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Efficacy and Tolerability of a Topical Gel Containing Milk-Derived miRNA-Enriched Exosomes in Androgenetic Alopecia and Telogen Effluvium: A 12-Week Randomized, Double-Blind, Placebo-Controlled Trial

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Abstract

Androgenetic alopecia (AGA) and chronic telogen effluvium (TE) are common hair loss disorders with substantial psychosocial impact and limited effective topical options beyond minoxidil. MicroRNAs (miRNAs) have emerged as promising modulators of hair follicle cycling via Wnt/β-catenin and related pathways. In this multicenter, randomized, double-blind, placebo-controlled clinical trial, adults aged 18–60 years with AGA, female AGA, or TE were allocated 1:1 to receive a topical gel containing bovine milk-derived exosomes enriched in specific miRNAs (MEV-miRNA gels) or placebo once weekly for 12 weeks. Primary endpoints were changes in anagen hair percentage, anagen/telogen ratio, and hair shaft diameter assessed by digital photo-trichogram at week 8 and week 12 versus baseline; secondary endpoints included patient-reported outcomes, global clinical assessment, and safety/tolerability. Of 40 randomized subjects, 39 (97.5%) completed the study. In the MEV-miRNA gel group, anagen hair percentage increased from 58% at baseline to 70% at week 8 and 78% at week 12, with corresponding improvements in anagen/telogen ratio (from 1.45 to 2.46 and 3.63) and mean hair shaft diameter (from 47.5 µm to 65.3 µm and 70.6 µm; all $p < 0.05$ vs. baseline and vs. placebo). The placebo did not show significant changes in trichoscopic parameters. At week 12, most MEV-miRNA-treated patients reported stronger hair, reduced shedding, and improved overall hair quality, with high satisfaction rates and no treatment-related serious adverse events. Once-weekly application of a topical gel containing bovine milk-derived microRNA-enriched exosomes significantly improved trichoscopic and patient-reported outcomes in AGA and TE over 12 weeks, with a favorable short-term tolerability profile. These findings support exosome-based microRNA delivery as a novel, non-pharmacologic therapeutic approach for hair loss disorders.

Keywords: androgenetic alopecia; telogen effluvium; hair loss; microRNA; exosomes; milk-derived exosomes; topical therapy; randomized controlled trial

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1. Introduction

Androgenetic alopecia (AGA) and chronic telogen effluvium (TE) represent multifactorial hair loss conditions with significant psychosocial burden affecting both males and females worldwide [1–3].

Recent epidemiological studies estimate that AGA prevalence impacts millions globally, with increasing incidence across diverse populations [4,5]. Pathophysiology involves complex interactions between genetic predisposition, androgen metabolism, inflammatory mediators, oxidative stress, and dysregulation of hair follicle cycling [4,5].

Current therapeutic options remain limited, with finasteride and minoxidil constituting the primary approved pharmacological treatments for AGA [6]. However, in clinical practice, these are increasingly complemented by off-label oral dutasteride, low-dose oral minoxidil, topical finasteride, and other topical antiandrogens, for which emerging evidence supports their use despite the lack of formal regulatory approval [7–10]. On the other ethichand, therapeutic management of chronic telogen effluvium is largely centered on identifying and eliminating triggering factors and correcting underlying nutritional or endocrine disturbances. At the same time, supportive measures may include off-label topical or oral minoxidil, corticosteroids in selected inflammatory or systemic contexts, and various cosmetic or nutritional formulations; however, robust clinical data are scarce, and non-specific targeted drugs have yet to receive formal approval for this indication [11]. However, concerns regarding side effects, treatment adherence, and incomplete response rates drive the need for complementary therapeutic strategies. Adjuvant procedures such as platelet-rich plasma injections, low-level light/laser therapy, microneedling, and, in selected cases, hair transplantation, alongside emerging approaches, including exosome-based products and androgen-receptor-targeted or siRNA-based therapies, are currently under investigation and not yet standard of care. Hair follicles are dynamic mini-organs that integrate genetic programs with hormonal, environmental, and epigenetic signals, and accumulating data highlight microRNAs as crucial post-transcriptional regulators that fine-tune their morphogenesis, cyclic regeneration, and overall homeostasis [12].

MicroRNAs are increasingly recognized as key post-transcriptional regulators of hair follicle biology, with effects on cell proliferation, differentiation, and pathways involved in follicular cycling, including Wnt/β-catenin signaling, insulin-like growth factor-1 (IGF-1), and other growth factor- and cytokine-mediated pathways [12–14].

MicroRNAs are increasingly recognized as central post-transcriptional regulators of hair follicle biology, coordinating stem cell activation, keratinocyte proliferation, and the transitions between anagen, catagen, and telogen [15–17].

In AGA, distinct microRNA expression patterns have been documented in the affected scalp, with several microRNAs associated with downregulation of Wnt/β-catenin signaling, reduced dermal papilla cell proliferation, and progressive follicular miniaturization [18,19]. Although microRNA signatures in telogen effluvium (TE) are less well defined, TE is characterized by an abrupt, synchronized shift of follicles into telogen, and emerging data suggest that stress- and inflammation-responsive microRNAs may contribute to this pathological shift by disrupting transcriptional programs that sustain anagen and protect matrix keratinocytes from apoptosis [20].

Collectively, this body of evidence provides a strong mechanistic basis for therapeutic strategies aimed at modulating microRNA pathways—particularly through exosome-mediated delivery of selected microRNAs—to counteract miniaturization in AGA and stabilize hair cycle dynamics in TE, supporting the evaluation of topical formulations enriched in specific microRNAs in these disorders.

Although microRNA-based gene therapy is highly promising, several key hurdles still limit its clinical application. Because microRNAs are polyanionic, they do not readily traverse cell membranes, and they also show poor stability in biological fluids, where they are rapidly degraded [21]. Consequently, designing safe, efficient delivery platforms is crucial to enhance cellular uptake and protect microRNAs, thereby maximizing the therapeutic impact of microRNA-based interventions. In this context, exosomes, especially

milk-derived ones, represent a naturally abundant nanocarrier system capable of protecting and delivering microRNAs to recipient cells, offering a promising platform for microRNA-based interventions, including in hair biology [22–24].

The primary aim of this study was to evaluate the efficacy and tolerability of a novel topical gel (MEV-microRNA gel) containing bovine milk-derived exosomes enriched in specific miRNAs (MEV-miRNAs) together with an SFRP1 (secreted Frizzled-Related Protein 1) inhibitor (iSFRP1), compared with placebo, in subjects affected by AGA and/or TE.

