DOUBLE BLIND CONTROLLED CLINICAL TRIAL FOR THE EVALUATION OF SAFETY, EFFICACY AND COMPLIANCE OF PATIENTS USING ANAGEN LOTION, INDICATED FOR THE PREVENTION AND TREATMENT OF HAIR LOSS

COMPARISON VERSUS ANAGEN 3, A PRODUCT AVAILABLE ON THE MARKET HAVING THE SAME INDICATION

" *Importance of non-pharmaceutical active principles in the treatment of androgenetic alopecia*

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1 – BACKGROUND

Androgenetic alopecia (male pattern and female pattern) is not considered a disease, but a genetic morphological alteration of the hair follicle, which leads to premature partial or total baldness, especially in male (according to Hamilton) and partially in women (according to Ludwig).

Certainly alopecia is a common problem which affects approximately 50 % of men and perhaps 30 % of women, over the age of 40. *(1)*

Although men and women have much different clinical presentation of androgenetic alopecia as well as potential systemic influences, the underlying cellular mechanisms are presumably the same. We can say that there is a genetically determined predisposition to alopecia, even if its expression is quite variable. *(2)*

The genetically programmed DNA loci of the cytosolic receptor of hair bulbs that are carriers of the alopecic character have already been partially demonstrated by the evidence of the gene responsible for the character. *(3)*

It is therefore clear that systemic or topical treatment cannot have a complete and lasting effect, in fact these treatments are often a complicate procedure, which frequently produces insignificant clinical results.

However, patients affected by androgenetic alopecia expect help from the dermatologists in order to relieve the discomfort, above all of psychological origin *(4),* which is caused by the alopecic process.

Once proved that it is impossible to have a real and permanent regrowth of hair, what we can expect from local or systemic treatment of androgenetic alopecia is an effective delay of its evolution (see classification according to Hamilton and Ludwig) by modifying the structure of the hair bulbs and shafts in the frontal-vertex area. *(5)*

An effective therapy should have the capacity to reduce the intrafollicular transformation of testosterone in dihydrotestosterone and prevent its receptorial captivation, whilst simultaneously exerting activity on the adenylcyclase . cAMP – proteinkinase – glycolysis and on the energetic metabolism of the trichokeratinocytes. *(6)*

Today there are many treatments of androgenetic alopecia reported in the international literature, and even more numerous are the substances that are simply supposed to exert an effect on this affection. A large part of these treatments doesn’t lead to successful results because they don’t really fight

the cause of hairloss, but they have simply a temporary effect on the hair appearance.

It should be also noted that a specific medical treatment of androgenetic alopecia is not always possible : in fact in about one third of the patients a systemic treatment is hindered by specific contraindications or incompatibility.

Two theories are today recognised as the major factors involved in androgenetic alopecia :

I. Influence of androgen
II. Role of the dermal papilla in the hair cycle control and influence of the proteoglycans in the dermal sheath and dermal papilla, helping to form a protective barrier which prevents the putative immune attack *(5)*

2 – MATERIALS AND METHODS

2.1) EXPERIMENTAL DESIGN

The investigation was performed under double blind randomized conditions, according to a randomization plan prepared by the sponsor.

2.2) SELECTION OF PATIENTS

A total of 100 patients was recruited for this investigation. The characteristics of the patient population were:

- 70 males and 30 females
- age between 25 and 50 years
  medium age: 28.7 males
  34.9 females

They were divided into 2 groups of 50 subjects : the first was treated with a new hair lotion named Anagen and the second received a treatment with Anagen 3, a product already available in some markets.

Only 97 patients completed the treatment cycle (49 for Anagen and 48 for Anagen 3).

