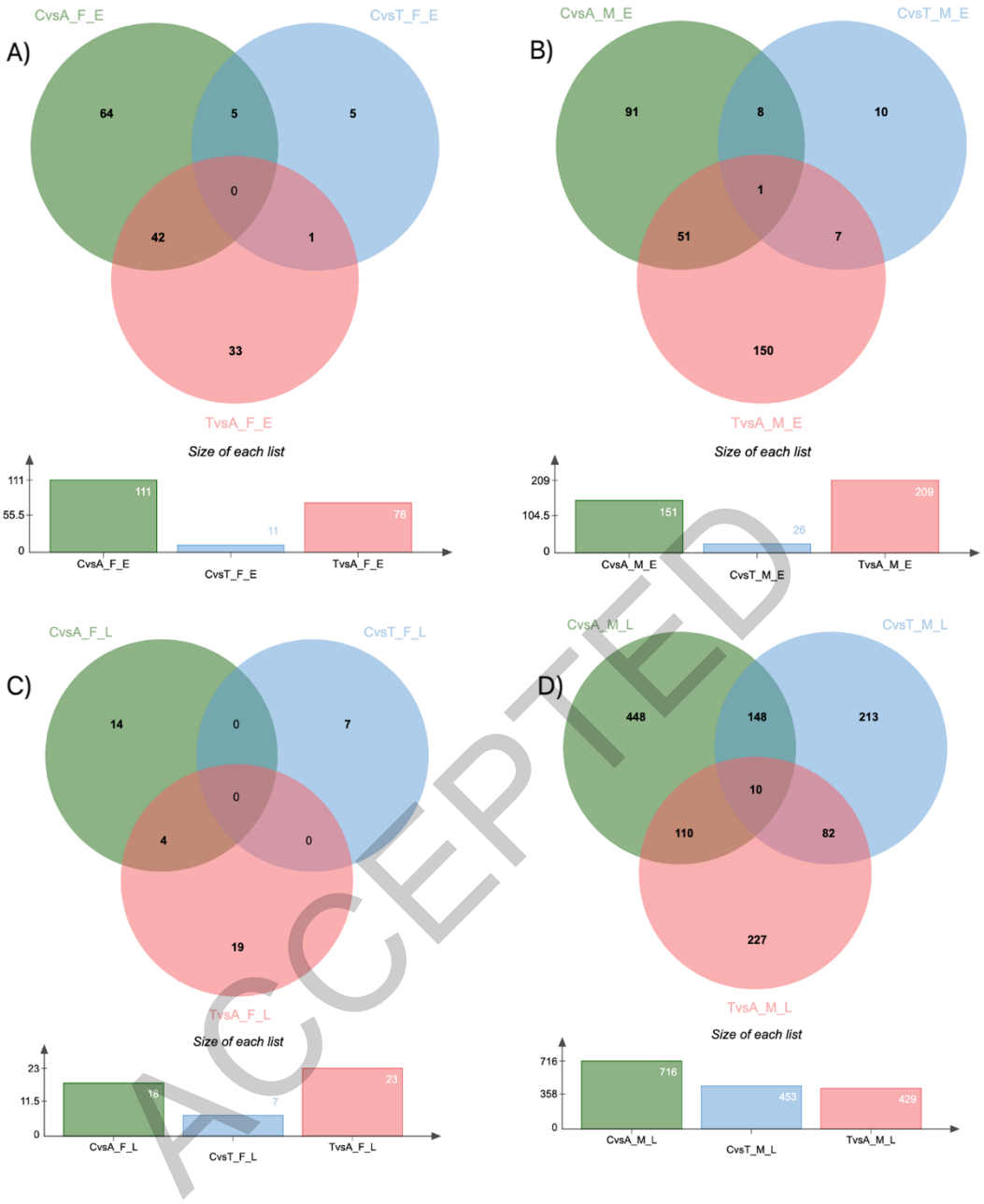




**Figure 7.** Venn diagrams that illustrate the different types of differentially expressed genes for each variety and sex in each phase. **A)** Female Eskisehir Angora goat group: CvsA_F_E: Catagen versus Anagen Female Eskisehir; CvsT_F_E: Catagen versus Telogen Female Eskisehir; TvsA_F_E:Telogen versus Anagen Female Eskisehir. **B)** Male Eskisehir Angora goat group: CvsA_F_E: Catagen versus Anagen Male Eskisehir; CvsT_F_E: Catagen versus Telogen Male Eskisehir; TvsA_F_E:Telogen versus Anagen Male Eskisehir. **C)** Female Lalahan Angora goat group: CvsA_F_L: Catagen versus Anagen Female Lalahan; CvsT_F_L: Catagen versus Telogen Female Lalahan; TvsA_F_L:Telogen versus Anagen Female Lalahan. **D)** Male Lalahan Angora goat group: CvsA_M_L: Catagen versus Anagen Male Lalahan; CvsT_M_L: Catagen versus Telogen Male Lalahan; TvsA_M_L:Telogen versus Anagen Male Lalahan.