2. Methods

2.1. Subjects

This multicenter, double-blind, randomized, controlled clinical trial was conducted across 2 dermatology clinics throughout Italy between May and November 2025.

In total, 40 subjects presenting with AGA, female androgenetic alopecia (FAGA), or chronic TE were screened and enrolled in the clinical trial. Participants were randomized in a 1:1 ratio using a computer-generated randomization sequence prepared by an investigator not involved in outcome assessments. Treatment allocation was concealed until study completion. Participants, treating investigators, and outcome assessors remained blinded to treatment allocation throughout the study. The investigational product and placebo were provided in visually identical formulations and packaging. Treatment allocation codes were maintained by study personnel not otherwise involved in study assessments and were not disclosed until completion of data collection and database lock. Trichoscopic image evaluations were performed without access to treatment assignment information.

The following inclusion and exclusion criteria were used (Figure 1):

Inclusion Criteria:

- Age between 18 and 60 years (inclusive);
- Clinical diagnosis of AGA (I-V Hamilton Scale for men and I-II Ludwig Scale for women) and/or TE (Savin Scale);
- No ongoing treatment for hair loss conditions at enrollment;
- Willingness to comply with study protocol and attend follow-up visits.

Exclusion Criteria:

- Presence of precancerous, systemic neoplastic, or cutaneous malignant conditions;
- Severe systemic disorders, including diabetes mellitus and cirrhosis;
- Pregnancy or breastfeeding;
- Current use of topical or systemic cosmetic or pharmacological treatments for AGA or TE;
- Known hypersensitivity to any study product ingredients.

2.2. Compliance with Ethics

All patients were evaluated and enrolled in the study after signing informed consent. This study was approved by the Ethical Independent Committee for Clinical, Non-Pharmacological Investigation, Genoa, Italy (Approval No. 2025/11, approved on 16 May 2025) and followed the ethical standards of the Declaration of Helsinki of 1964. As an observational investigation, the study was not registered in a public clinical trial registry.

2.3. Study Design

Participants were instructed to apply the assigned gel once weekly to the affected scalp area for 12 consecutive weeks according to standardized written instructions. The product was applied to dry scalp skin and left in place without rinsing, and participants were asked to avoid washing the scalp immediately after application. Usual hair-care practices were maintained throughout the study, provided that no additional hair-loss treatments were

used. The placebo gel was formulated to match the investigational product in appearance, texture, and cosmetic acceptability while lacking the active ingredients. Comprehensive clinical and dermoscopic evaluations were performed by qualified dermatologists at baseline (T0, recruitment visit), after two months of treatment (T1, 60 days), and at the end of the treatment period visit at Month 3 (T2, 90 days). At baseline, subjects were assessed for the severity of symptoms using the Norwood–Hamilton Scale (males with AGA) [25], the Ludwig Scale (females with FAGA) [26], or the Savin Scale (TE) [27]. Efficacy was assessed using digital photo-trichogram analysis and epiluminescence microscopy in order to evaluate total hair count, the percentage of anagen hair follicles, anagen/telogen ratio, and hair shaft diameter. Participant-reported satisfaction, treatment tolerability, and adverse event incidence were used as secondary endpoints. Standardized global scalp photographs were acquired at each study visit using consistent lighting, camera settings, and subject positioning. Corresponding trichoscopic images were obtained from the same pre-identified target area of the scalp using a fixed 20× magnification throughout the study period. A self-assessment questionnaire by the enrolled subjects was also used to evaluate the efficacy of the treatment in both groups. Tolerability assessment was based on the medical examination by both the investigators and the enrolled subjects after two months (T1, 60 days) and at the end of treatment (T2, 90 days).

The active product was formulated as a topical gel containing MEV-miRNAs and an inhibitor of secreted frizzled-related protein 1 (iSFRP1) as active ingredients. SFRP1 is a physiological antagonist of Wnt signaling and has been implicated in the regulation of hair follicle cycling and follicular miniaturization [28]. The milk-derived exosomes were enriched in microRNAs belonging predominantly to the Let-7, miR-21, miR-30, miR-200, and miR-148/152 families, as previously described [29]. The placebo was formulated without the above-mentioned ingredients using the same ingredients to build the gel.

2.4. Efficacy Endpoints

Treatment efficacy was assessed by analyzing changes in quantitative trichoscopic parameters—anagen hair percentage, anagen/telogen ratio, and hair shaft diameter—between baseline (T0), Week 8 (T1), and Week 12 (T2), and by comparing these changes between the MEV-miRNA gel and placebo groups. Complementary patient-reported outcomes at Week 12 were used to evaluate perceived improvements in hair strength, density, and quality.

2.5. Statistical Analysis

Statistical analyses were performed using GraphPad Prism software (version 10.4.2, March 2025). TrichoScan software (v. 3.0; Trichology GmbH, Freiburg, Germany) was used for the quantitative assessment of hair cycle parameters. TrichoScan analyses were performed using standardized acquisition procedures and calibrated equipment according to the manufacturer's recommendations. All measurements were obtained under identical evaluation conditions and from the same predefined target scalp area throughout the study to minimize measurement variability and improve the reproducibility of longitudinal assessments. Continuous variables were summarized using descriptive statistics and are presented as mean ± standard deviation (SD). Graphical representations are shown as mean ± standard error of the mean (SEM). For the primary efficacy endpoints, 95% confidence intervals (95% CIs) were calculated to provide an estimate of the precision of the observed effects. Within-group changes from baseline (T0) to Month 2 (T1) and Month 3 (T2) were assessed using paired-samples *t*-tests for anagen hair follicle percentage, anagen/telogen ratio, and hair shaft diameter. Between-group comparisons at T1 and T2 were initially evaluated using independent-samples *t*-tests.

Because baseline differences were observed for selected efficacy variables, confirmatory between-group analyses were additionally performed using analysis of covariance (ANCOVA), with treatment group included as a fixed factor and the corresponding baseline value as a covariate. Adjusted treatment differences, corresponding 95% confidence intervals, and associated *p*-values were estimated to assess treatment effects independently of baseline values. To account for multiple predefined comparisons across efficacy endpoints and timepoints, *p*-values were adjusted using the Holm–Bonferroni procedure where appropriate. A two-sided p-value < 0.05 was considered statistically significant. Detailed descriptive statistics, percentage changes from baseline, and corresponding 95% confidence intervals for the primary efficacy endpoints are reported in Supplementary Table S1. Given the exploratory nature of this pilot study, the sample size was considered adequate to provide preliminary estimates of efficacy and safety and to support the design of future confirmatory trials.

