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**RESEARCH**

# EZH2 expression in the skin of developing human fetuses and adults: a comparative study

## Gelişmekte olan insan fetüslerinin ve yetişkinlerin derisinde EZH2 ekspresyonu: karşılaştırmalı bir çalışma

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**Abstract**

**Purpose:** In animals, the appropriate levels of Enhancer of zeste homolog 2 (EZH2), a well-established epigenetic regulator, are essential for the embryonic development of skin and appendages. We aimed to explore the EZH2 expression patterns in the skin of human fetuses and compare them with adults.
**Materials and Methods:** We performed EZH2 immunohistochemical staining in skin samples from the scalp region of 67 fetuses and from the farthest surgical margin of the lip wedge resection, where no lesions were found, of 23 adults. EZH2 scores were evaluated according to the literature.
**Results:** Epidermal ($r = -0.528$), dermal ($r_s = -0.509$), and hair follicle ($r = -0.576$) EZH2 scores were inversely correlated with gestational age. Epidermal and hair follicle EZH2 scores were significantly higher in adults compared to fetuses. There were significant and positive correlations between epidermal, dermal, and hair follicle EZH2 scores among fetuses and adults.
**Conclusion:** Lower levels of EZH2 may be necessary for final cutaneous differentiation and maturation before birth. Our findings may have a therapeutic impact on cutaneous disorders with differentiation defects, chronic wounds, and alopecias.
**Keywords:** Fetus, EZH2, skin, epidermis, dermis, hair follicle

**Öz**

**Amaç:** Epigenetic düzenleyici olan Enhancer of zeste homolog 2 (EZH2), hayvanlarda deri ve eklerinin embriyonik gelişimi için gereklidir. Bu çalışmada, insan fetüslerinin deri örneklerinde EZH2 ekspresyon paternlerini, erişkinler ile karşılaştırmalı olarak araştırmayı amaçladık.
**Gereç ve Yöntem:** Altmış yedi fetüsün skalp bölgesinden ve 23 yetişkinin dudak wedge rezeksiyonunun en uzak cerrahi sınırından alınan, herhangi bir lezyon bulunmayan, deri örneklerinde EZH2 ile immünohistokimyasal boyama uygulandı. EZH2 skorları literatüre göre değerlendirildi.
**Bulgular:** Epidermal ($r = -0,528$), dermal ($r_s = -0,509$) ve kıl folikülü ($r = -0,576$) EZH2 skorları gebelik yaşı ile ters orantılıydı. Epidermal ve kıl folikülü EZH2 skorları erişkinlerde fetüslere göre anlamlı olarak daha yüksekti. Hem fetüs hem de erişkin dokularda epidermal, dermal ve kıl folikülü EZH2 skorları arasında anlamlı ve pozitif korelasyonlar saptandı.
**Sonuç:** Doğumdan önce nihai kutanöz farklılaşma ve olgunlaşma için daha düşük EZH2 seviyeleri gerekli olabilir. Bulgularımız, farklılaşma kusurları izlenen deri hastalıkları, kronik yaralar ve alopesiler üzerinde terapötik bir etkiye sahip olabilir.

**Anahtar kelimeler**: Fetüs, EZH2, deri, epidermis, dermis, kıl folikülü

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## INTRODUCTION

It is still unclear which molecular mechanisms exactly play a role in orchestrating the process of skin development and regeneration in fetal and postnatal periods of life. Understanding these mechanisms would help to improve the therapeutic approaches for various skin diseases, such as cutaneous disorders with differentiation defects, chronic wounds, alopecias, or cancers.

Polycomb-group proteins, composed of Polycomb Repressive Complex (PRC) 1, PRC 2, and Polycomb Repressive Deubiquitinase, regulate gene expression through chromatin remodeling and cooperate in the maintenance of gene silencing[1]. Enhancer of zeste homolog 2 (EZH2), one of the core components of PRC2 in mammals, possesses the catalytic and methyltransferase activity for trimethylation of histone 3 at lysine 27[1].