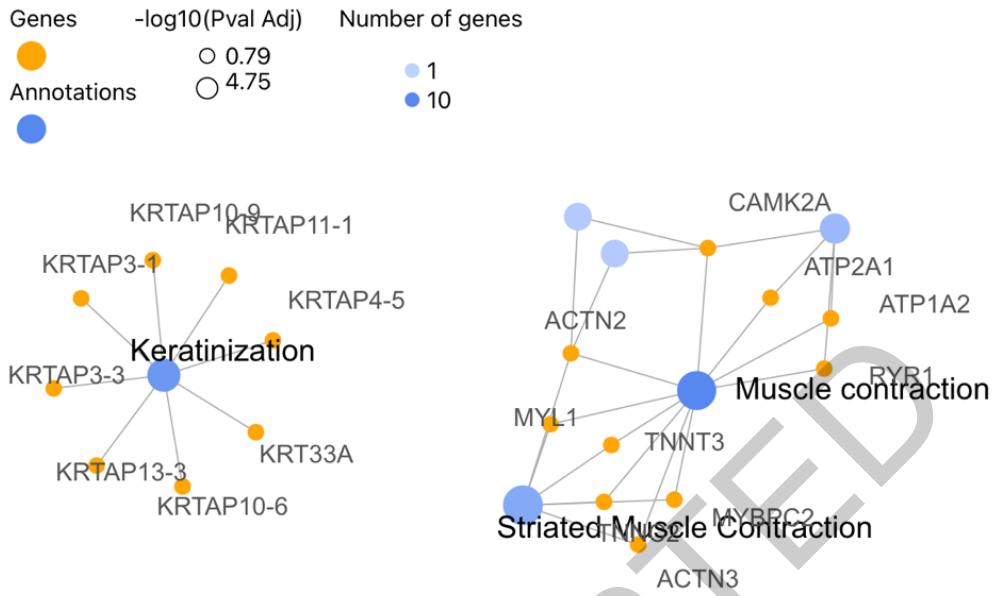


**Figure 8.** The Reactome pathway analysis of the differentially expressed genes that were found to be statistically significant in the comparison of Female Anagen vs Catagen revealed that the "Keratinization" pathway was significantly enriched in Eskisehir Angora goat.

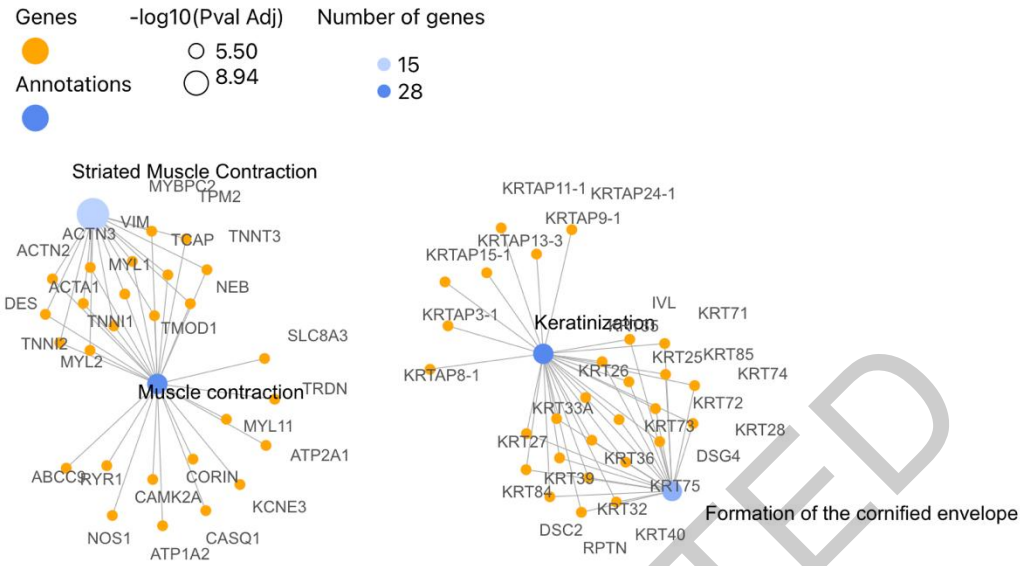


**Figure 9.** The Reactome pathway analysis of the differentially expressed genes that were found to be statistically significant in the comparison of Lalahan Male Anagen vs Catagen.