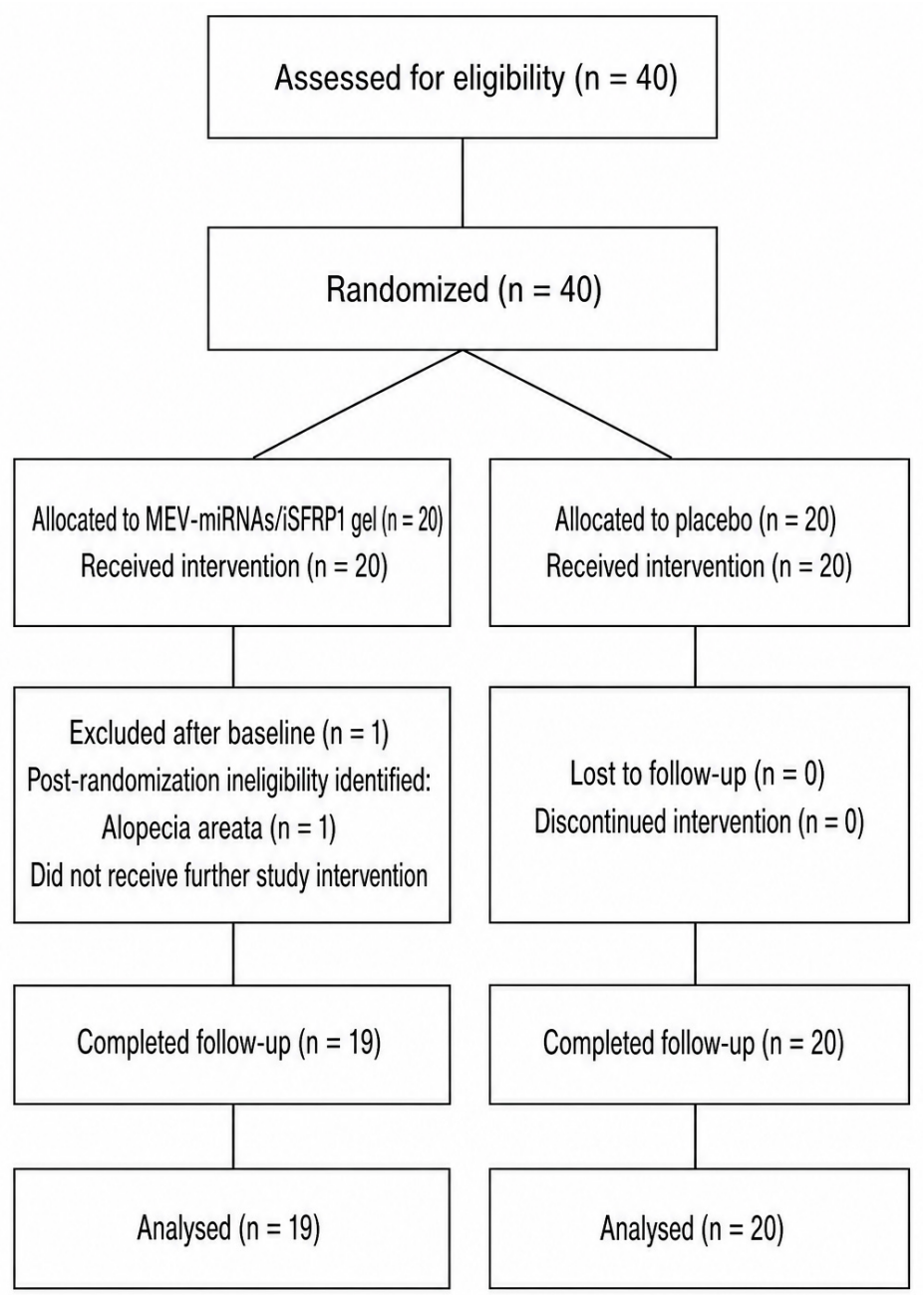


Figure 1. CONSORT flow diagram of participant screening, randomization, follow-up, and analysis.

3. Results

All baseline clinical and demographic characteristics of the randomized subjects are summarized in Table 1. Forty subjects were randomized and allocated to treatment. One participant assigned to the MEV-miRNA/iSFRP1 gel group was excluded after baseline evaluation because a diagnosis of alopecia areata, representing a post-randomization ineligibility criterion, was identified. Consequently, 39 subjects (97.5%) completed the study and constituted the final efficacy and safety analysis population.

Table 1. Subjects' demographic and clinical characteristics at baseline.

Characteristics of the Included Panel	MEV-miRNA /iSFRP1 Gel	PLACEBO
Sex	M = 10; F = 9	M = 8; F = 12
Age	38.84 ± 12.24	40.45 ± 11.86
AGA (II-IV Hamilton Scale for men)		
Grade I	1 (5.26%)	2 (10.0%)
Grade II	3 (15.8%)	2 (10.0%)
Grade III	1 (5.26%)	2 (10.0%)
Grade IV	1 (5.26%)	2 (10.0%)
Grade V	1 (5.26%)	/ (0.0%)
Grade VI	/ (0.0%)	/ (0.0%)
AGA (I-II Ludwig Scale for women)		
Grade I	2 (10.53%)	3 (15.0%)
Grade II	3 (15.8%)	4 (20.0%)
Grade III	/ (0.0%)	/ (0.0%)
TE (Savin Scale)		
M1	2 (10.53%)	1 (5.0%)
M2	2 (10.53%)	/ (0.0%)
M3	3 (15.8%)	1 (5.0%)
M4	/ (0.0%)	2 (10.0%)
M5	/ (0.0%)	/ (0.0%)

In the MEV-miRNA/iSFRP1 gel group, a significant improvement in all trichoscopic parameters was observed at both week 8 and week 12. The MEV-miRNA/iSFRP1 gel significantly increased the percentage of anagen hair follicles from 58.15 ± 1.67 (mean ± SEM) at baseline to 70.37 ± 1.05 at Week 8 and 77.73 ± 0.86 at Week 12 (both $p < 0.001$ vs. baseline) (Figure 2). In contrast, the placebo group showed no statistically significant changes despite minimal numerical increases over time (Figure 2). Notably, baseline values differed between groups (58.15% vs. 68.31% in placebo), warranting adjusted analyses. Compared with placebo, the mean change from baseline in anagen hair percentage was greater with MEV-miRNA/iSFRP1 gel at both Week 8 (+23 ± 4% vs. +4 ± 2%) and Week 12 (+36 ± 5% vs. +3 ± 2%). Between-group differences reached statistical significance at Week 12 in both unadjusted and adjusted analyses (adjusted $p < 0.001$), whereas at Week 8 significance was observed only after adjustment for baseline values using ANCOVA with Holm–Bonferroni correction (adjusted $p < 0.001$).

