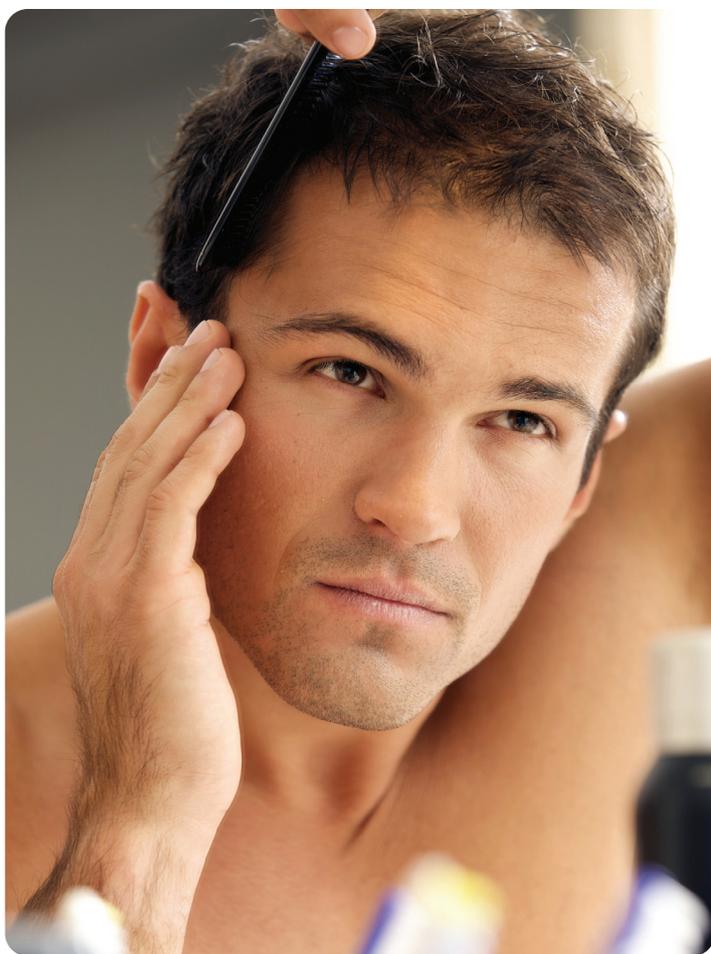


# Stimulation of hair growth by enhancing the expression of FGF7 and noggin genes

Mibelle Biochemistry developed AnaGain™, a new anti-hair loss ingredient



Plucked hair follicles before and after treatment with a scalp product were analysed for the expression of 30 genes important in hair physiology. An extract of pea sprouts (AnaGain™) was found to strongly upregulate the FGF7 and noggin genes, two important triggers for a new hair growth phase. In an anti-hair loss study, phototrichograms confirmed the efficacy of AnaGain™ to significantly increase the ratio of anagen to telogen hair and thus to improve hair growth.

## The hair growth cycle is regulated by dermal papilla signals

The human hair follicle is a complex structure consisting of an outer root sheath, an inner root sheath, the hair shaft, the bulge and the sebaceous

gland. The swollen, bottom part of the hair follicle is called the hair bulb. There highly proliferative matrix keratinocytes produce the keratinized hair shaft. The ectodermal matrix encloses a zone of mesenchymal cells, called the dermal papilla. This well vascularised zone transfers nutrients to the hair bulb and plays an important role in the hair growth cycle.

After hair follicles are generated in embryogenesis, the follicles undergo a cyclical growth. A hair growth cycle consists of three phases: anagen, catagen and telogen. Anagen is the growth phase that lasts about three to five years. Afterwards catagen, the involution phase, follows, lasting a couple of weeks. The cycle terminates with the telogen phase, a resting period of up to 4 months.