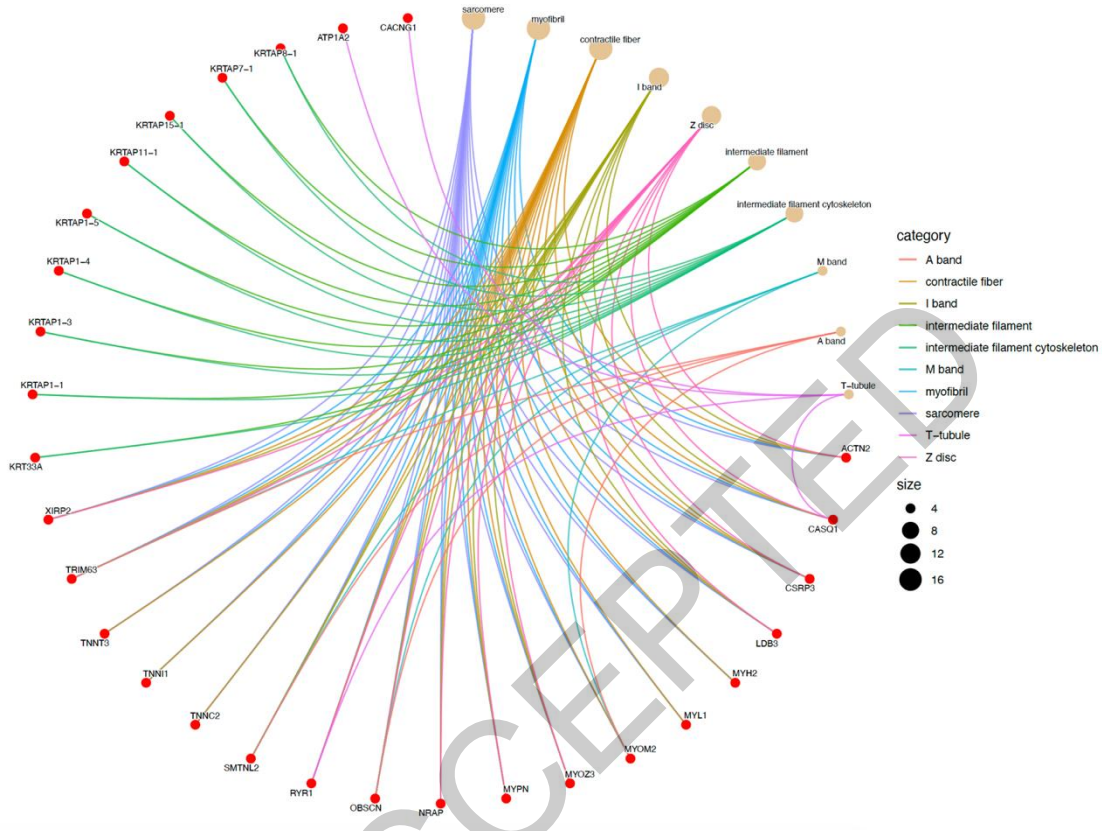


**Figure 10.** The cnetplot of the GO enriched terms of DEGs in the Eskisehir Female Anagen vs Telogen group.

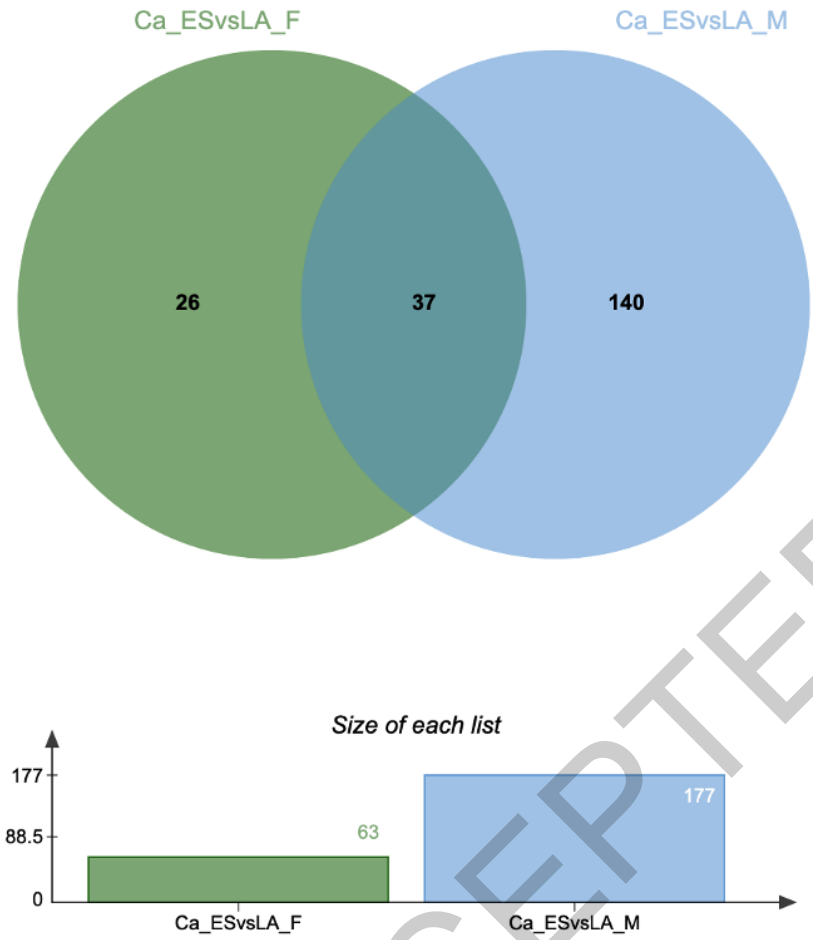




**Figure 11.** Venn diagrams that illustrate the different types of differentially expressed genes for each gender for each goat variety in catagen phase of hair growth. Ca_ESvsLA_F: Catagen Female Eskisehir vs Lalahan; Ca_ESvsLA_M: Catagen Male Eskisehir vs Lalahan.