The anagen/telogen ratio increased significantly with MEV-miRNA/iSFRP1 gel, from 1.45 ± 0.09 at baseline to 2.46 ± 0.13 at Week 8 and 3.63 ± 0.20 at Week 12 ($p < 0.001$ for all within-group comparisons), corresponding to relative increases of +80 ± 14% and +173 ± 28%, compared with +6 ± 3% and +11 ± 7% in the placebo group. Between-group differences remained statistically significant at both time points after ANCOVA adjustment for baseline values and Holm–Bonferroni correction (adjusted $p < 0.001$) (Figure 3).

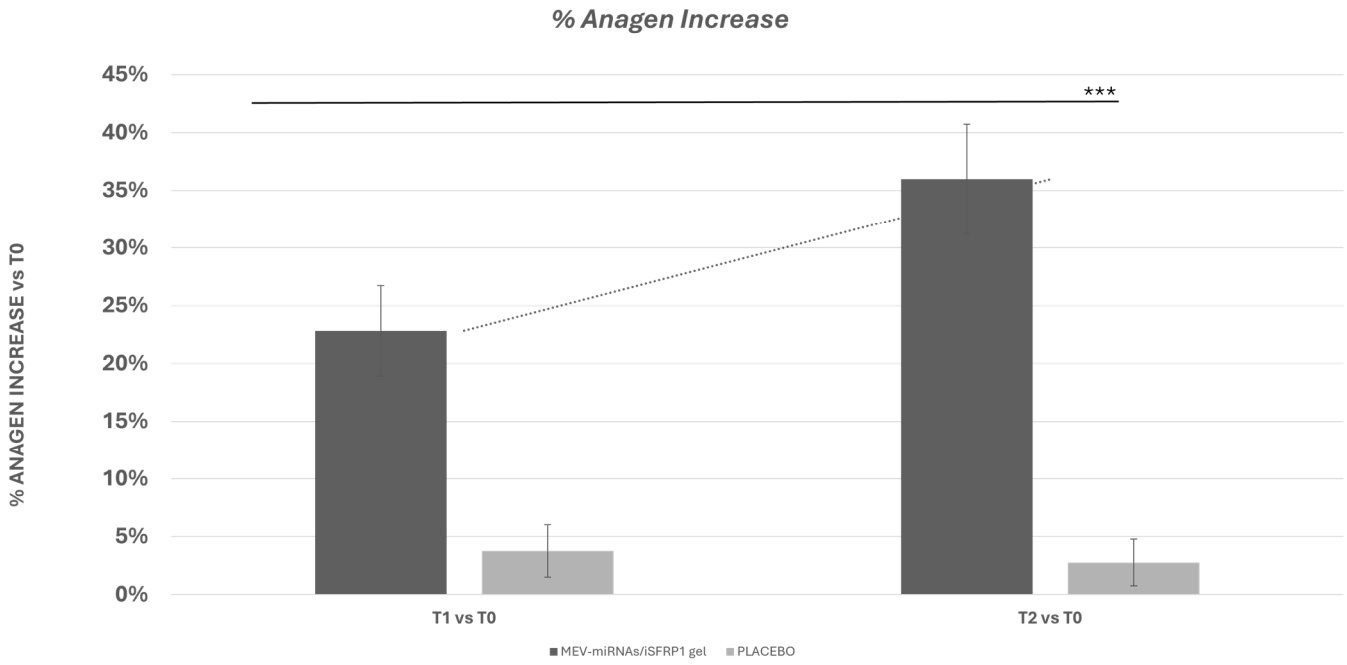


Figure 2. Percentage increase in anagen hair follicle percentage relative to baseline (T0) at Week 8 (T1) and Week 12 (T2) following treatment with MEV-miRNA/iSFRP1 gel or placebo. The bars represent percentage change from baseline; the line connects mean values across assessment timepoints for visual comparison. Data are presented as mean ± SEM. *** Adjusted $p < 0.001$ versus placebo (ANCOVA with baseline adjustment; Holm–Bonferroni correction). Abbreviations: SEM, standard error of the mean.

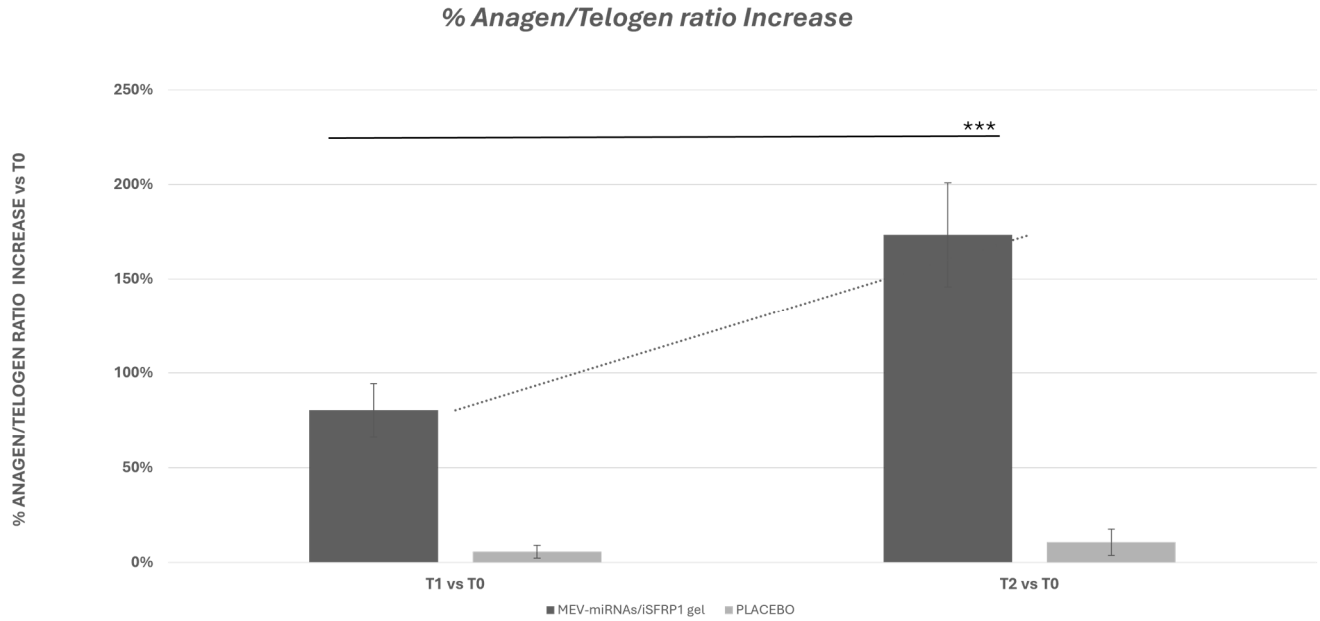


Figure 3. Percentage increase in the anagen/telogen ratio relative to baseline (T0) at Week 8 (T1) and Week 12 (T2) following treatment with MEV-miRNA/iSFRP1 gel or placebo. The bars represent percentage change from baseline; the line connects mean values across assessment timepoints for visual comparison. Data are presented as mean ± SEM. *** Adjusted $p < 0.001$ versus placebo (ANCOVA with baseline adjustment; Holm–Bonferroni correction). Abbreviations: SEM, standard error of the mean.