The importance of the appropriate levels of EZH2 for embryonic development was shown in various animal studies[2-5]. Dermal EZH2 is required to regulate dermal fibroblast differentiation and initiation of secondary hair follicles (HFs)[2]. In addition, dermal fibroblast differentiation and the concomitant reciprocal dermoepidermal signaling are essential components of skin patterning, the development of skin appendages, and wound healing[2,6-8].

Ezhkova et al. showed that EZH2 expression in epidermal progenitors of mice wanes concomitant with embryonic development and postnatally[5]. In addition, loss of EZH2 in basal keratinocytes was associated with precocious barrier formation and epidermal differentiation[5,9,10]. Moreover, Ezhkova et al. reported that loss of EZH1 and EZH2 arrested HF morphogenesis[11]. Tan et al. observed in pig fetuses that EZH2 expression significantly decreased in the spleen and lung from the gestational day 65 to 90, yet there were no significant differences in the heart, liver, muscle, and kidney[3].

EZH2 has an important role in the epigenetic regulation of skin and hair regeneration after wounding[11-13]. Ezhkova et al. reported that EZH1/2-null skin has slow wound closure[11]. Because there are also epigenetic mechanisms other than EZH2, the balance of epigenetic modifications is vital during wound repair. Shaw and Martin found that EZH2 and EED, PRC2 components, expressions are downregulated in wound-edge epithelium after the injury, whereas, concomitantly, UTX and JMJD3, histone demethylases, expressions are transiently upregulated[12].

Previous studies have shown the abnormal levels of epidermal EZH2 in dermatitis or psoriasis and the role of EZH2 in the pathogenesis of collagen vascular diseases, such as systemic sclerosis (SSc) and systemic lupus erythematosus (SLE)[14-20]. In addition, overexpression of EZH2 has been associated with various cutaneous, such as malign melanoma, Merkel cell carcinoma, head and neck squamous cell carcinoma, and visceral malignancies[21-24]. Therefore, EZH2 inhibitors have been studied recently as a novel therapeutic target in those diseases.

To our knowledge, there has been no report regarding the cutaneous EZH2 expression in developing human fetuses in the literature. Understanding the difference in cutaneous EZH2 expression between fetal and postnatal periods would contribute to achieving a new diagnostic biomarker or therapeutic approach for various skin diseases such as chronic wounds, alopecias, differentiation defects, or collagen vascular diseases. We aimed to explore the EZH2 expression pattern in the skin of human fetuses and compare it with adults, hypothesizing declining levels of EZH2 in the later developing stages of skin.

## MATERIALS AND METHODS

### Sample

Data from a total of 110 fetus autopsies performed between September 2019 and June 2022 in the pathology departments of two tertiary referral hospitals, Tekirdağ Namık Kemal University Hospital and Çukurova University Hospital, were retrospectively analyzed via digital archive records. In those centers, fetal autopsies are performed regularly by experienced pathologists and pathology residents. Data from fetal autopsies, including prenatal and postnatal history and external and internal examination, is precisely kept in the medical archive files.

Of 110 fetuses, only 67, whose whole-body parts were preserved in formalin, enrolled in the study. Of 67 fetuses, 1 cm skin samples were excised from the scalp regions by pathology residents. The fetuses not preserved in formalin or with macerated or autolytic skin were excluded. < 16 gestational week fetuses were also excluded owing to being aborted through curettage and macerated or autolytic skin nature. We obtained autopsy informed consent forms from the

parents of > 16-week fetuses before all autopsy procedures following local policies. Accompanying fetal anomalies, sex, and gestational age of abortion were retrieved from medical archive records.

We included 23 adult samples from the farthest surgical margin of lip wedge resections that were histopathologically normal and undamaged in the adult group. Formalin-fixed paraffin-embedded (FFPE) tissue blocks and hematoxylin-eosin-stained slides were obtained from the archive files of the pathology department at Tekirdağ Namık Kemal University Hospital.

The study was approved by the Tekirdağ Namık Kemal University Noninterventional Clinical Research Ethics Committee (approval number: 2022.147.07.14 and date: 26.07.2022) and was carried out following the principles of the 1975 Declaration of Helsinki as revised in 2000.