Dermal papilla cells generate signals that regulate the activity of keratinocytes in the follicular matrix. These keratinocytes stop proliferation at the end of the anagen phase and undergo apoptosis in the catagen phase. The onset of a new growth phase and thus the length of the anagen phase are defined by the release of growth factors from dermal papilla cells. Adjacent, quiescent keratinocyte progenitor cells start to proliferate and to generate a new hair follicle.

Hair follicles cycle independently leading to growing, resting and shedding hair at the same time. The density and total number of scalp hairs does not change. The proportion of telogen hair is normally 10% to 15%. Diffuse hair loss is characterised by a uniform reduction of hair density. Telogen effluvium is

a diffuse hair loss during stress with premature development of catagen and telogen follicles and premature termination of anagen follicles. In this case, a trichogram analysis shows a significant reduction in the anagen:telogen ratio leading to >25% of telogen hair. Triggers for diffuse hair loss include physiologic or emotional stress, a hormonal imbalance or nutritional deficiencies.

## Use of pea sprouts for a new anti-hair loss ingredient

AnaGain™ is based on an extract of pea sprouts (NCI name: Pisum Sativum Sprout Extract, Phenoxyethanol, Sodium Benzoate, Aqua). Many health benefits are attributed to pulses, including peas. Pulses belong to the family of fabaceae and are thus rich in isoflavones, a class of polyphenolic secondary plant metabolites. These are plant compounds that are not required for the primary metabolic processes like growth and reproduction. The role of secondary metabolites in plants is to protect them from disease, damage, pathogens, drought, salinity, extreme ultraviolet and pollutants. Many of these phytochemicals are known to exert beneficial effects on human health or to play an active role in the amelioration of disease. Isoflavones for example are known to act as phytoestrogens or to exert antioxidant activity. Sprouts are the young shoots that develop from germinating seeds. Because the shoots of a plant are especially vulnerable, secondary metabolites are very abundant at this stage.

## Effect of AnaGain™ on Gene Expression in Plucked Hairs

A clinical trial was designed to study the effect of AnaGain™ on gene expression in hair follicles. AnaGain™ was formulated at 2% into a neutral scalp product and applied in the morning and evening over two weeks on 10 subjects. A pool of 20 follicles, plucked before and after treatment, was sufficient to extract enough RNA for a quantitative gene expression profile. Thirty genes important for hair physiology were followed. Besides a modulation of the expression of genes involved in hair pigmentation, the results showed mainly modulation of the expression of fibroblast growth factor 7 (FGF7) and of noggin (Figure 1). The expression of FGF7 was increased by 56% and of noggin by 85% on average after two weeks'

treatment. FGF7 was stimulated in 8 and noggin in 7 out of the 10 volunteers. The expression products of the genes FGF7 and noggin, the FGF7 and noggin proteins, are well known signalling compounds, important for the induction of a new hair growth phase. Both proteins are mainly synthesised in dermal papilla cells. The plucked hair follicles used for the gene expression analysis contained the entire hair bulb enclosing the dermal papilla. So, the extracted RNA came not only from the matrix keratinocytes but also from the neighbouring mesenchymal papilla cells. Figure 2 shows the roles of noggin and FGF7 in the hair follicle cycle. Noggin has an indirect function. It inhibits the activity of the bone morphogenetic protein 4 (BMP4) that has a suppressive role in telogen-anagen transition. Over-expression of noggin results in a shortened refractory phase. FGF7 is a dermal papilla signal that participates in instructing hair germ cells to proliferate and initiate the new hair cycle. The results of the gene expression profiles before and after treatment indicates a stimulatory effect of the pea sprout extract on hair growth.