Hair shaft diameter increased from 47.54 ± 2.64 µm at baseline to 65.26 ± 3.10 µm at Week 8 and 70.63 ± 2.27 µm at Week 12 with MEV-miRNA/iSFRP1 gel, whereas only mini-

mal, non-significant changes were observed with placebo (46.00 ± 2.11 µm, 47.00 ± 1.74 µm, and 48.44 ± 2.52 µm, respectively) (Figure 4). This corresponded to relative increases of +50 ± 12% and +62 ± 10% with MEV-miRNA/iSFRP1 gel versus +3 ± 2% and +7 ± 4% with placebo, with statistically significant between-group differences at both Week 8 (adjusted $p = 0.001$) and Week 12 (adjusted $p < 0.001$) following ANCOVA adjustment and Holm–Bonferroni correction (Figure 4). Detailed descriptive statistics, percentage changes from baseline, and corresponding 95% confidence intervals for the primary efficacy endpoints are reported in Supplementary Table S1.

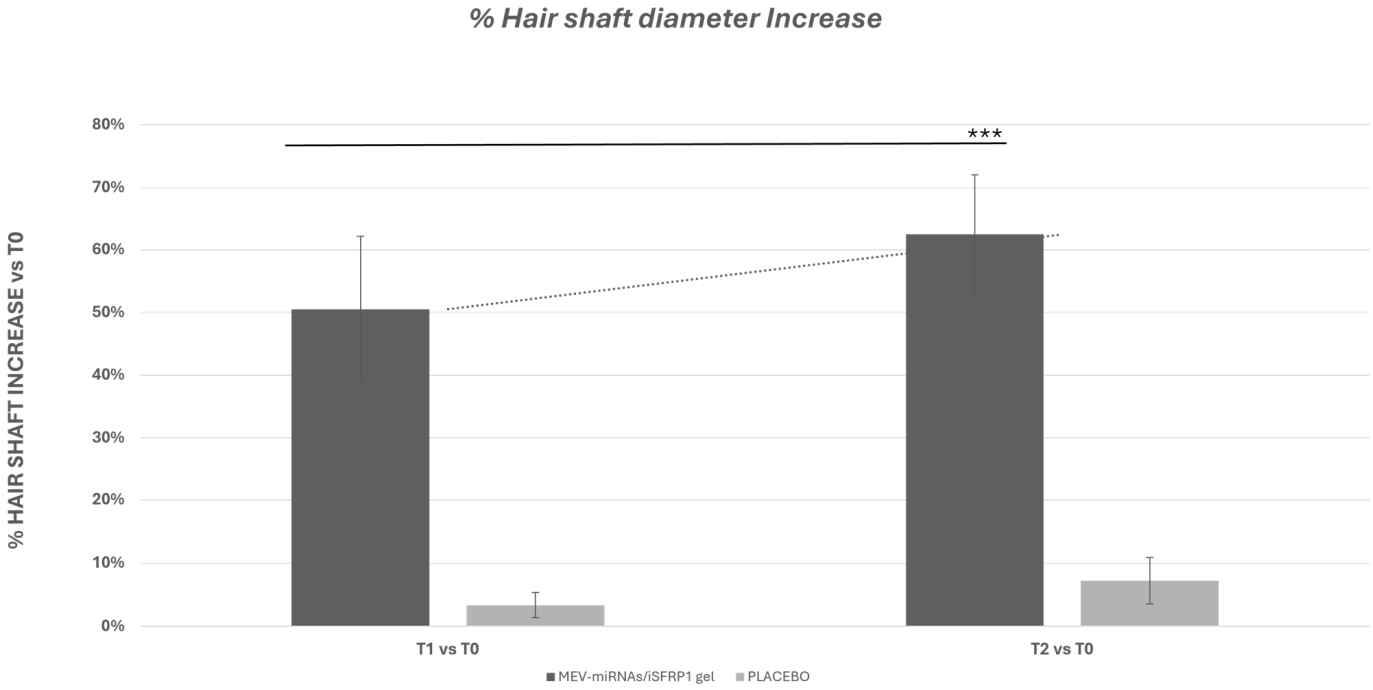


Figure 4. Percentage increase in hair shaft diameter relative to baseline (T0) at Week 8 (T1) and Week 12 (T2) following treatment with MEV-miRNA/iSFRP1 gel or placebo. The bars represent percentage change from baseline; the line connects mean values across assessment timepoints for visual comparison. Data are presented as mean ± SEM. *** Adjusted $p < 0.001$ versus placebo (ANCOVA with baseline adjustment; Holm–Bonferroni correction). Abbreviations: SEM, standard error of the mean.

At Week 12, subject-reported outcomes favored MEV-miRNA/iSFRP1 gel, with 89.47% of participants reporting stronger hair, 89.47% higher hair density, 94.74% improved hair quality, and 89.47% overall satisfaction (Figure 5). No product-related clinical signs or discomfort were reported in either group throughout the study. Figures 6 and 7 illustrate the images of representative subjects in the MEV-miRNA/iSFRP1 gel at baseline and after 3 months of treatment. Improvements were observed in the overall heterogeneous study population; the study was not powered to establish diagnosis-specific efficacy.

These structural improvements were mirrored by patients' perceptions, with most treated subjects reporting stronger, thicker hair, reduced shedding, and high satisfaction with the cosmetic outcome. Cosmetic acceptability and tolerability were high in both groups. No treatment-related adverse events, allergic *reactions*, erythema, edema, or local intolerance reactions were observed during the study period.