## Immunohistochemical (IHC) staining procedure and evaluation

All staining procedures were conducted by an experienced pathologist and two pathology technicians in the Pathology Department at Tekirdağ Namık Kemal University Hospital. We obtained FFPE tissues from 1 cm skin samples excised from the scalp regions of 67 fetuses. Hematoxylin-eosin staining was performed on the fetal samples. In addition, for IHC staining, 3 µm-thick serial sections were made from FFPE tissues of 67 fetuses and 23 adults. Then, the sections were taken onto positively charged slides to avoid tissue shedding. After keeping the slides on a slide warmer at 60°C for an hour, the sections were deparaffinized by exposure to xylene for 15 minutes. The sections were then rehydrated using a descending series of alcohol and rinsed with distilled water. Then, the sections were immunostained with monoclonal rabbit anti-EZH2 antibodies (clone IHC770, GeneAb, Richmond, BC, Canada, 1:20) on the BenchMark XT autostainer (Ventana, Tucson, AZ, USA). Stained slides were sealed with water-based sealing agents. Tonsil tissue was the positive and negative control for EZH2 staining. We evaluated the results using an Olympus BX51 microscope.

Evaluation of IHC staining of each case, determined at × 40 magnification in five random fields per section, was simultaneously performed by two blinded pathologists who had no knowledge of the case or each others' results. After the evaluation was over, when there was an inconsistency among the pathologists, they reached a consensus and determined the most appropriate score for the case. EZH2 staining score, which was evaluated for the epidermis, HFs, and dermis separately, from 0 to 9 was calculated by multiplying the staining intensity (0, negative; 1, weak; 2, moderate; 3, very strong) with the mean percentage of cells stained with EZH2 (0, 0-10%; 1, 11-25%; 2, 26-75%; 3, 76-100%)[25]. Only fibroblasts were evaluated for dermal EZH2 scoring.

## Statistical analysis

The sample sizes of fetus and adult groups were calculated with the help of the G*power 3.1.9.4 program, using the t-test family, with a desired effect size of 1, an alpha-type error of 0.02, and 97% statistical power. Power analysis revealed that at least 63 samples in the fetus group and 21 in the adult group were required. We included 67 fetal and 23 adult samples to strengthen the statistical analysis.

We assessed whether the variables follow a normal distribution with the Shapiro–Wilk test. According to the normality test results, the independent samples t-test or the Mann–Whitney U test was used for comparisons between two groups for normally (epidermal and HF EZH2 scores) or non-normally distributed (dermal EZH2 scores) variables, respectively. One–way ANOVA or Kruskal–Wallis test was used if the number of groups was more than two for normally (epidermal and HF EZH2 scores) or non-normally (dermal EZH2 scores) distributed variables, respectively. Multiple comparison procedures were performed using the post-hoc analysis after one-way ANOVA and the Dunn–Bonferroni approach to identify different groups after the Kruskal–Wallis test. We presented continuous variables as mean ± standard deviation or median (minimum and maximum) and categorical variables as n (%). According to the normality test results, the Pearson correlation coefficients were calculated to analyze the correlation of epidermal and HF scores with gestational ages, and Spearman correlation coefficients to examine the correlation of dermal scores with gestational ages, and epidermal, dermal, and HF scores with each other.

We performed the analysis using SPSS v.25 software (IBM Corp., Armonk, NY, USA). $p < 0.05$ was considered statistically significant.

## RESULTS

Of the fetuses (n = 67), 32 (49.2%) were female, and 33 (50.8%) were male. Of the adults (n = 23), 4 (17.4%) were female, and 19 (82.6%) were male.

Among fetuses, the mean gestational week was 22.77 ± 4.876 (median, minimum–maximum: 22.0, 14–39). Fifty-four (81.8%) abortions occurred at the 14-27$^{th}$ weeks (second trimester) and 12 (18.2%) at the 28-40$^{th}$ weeks (third trimester).