**Clinical Study Demonstrates Anti-hair Loss Effect of AnaGain™**

AnaGain™ formulated at 4% into a gel base, was tested on twenty, mainly female subjects. The test product was applied on a defined scalp zone, twice a day, over 3 months. Before and after treatment a phototrichogram was performed to analyse hair

growth. To perform a phototrichogram, a defined scalp zone is shaved and then photographed. The number of small dots corresponds to total hair follicles. Two days later, the shaved zone is photographed again in order to verify the amount of hair follicles in the growing phase (anagen hair). As shown in Figure 3, treatment of the scalp with the pea sprout extract clearly reduced the density of telogen hair (- 28.3%) and increased the density of anagen hair (+ 7.9%). Consequently, the hair growth coefficient which is defined as the ratio of anagen to telogen hair, increased from 4 to 7.2 (Figure 4). The slightly enhanced hair loss at the beginning of

the study (about 20% telogen hair) of the subjects could be normalised by a three month treatment with the pea sprout extract (about 12% telogen hair at the end of the study).

At the end of the study, the volunteers were asked to evaluate the efficacy of the treatment in a questionnaire. 95% of the volunteers noticed a slight to strong reduction in hair loss and a slight to strong improvement of the general hair condition (Figure 5). 85% of the volunteers noticed a regrowth of their hair.

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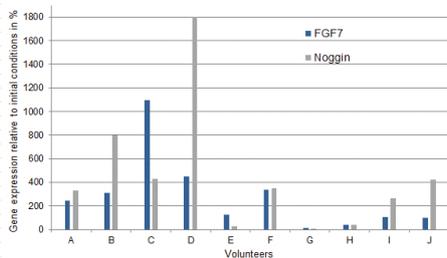


Figure 1: Effect of AnaGain™ on expression of fibroblast growth factor 7 and noggin in volunteers A to J after two weeks application

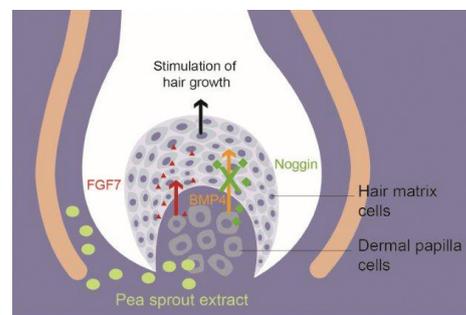


Figure 2: The Role of the noggin and fibroblast growth factor 7 proteins as terminator of the telogen phase and inducer of a new anagen phase, respectively

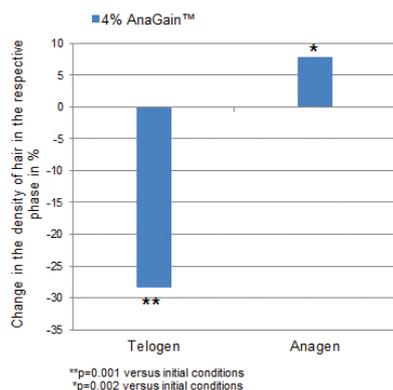


Figure 3: After three months' treatment, AnaGain™ clearly reduced the number of telogen hair and increased the number of anagen hair

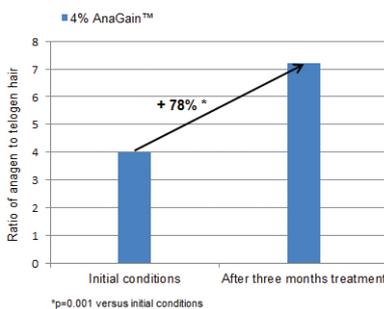


Figure 4: Effect of AnaGain™ on the hair growth coefficient (ratio of anagen to telogen hair) after application for three months

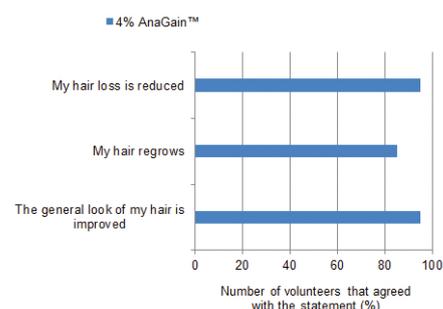


Figure 5: Evaluation questionnaire shows positive effects of AnaGain™ on hair growth