The admission criteria were a well-defined degree of alopecia, according to Hamilton and Ludwig's classification; the patients who completed the treatment were so subdivided :

	Nr. of cases	Degree of severity
Males	37	2
	32	3

	Nr. of cases	Degree of severity
Females	13	1
	15	2

Patients selection comprised also some exclusion criteria, all of the subjects responding to one or more of the following points were excluded from the study :

- androgenetic alopecia with a degree of severity not within the experimental range established for the study

- pathological affection of the scalp
- congenital or acquired pathological affections of the hair shafts, excluding symptoms compatible with androgenetic alopecia
- use of topical preparations for the treatment of hair loss (excluding shampoos and cosmetics) during the two months preceding the beginning of the trial period
- hormone therapy and use of any other preparation with an anti-androgenic activity
- use of oral contraceptives
- pregnancy
- iron deficiencies
- serious renal or hepatic pathologies
- diabetes
- non-compliance

2.3) TEST PREPARATION

The test solution (Anagen) and the control solution (Anagen 3) used for the investigation were supplied by the sponsor (Farmaka) packed in identical boxes and numbered in chronological order according to a randomization plan that was opened only at the end of the trial period.

Formulations of the products are the following (as per sponsor's statement) :

ANAGEN (content of a 5 ml vials)		
Alcohol denat.	ml	1.100
Capigen (hydrolized wheat protein + aminoacids)	mg	75.000
Sodium chondroitin sulfate	"	10.000
Serenoa repens	"	7.500
Panthenol	"	5.000
Biotin	"	0.150
Carboxymethylcisteine	"	10.000
Arginine	"	5.000
Lysine	"	5.000
Thurfylnicotinate	"	1.500
Methylparaben	"	6.750
Propylparaben	"	2.250
Potassium sorbate	"	10.000
PEG-40 Hydrogenated castor oil	"	10.000
Disodium EDTA	"	5.000
Fragrance	"	0.500

ANAGEN 3 (content of a 7 ml vials)		
Distilled water	ml	7.000
Alcohol denat.	“	0.511
Propylene glycol	“	0.233
Oxylastil (hydrolized soy protein)	mg	210.000
Glycosaminoglycans	“	10.500
Panthenol	“	7.000
Sorbic acid	“	7.000
Methylparaben	“	6.419
Thurfyl nicotinate	“	5.250
Propylparaben	“	1.400
Biotin	“	0.175
Fragrance	“	0.004

2.4) TRIAL PERIOD

The patients who completed the trial had their 3 months treatment during the period from March 1998 to June 1998

2.5) TREATMENT

The patients were requested to use the products for a period of three month. Compatibly with the high number of recruited patients, the treatment was performed during the same period of the year, in order to avoid physiological variations of the telogen phase hairs. *(7), (8)*

The dose regimen was of one vial once a day for the first month and, for the rest of the period, one vial every two days.

The patients were instructed to apply the solution mainly to the affected zones, massaging the whole scalp in order to enhance absorption.

As far as the scalp hygiene, the patients were instructed to use the same brand of shampoo (a product commonly available on the market) with their usual washing frequency, in any case not greater than three times a week.

2.6) GOOD CLINICAL PRACTICE

Before admission each subject was carefully informed about the purpose of the study and was given an information leaflet, in order to comply the good

clinical practice criteria regarding the quality of scientific studies. All subjects who accepted to participate to the study gave a verbal consent

2.7) GENERAL JUDGEMENT OF THE EFFICACY

The efficacy of the test product ANAGEN versus the reference product ANAGEN 3 was assessed through the tests listed in the next paragraph.
These subjective tests were performed at baseline, after 45 days of treatment and at the end of the trial period.

The parameters considered in order to give a complete evaluation were the following :

- ✓ controlled and/or delayed hair loss
- ✓ promotion of the hair in anagen phase, through the evaluation of increased anagen bulbs, decreased dystrophic hairs, Anagen / non anagen ratio, increased diameter of the shaft, increased total trichological count per cm^2, resistance of anagen hairs to the traction test
- ✓ improved diameter of the shaft and bulb of the hair miniaturized by the effect of the androgenetic alopecia
- ✓ stimulation of hair growth

The results of the whole test were quantified by expressing an overall clinical judgement according to the following numerical scale :

0	Unsatisfactory/ insignificant
1	Satisfactory/good
2	Very satisfactory

2.8) OBJECTIVE TESTS

- ✓ sebumetry of scalp and wrist
- ✓ cutaneous pH of scalp and wrist
- ✓ trichogram
- ✓ traction test

2.9) TOLERABILITY

The patients were asked to report signs of pruritus, burning and any signal of a general intolerance toward he product.