Epidermal, dermal, and HF EZH2 scores of the fetus and adult groups are presented in Table 1. We observed a nuclear staining pattern of EZH2. In the epidermis, EZH2 staining was strong in the basal layer and decreased towards the stratum corneum. In the dermis, the staining pattern was similar in the papillary and reticular dermis. In addition, EZH2 strongly stained lymphoplasmacytic cells, besides vascular endothelial cells. However, we evaluated only fibroblasts while dermal EZH2 scoring. The staining intensities of mature and progenitor fibroblasts were similar. It was noteworthy that dermal fibroblasts were not stained with EZH2 in any sample in the third trimester and adult group. On the other hand, EZH2 strongly stained mainly the hair bulb areas, where undifferentiated and actively dividing matrix cells are located, excluding dermal papillae. In some HFs, outer root sheaths (ORSs) were also moderately stained with EZH2 (Figures 1 and 2). The vast majority of HFs of both fetuses and adults were in the anagen phase.

**Table 1. Epidermal, dermal, and hair follicle EZH2 scores of fetus and adult groups.**

| EZH2 Scores | Mean ± SD (median, min–max) | p |
|---|---|---|
| Epidermal EZH2 score | | < 0.001[a] |
| Fetus | 2.46 ± 2.6 (2, 0–9) | |
| Adult | 6.43 ± 2.17 (6, 2–9) | |
| Dermal EZH2 score | | 0.018[b] |
| Fetus | 0.67 ± 1.7 (0, 0–9) | |
| Adult | 0 | |
| Hair follicle EZH2 score | | < 0.001[a] |
| Fetus | 3.22 ± 3.584 (2, 0–9) | |
| Adult | 6.60 ± 2.817 (7.5, 0–9) | |

min: minimum, max: maximum, SD: standard deviation, [a]: Independent t-test, [b]: Mann–Whitney–U test.

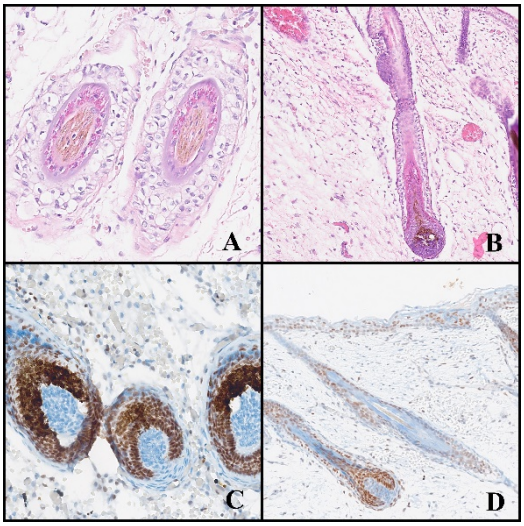


**Figure 1. Histopathological sections of skin in the fetal period. (A) Terminal follicles (Hematoxylin and eosin (H&E), × 200). (B) The upper and mid dermis, the terminal follicle (H&E; × 200). (C) EZH2 staining in hair bulb regions (intensity, 3; percentage, 80; score, 9). None of the dermal papillae were stained with EZH2 (× 200). (D) EZH2 staining in the epidermis (intensity, 2; percentage, 80; score, 6), dermis (intensity, 1; percentage, 15; score, 1), and outer root sheaths and bulbs of hair follicles (intensity, 2; percentage 90; score, 6) (× 100).**

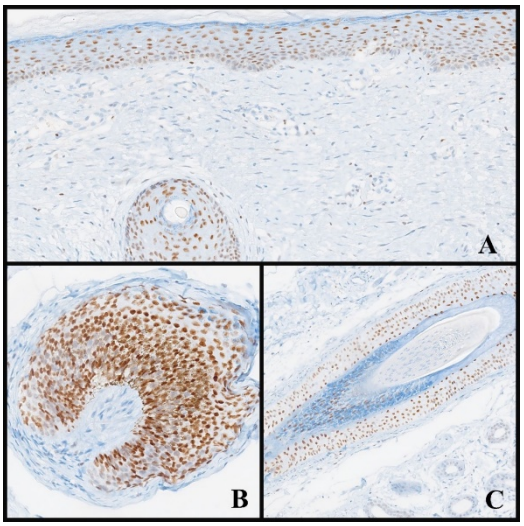


**Figure 2. Histopathological sections of skin in the adult group. (A) EZH2 staining in the epidermis (intensity, 3; percentage, 95; score, 9), dermis (intensity, 0; percentage, 0; score, 0), and hair follicle (intensity, 3; percentage, 85; score, 9) (× 200). (B) EZH2 staining in the hair bulb (intensity, 3; percentage, 75; score, 6) (× 200). (C) EZH2 staining in the hair follicle (intensity, 2; percentage, 80; score, 6) (× 100).**