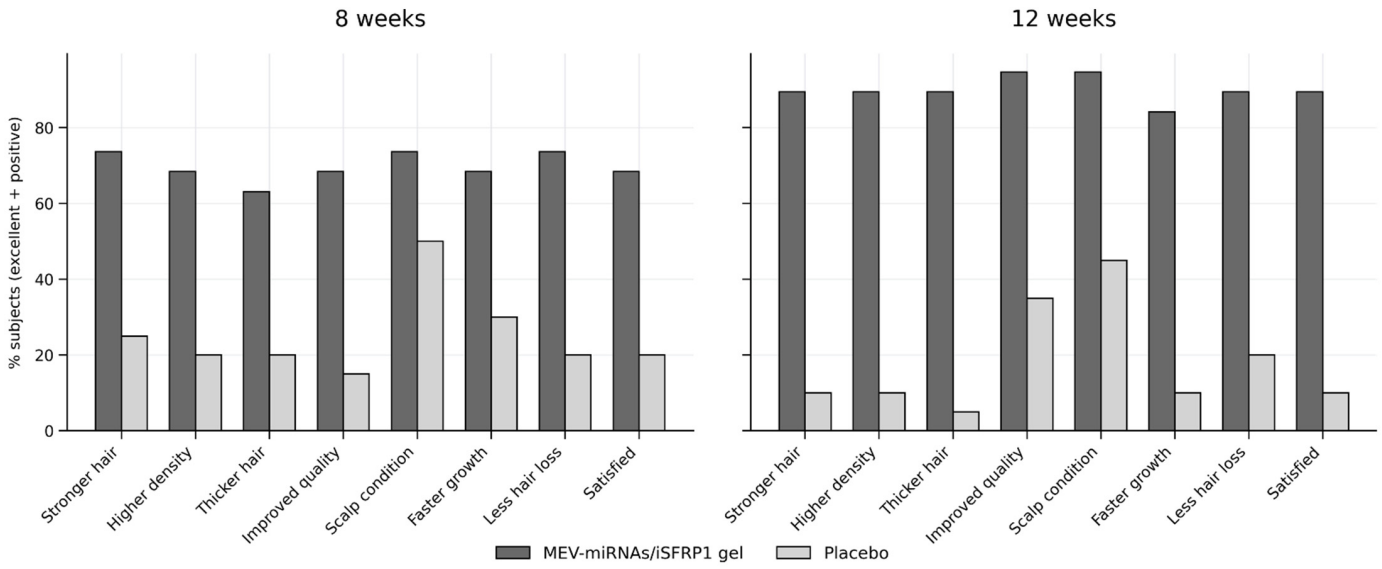


Figure 5. Patient self-assessment after 12 weeks of treatment. Percentage of subjects reporting "excellent" or "positive" outcomes across different parameters. MEV-miRNA/iSFRP1 gel showed consistently higher satisfaction scores compared to placebo.

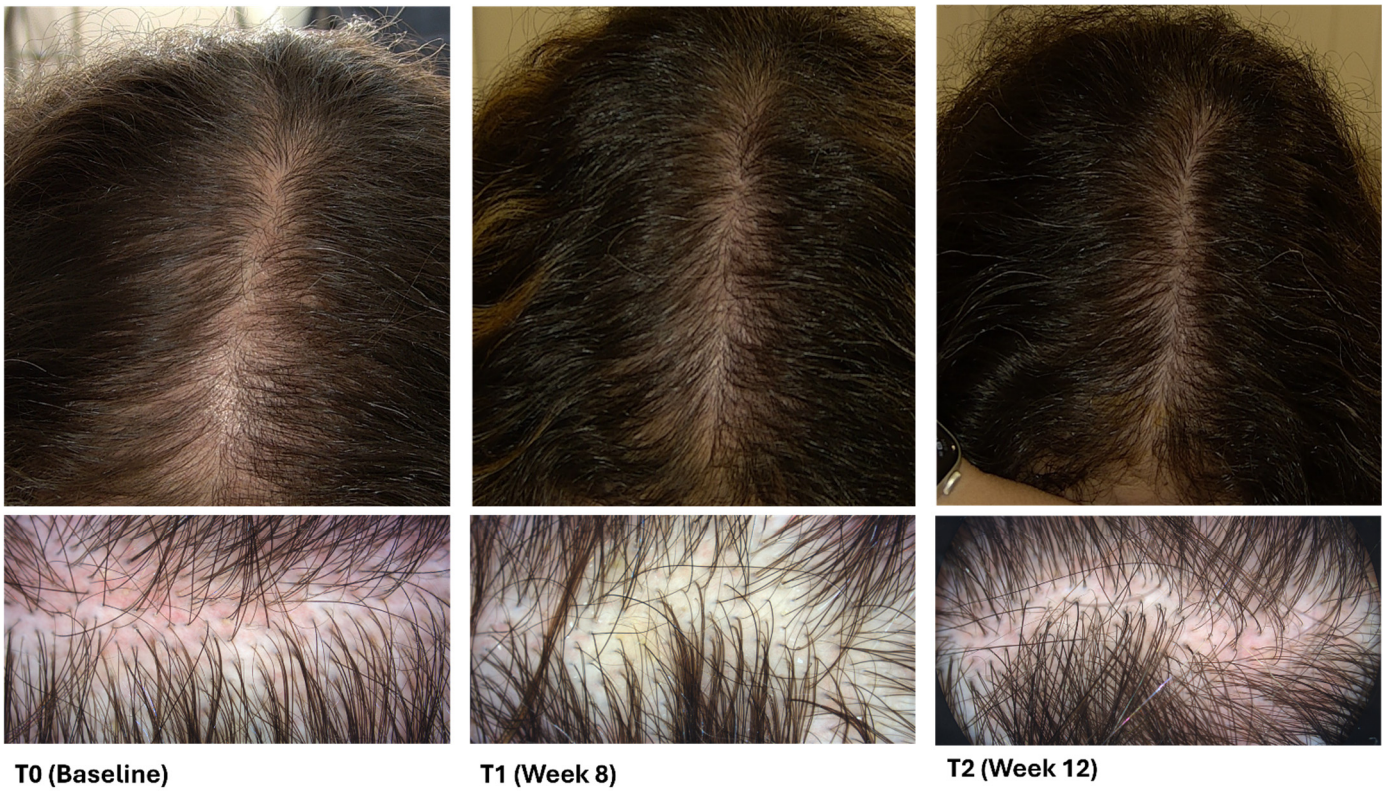


Figure 6. Representative images of a subject with androgenetic alopecia (AGA) treated with MEV-miRNA/iSFRP1 gel at baseline (T0), Week 8 (T1), and Week 12 (T2). Upper panels show standardized global clinical photographs obtained under identical acquisition conditions using consistent lighting, camera settings, and scalp positioning. Lower panels show corresponding trichoscopic images acquired at 20× magnification from the same scalp target area at each study visit.