The correlations of the epidermal, dermal, and HF EZH2 scores with gestational ages in fetuses are presented in Table 2. There were moderate negative and significant correlations between all EZH2 scores and the gestational ages.

**Table 2. The correlation of the epidermal, dermal, and hair follicle EZH2 scores with gestational ages in fetuses.**

| EZH2 scores | Gestational age |
|---|---|
| Epidermal | $r = -0.528$, $p < 0.001$ |
| Dermal | $r_s = -0.509$, $p < 0.001$ |
| Hair follicle | $r = -0.576$, $p < 0.001$ |

r: Pearson's correlation coefficient, $r_s$: Spearman's rank correlation coefficient

The comparisons of EZH2 scores between the second and third trimesters, and adults are presented in Table 3. The differences in epidermal scores between the second and third trimesters, second trimester and adults, and third trimester and adults were statistically significant ($p = 0.003$, $p < 0.001$, and $p < 0.001$, respectively). The differences in HF scores between groups were also significant (second and third trimesters, second trimester and adults, and third trimester and adults; $p = 0.003$, $p = 0.005$, and $p < 0.001$, respectively). However, in the case of dermal scores, the significant differences were between the second and third trimesters ($p = 0.027$) and the second trimester and adults ($p = 0.005$) (Figure 3).

**Table 3. The comparison of epidermal, dermal, and hair follicle EZH2 scores between the second and third trimesters, and adults.**

| | 2nd Trimester | 3rd Trimester | Adults | p |
|---|---|---|---|---|
| Epidermal | | | | |
| mean ± SD | 2.94 ± 2.631 | 0.33 ± 1.155 | 6.43 ± 2.171 | < 0.001[a] |
| median, min–max | 4, 0–9 | 0, 0–4 | 6, 2–9 | |
| Dermal | | | | |
| mean ± SD | 0.83 ± 1.83 | 0 | 0 | 0.005[b] |
| median, min–max | 0, 0–9 | 0 | 0 | |
| Hair Follicle | | | | |
| mean ± SD | 3.85 ± 3.662 | 0.33 ± 1.155 | 6.60 ± 2.817 | < 0.001[a] |
| median, min–max | 4, 0–9 | 0, 0–4 | 7.5, 0–9 | |

SD: standard deviation, min: minimum, max: maximum, [a]: One-way ANOVA test, [b]: Kruskal–Wallis test


**Figure 3. EZH2 staining scores in the second and third trimesters, and adults.**

A moderate positive and significant correlation was found between dermal and HF EZH2 scores among all cases or only fetuses. There was a moderate positive and significant correlation between epidermal and dermal EZH2 scores in only fetuses. In addition, a strong positive and significant correlation was found between epidermal and HF EZH2 scores among all cases or only fetuses (Table 4).

**Table 4. The correlation of the epidermal, dermal, and hair follicle EZH2 scores with each other.**

| **Among all cases** | | |
|---|---|---|
| | **Dermal** | **Hair follicle** |
| Epidermal | $r_s = 0.204$, $p = 0.053$ | $r_s = 0.871$, $p < 0.001$ |
| Dermal | | $r_s = 0.425$, $p < 0.001$ |
| Among only fetuses | | |
| | **Dermal** | **Hair follicle** |
| Epidermal | $r_s = 0.477$, $p < 0.001$ | $r_s = 0.879$, $p < 0.001$ |
| Dermal | | $r_s = 0.604$, $p < 0.001$ |