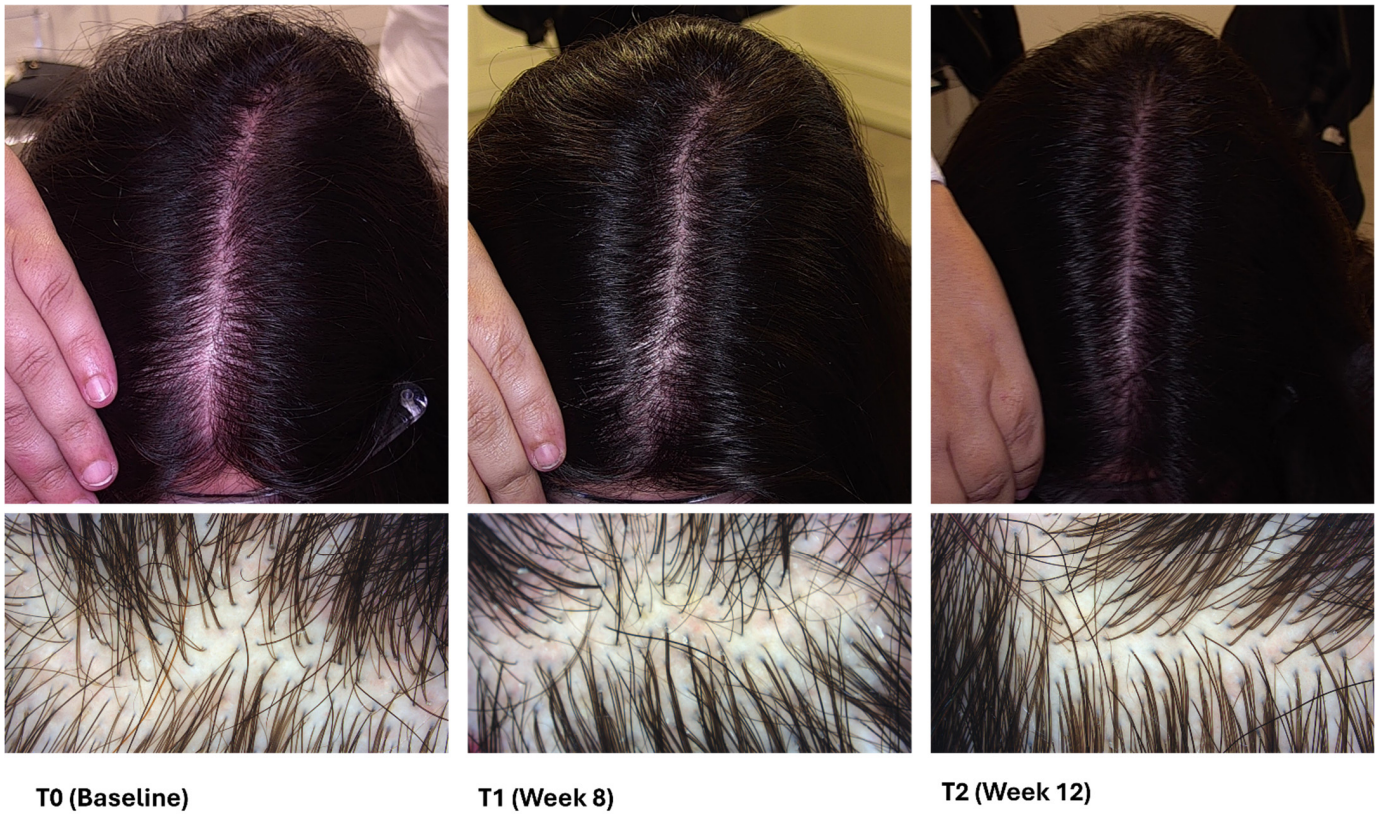


Figure 7. Representative images of a subject with telogen effluvium (TE) treated with MEV-miRNA/iSFRP1 gel at baseline (T0), Week 8 (T1), and Week 12 (T2). Upper panels show standardized global clinical photographs obtained under identical acquisition conditions using consistent lighting, camera settings, and scalp positioning. Lower panels show corresponding trichoscopic images acquired at 20× magnification from the same scalp target area at each study visit.

4. Discussion

The present study demonstrated concordant changes in objective parameters reflecting both hair-cycle dynamics and hair-fiber production. These clinical observations extend our previous experimental findings, in which the MEV-miRNA platform, evaluated independently of SFRP1 inhibition, showed biological activity in human follicular models and modulated multiple components of WNT-associated signaling. This convergence provides a translational link between experimentally demonstrated activity of the MEV-miRNA platform and the clinical response observed in vivo. However, because the clinical formulation also contained an SFRP1 inhibitor, the present study cannot determine the relative contribution of the two components or establish that miRNAs alone were responsible for the clinical effect.

The present randomized, double-blind, placebo-controlled study provides clinical proof-of-concept that a topical formulation containing MEV-miRNAs together with iSFRP1 can induce measurable changes in objective parameters of hair follicle function in subjects with AGA and TE. Importantly, the present trial establishes the efficacy of the complete formulation and cannot attribute the observed clinical effects to either MEV-miRNAs or iSFRP1 individually.

Because significant baseline differences were observed for some efficacy variables, additional ANCOVA analyses were performed using baseline values as covariates. These analyses confirmed the superiority of the active formulation over placebo for the primary efficacy outcomes, indicating that the observed effects were not attributable to baseline

imbalances between groups. Detailed ANCOVA-adjusted treatment effects are reported in Supplementary Table S1.

These *in vivo* findings are coherent with previous *in vitro* data showing that the same milk-derived exosome preparation enhances human dermal papilla cell viability and up-regulates Wnt-associated genes in organ-cultured human hair follicles [28]. However, no molecular assessments were performed in study participants; therefore, the involvement of the Wnt/β-catenin pathway should be considered a biologically plausible hypothesis rather than a mechanism directly demonstrated in vivo within the present clinical trial.

The observed increase in anagen hair percentage, improvement of the anagen/telogen ratio, and increase in hair shaft diameter are consistent with the biological effects previously documented in experimental follicular models. Nevertheless, direct confirmation of these molecular mechanisms in humans was beyond the scope of the present study.

A central element of this strategy is the specific microRNA fingerprint carried by milk-derived exosomes, dominated by Let-7, miR-21, miR-30, miR-200, and miR-148/152 families, which have conserved human orthologs and are involved in hair follicle development and cycling [12,16]. Experimental and translational studies indicate that these microRNAs can regulate proliferation, apoptosis, and differentiation of hair follicle stem cells and dermal papilla cells through pathways including Wnt/β-catenin, TGF-β, and BMP signaling, which are known to be altered in androgenetic alopecia and telogen effluvium [12,14–16]. By delivering a coordinated microRNA signature within a physiological vesicular carrier, milk-derived exosomes may facilitate the stability and cellular delivery of regulatory microRNAs [22–24]. This provides a biologically plausible rationale for the observed clinical findings, although direct target engagement was not investigated in the present study.