$r_s$: Spearman's rank correlation coefficient

Nervous system anomalies were observed in 13 (20.0%), cardiovascular anomalies in 12 (18.5%), spontaneous abortion in 10 (15.4%), hydrops fetalis in 7 (10.8%), multisystem anomalies in 5 (7.7%), renal anomalies in 4 (6.2%), intrauterine fetal demise in 4 (6.2%), trisomy 21 in 3 (4.62%) fetuses, and trisomy 18 in only one (1.54%) fetus. The other congenital anomalies (9.2%) observed in our study were sacral teratoma, cleft lip-palate, fetal skeletal dysplasia, omphalocele, chorioamnionitis, pulmonary hypoplasia, and gastroschisis. There were no statistically significant associations between fetal anomalies and none of the EZH2 scores ($p = 0.096$, $p = 0.130$, and $p = 0.332$, epidermal, dermal, and HF, respectively). There were no statistically significant differences between any EZH2 scores and sex, both in the fetus and adult groups.

## DISCUSSION

All EZH2 scores were significantly lower in the third trimester. In addition, epidermal and HF EZH2 scores were significantly higher in adults than in fetuses. A significant correlation between epidermal, dermal, and HF EZH2 scores indicates the importance of dermoepidermal signaling in proper skin development and function. It was noteworthy that among fetuses aborted in the third trimester and the adult group, EZH2 did not stain dermal fibroblasts in any samples. Lower EZH2 levels may be necessary for final skin maturation before birth. EZH2 may be a novel molecular target for skin disorders with differentiation defects, chronic wounds, or alopecias. Why epidermal and HF EZH2 scores are higher in adults while there are no EZH2-stained dermal fibroblasts needs further investigation.

EZH2 is mainly expressed in proliferating cells[1]. Several studies have shown the overexpression of EZH2 in various tumors[21-24]. However, the exact role of EZH2 in tumorigenesis is still unknown. The proliferation rate is also high in fetuses, similar to the behavior of tumor cells. Ezhkova et al. reported that EZH2 expression in epidermal progenitors decreased with embryonic development and postnatally in mice[5]. In addition, loss or inactivation of EZH2 in epidermal basal keratinocytes was shown to induce precocious barrier formation and epidermal differentiation[5,9,10]. Similarly, we observed that EZH2 expressions in the epidermis, dermis, and HFs declined towards the third trimester, suggesting that lower levels of EZH2 may be necessary for final cutaneous differentiation and maturation before birth and may be a therapeutic target in human cutaneous disorders with differentiation defects. On the other hand, Wnt/β-catenin, retinoic acid, and BMP signaling pathways were also found to be involved in the differentiation of dermal fibroblast progenitors and maintaining dermal papillae's ability to induce HFs[2]. Wurm S et al. reported that the terminal epidermal differentiation is regulated by Fra-2/AP-1 in interaction with EZH2 and ERK1/2[9]. In addition, the interactions of EZH2 with TGFβ/SMAD and CDK4/6 pathways were reported in psoriasis[14,26]. Therefore, probably there are other molecular factors, signaling pathways, and epigenetic modifiers contributing to the skin differentiation process. How and to what extent epigenetic modifiers regulate this

complicated coordination and interactions remains unclear[27].

EZH2, as an epigenetic repressor, has been implicated in various immune gene regulation and cell functions such as T and B lymphocytes, leucocytes, interleukin 4 and 13, neutrophils, dendritic and Langerhans cells[4,16,20,28,29]. In addition, the potential role of EZH2 in controlling skin tolerance has been speculated[4]. Abnormal levels of epidermal EZH2 expression in patients with dermatitis or psoriasis compared with healthy people have been reported[14,15]. Moreover, a central role of EZH2 in the pathogenesis of SSc, comprised of autoimmunity, excessive fibrosis, and vascular dysfunction, was shown[16-19]. Overexpression of EZH2 causes profibrotic and antiangiogenic effects in SSc[16-19]. In SLE, as another autoimmune disease, EZH2 was found to be involved in the pathogenesis through different immune cells[20]. EZH2 inhibitors have still been studied in the treatment of SSc and SLE[16-20]. Therefore, EZH2 may be a diagnostic biomarker and a novel therapeutic target in that field. All these studies indicate the role of EZH2 in cutaneous health. Furthermore, the role of EZH2 during dermal fibroblast differentiation was suggested in murine skin[2]. We observed that EZH2 strongly stained lymphoplasmacytic cells in both fetuses and adults. The higher levels of epidermal and HF EZH2 expression in the adult group compared with fetuses may be related to the improved immune system and skin tolerance and continuing skin regeneration in adulthood. However, further studies are necessary to lighten the potential unknown functions of EZH2 in healthy skin and the reason why EZH2 did not stain skin fibroblasts in fetuses aborted in the third trimester and adults.

The reciprocal dermoepidermal signaling is integral to skin maturation, the initiation of HF formation, hair regeneration, and also the development of the other skin appendages[2,6,7,30,31]. In our study, the significant correlation between epidermal, dermal, and HF EZH2 expressions in both fetuses and adults indicates the importance of the dermoepidermal signal network in skin development and function.

After the formation of hair placode in the epidermis, it subsequently extends to the underlying dermis and differentiates to mature HF. Reservoir stem cells of HFs are located in the bulge region, below the sebaceous gland in the ORS, and are periodically activated for HF regeneration[11,31]. Activated stem cells produce ORS cells extending from the bulge to the bulb region, where the ORS forms transit-amplifying matrix cells[11]. The hair bulb contains the actively dividing hair matrix where the metabolical activation is very high[32]. In our study, EZH2 staining was mainly observed in the hair bulb and ORS regions, indicating EZH2 expression in the actively proliferating cells and the potential association between EZH2 and HF stem cells. However, because EZH2 did not specifically stain the bulge regions, there may be other stem cell locations besides the hair bulge. Why EZH2 did not stain any dermal papillae, providing communication with the mesenchymal cells, needs further investigation.

In alopecia areata, there is lymphocyte-predominant inflammation in peribulbar areas. In addition, peri-infundibular or perifollicular inflammations are seen in the other non-cicatricial or cicatricial alopecias[33]. Moreover, loss of EZH1 and EZH2 arrests the HF and sebaceous gland development and progressive degeneration[11]. We speculated that EZH2 may be a therapeutic target for alopecias, especially alopecia areata, based on the EZH2 staining pattern in our study.

Intriguingly, EZH2 did not stain dermal fibroblasts in the third trimester and adults. Our results indicate that the other tissue-specific compensatory mechanisms or epigenetic modifiers are vital for the skin regeneration and maintenance of skin homeostasis in adulthood.

The limitations of this study were the limited sample size and autopsy-based nature. Although the fetuses with autolytic features were excluded from the study, the results might still be affected by the autopsy nature. Since we did not include the first-trimester abortions due to autolytic features or neonates, the EZH2 staining scores of the first trimester or postnatal periods could not be analyzed. The other thing is we only performed the IHC staining method. Therefore, in order to generalize, our findings need to be verified by studies of larger sample sizes, in which the first trimester and neonatal periods are also evaluated, and more specific methods.

In conclusion, the cutaneous expression level of EZH2 was inversely correlated with the gestational age during human fetal development. Why dermal fibroblasts of fetuses aborted in the third trimester and of adults were not stained with EZH2 needs further investigation. Lower levels of EZH2 may be necessary for final cutaneous differentiation and maturation before birth. Our findings may have a

therapeutic impact on cutaneous disorders with differentiation defects, chronic wounds, hair disorders, and inflammatory skin diseases. Because it is the first study about EZH2 expression in developing human fetuses in the literature, our results are significant. Our results would lead to further more comprehensive studies exploring the role of EZH2 in specific skin disorders or investigating the interplay between EZH2 and other molecular factors.

**Author Contributions:** Concept/Design : ÖZ, SK; Data acquisition: ÖZ, SK, KEE, EK, İÖA; Data analysis and interpretation: ÖZ, SK; Drafting manuscript: ÖZ, SK, KEE, EK, İÖA; Critical revision of manuscript: ÖZ, SK; Final approval and accountability: ÖZ, SK, KEE, EK, İÖA; Technical or material support: -; Supervision: ÖZ, SK; Securing funding (if available): n/a.
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