Clinically, the pattern of response we observed—a higher proportion of anagen follicles, an improved anagen/telogen ratio, and increased hair shaft diameter, coupled with a marked reduction in perceived hair loss—is consistent with modulation of biological pathways involved in hair follicle cycling and dermal papilla function [4,30]. In AGA, impaired Wnt signaling and reduced dermal papilla proliferative capacity are recognized contributors to progressive follicular miniaturization, whereas in TE, an abrupt shift of follicles into telogen has been associated with stress-responsive pathways, including microRNA-mediated regulatory mechanisms that may destabilize anagen maintenance [30–32]. Although the underlying molecular mechanisms were not directly investigated in the present clinical study, the observed clinical improvements are consistent with the hypothesis that modulation of follicle-associated regulatory networks may contribute to normalization of hair-cycle dynamics. The high levels of patient-reported satisfaction observed in this study, together with the favorable short-term tolerability profile, support further investigation of this approach in larger, controlled clinical trials. Because no active comparator arm was included, direct comparisons with established pharmacological, cosmetic, or regenerative interventions cannot be made. Importantly, no treatment-related adverse events, allergic reactions, or discontinuations due to local intolerance were observed during the 12-week treatment period.

However, this study has limitations. The sample size was modest and the treatment duration relatively short, which may underestimate the full potential of follicular remodeling, particularly in long-standing AGA. An additional limitation is that the study was not prospectively registered. Although the protocol, randomization procedures, blinding methods, and statistical analyses are described in detail, prospective registration would have further strengthened methodological transparency. Future confirmatory studies will incorporate prospective trial registration before participant enrollment. In addition, no scalp biopsies or molecular biomarker assessments were performed; therefore, direct confirmation of the biological mechanisms underlying the observed clinical effects was beyond the scope of the present study. The inclusion of both AGA and TE reflects clinical reality but does

not allow definitive conclusions about differential efficacy in each phenotype. Although these conditions share alterations in hair cycling and follicular function, they differ in their underlying pathophysiology and may exhibit different responses to treatment. Because the study was designed as an exploratory proof-of-concept trial and was not powered for subgroup comparisons, efficacy outcomes were not analyzed separately according to alopecia subtype. Consequently, the results should be interpreted as preliminary evidence in a heterogeneous population of non-scarring alopecia patients. Future adequately powered studies stratified by diagnosis are needed to better characterize indication-specific treatment effects. While efficacy assessments were performed at clinically relevant timepoints (Weeks 8 and 12), additional intermediate evaluations may help to further characterize the temporal dynamics of treatment response and determine the earliest detectable clinical effects in future studies. In addition, the investigational formulation contained both microRNA-enriched exosomes and iSFRP1. Accordingly, the present trial establishes the efficacy of the complete formulation rather than the efficacy of either active component considered individually. Therefore, the relative contribution of each component to the observed clinical effects cannot be determined from the current study design. In addition, the study did not include a post-treatment follow-up period; therefore, the persistence of clinical benefits after treatment discontinuation remains unknown.

Previously published *in vitro* data support the mechanistic rationale of the formulation. Further studies incorporating molecular biomarkers, *ex vivo* follicle models, or scalp tissue analyses are warranted to confirm pathway modulation in humans. Larger and longer clinical studies, ideally incorporating diagnosis-specific stratification and integrated biomarker programs (including scalp transcriptomics, microRNA profiling, and immunostaining of pathway-related targets), will be important to further investigate the biological mechanisms underlying the observed clinical effects and to refine their interpretation.

Despite these limitations, the convergence between experimental and clinical observations supports further investigation of microRNA-based regulatory strategies in hair-loss disorders. Because microRNAs are capable of modulating multiple interconnected signaling networks, they represent an attractive area for translational research in hair follicle biology. In this context, milk-derived exosomes may offer several theoretical advantages as delivery vehicles, including biocompatibility, protection of their molecular cargo from degradation, and the potential for topical administration.

As knowledge of microRNA signatures involved in AGA and TE continues to evolve, exosome-based formulations may provide useful opportunities for the development of novel topical approaches targeting hair follicle function. However, the clinical relevance of specific molecular pathways and the independent contribution of the individual formulation components require further investigation.

5. Conclusions

In this multicenter, randomized, double-blind, placebo-controlled trial, a once-weekly topical gel containing bovine milk-derived microRNA-enriched exosomes (MEV-miRNA/iSFRP1) significantly improved quantitative trichoscopic parameters, including anagen hair percentage, anagen/telogen ratio, and hair shaft diameter, in adults with androgenetic alopecia and chronic telogen effluvium compared with placebo. These objective changes were paralleled by high rates of patient-reported improvement in hair strength, density, and overall hair quality, together with a favorable short-term tolerability profile and the absence of treatment-related adverse events.

Overall, these findings support further investigation of exosome-based microRNA delivery strategies in androgenetic alopecia and telogen effluvium. However, larger studies incorporating longer follow-up, active comparators, and mechanistic assessments will

be required to better define their clinical role and therapeutic positioning. Because the investigational formulation contained both microRNA-enriched exosomes and iSFRP1, the present study establishes the efficacy of the complete formulation but cannot determine the relative contribution of either active component individually.

Although previous preclinical studies support a potential role of hair follicle signaling pathway modulation, the underlying biological mechanisms were not directly investigated in the present clinical trial. Future randomized studies with larger sample sizes, longer follow-up periods, active comparator arms, molecular biomarker assessments, and predefined stratification according to alopecia subtype are warranted to confirm these findings and to determine whether treatment responses differ between AGA/FAGA and TE patients. Overall, these findings support further investigation of exosome-based microRNA delivery strategies in hair disorders. Further adequately powered studies incorporating longer follow-up, active comparators, and mechanistic assessments will be required to better define their clinical role and therapeutic positioning.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cosmetics13050261/s1, Table S1: Descriptive statistics and 95% confidence intervals.

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Abbreviations

The following abbreviations are used in this manuscript:

AGA	Androgenetic alopecia
ANCOVA	Analysis of covariance
BMP	Bone morphogenetic protein

FAGA	Female androgenetic alopecia
HF	Hair follicle
iSFRP1	Inhibitor of Secreted Frizzled-Related Protein 1
MEV-miRNAs	Milk-derived exosome vesicles enriched in specific miRNAs
miRNA	Micro-RNA
SEM	Standard error of the mean
TE	Telogen effluvium

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