In order to verify the allergenic potential of the test product ANAGEN versus the control product ANAGEN 3, a patch test was performed at the end of the study, after the opening of the randomization table, on ten subject randomly chosen from each group.

The test was performed according to the Finn Chamber method, with observations after 48 and 72 hours. The solutions were used at the following concentrations :

ANAGEN	:	concentration normally used
ANAGEN 3	:	concentration normally used
HISTAMINE	:	control concentration

2.10) ACCEPTABILITY / PATIENT COMPLIANCE

During the last test control, the patients were asked to give a general opinion about the product.

3 – RESULTS

Three patients, one in the test group and two in the control group, did not complete the treatment cycle for unknown reasons and they were did not replaced.

As far as the statistical analysis, the composition of each group was homogeneous with regard to age, sex, number of cases and other parameters which allowed the statistical analysis to be reliable.

3.1) GENERAL CLINICAL JUDGEMENT ON EFFICACY

With regard to the parameters previously described, a general clinical judgement was given on the efficacy of the two substances, and the results are reported in the following table :

ANAGEN	ANAGEN 3
Score 0 = unsatisfactory results	
4 (8.16 %)	14 (29.17 %)
Score 1 = satisfactory / good results	
9 (18.37 %)	25 (52.08 %)
Score 2 = very satisfactory results	
36 (73.47 %)	9 (18.75 %)

Significance $p < 0.05$

From a general point of view, it can be seen that the test product ANAGEN gave 91.84 % of positive results, while in the control group of ANAGEN 3 the positive results were 70.83 %

3.2) TRICHOGRAM

The widely used trichogram technique allowed us to have a reliable evaluation of the ratio between anagen and non-anagen hair before and after the treatment in both groups.
The normal values of a trichogram are the following :

	FRONTAL AREA	OCCIPITAL AREA
Anagen	81 – 85 %	83 – 85 %
Catagen	0 – 2 %	0 – 2 %
Telogen	13 – 15 %	12 – 15 %
Dystrophic anagen (as part of anagen)	2 – 5 %	2 – 5 %

The results obtained with the trichogram performed on the frontal area are reported in the following tables :

	ANAGEN	
	BEFORE	AFTER
Anagen	47 %	80 %
Catagen	25 %	7 %
Telogen	2 %	2 %
Dystrophic anagen	26 %	11 %

$p < 0.01$

	ANAGEN 3	
	BEFORE	AFTER
Anagen	48 %	78 %
Catagen	24 %	10 %
Telogen	2 %	2 %
Dystrophic anagen	26 %	10 %

$p < 0.05$

The best data in order to evaluate the real improvement of the hairs' condition are the ratios between anagen and non-anagen hair, as reported in the following table :

	ANAGEN / NON ANAGEN RATIO	
	Before	After
ANAGEN	2.66	9.66
ANAGEN 3	2.76	6.50

The total count of the anagen hair includes also the dystrophic anagen.

The data obtained from the trichogram performed in the occipital area are not reported because they did not give a statistically significant difference

3.3) DIAMETER OF THE HAIR SHAFT

The mean diameter of the hair shafts before and after the three months treatment are reported in the following table :

	HAIR SHAFTS MEAN DIAMETER	
	Before	After
ANAGEN	0.040	0.095 $p < 0.05$
ANAGEN 3	0.040	0.065 p = n. s.

3.4) TOLERABILITY

No patients appeared to have dermatological or systemic affection at the baseline control and during the following examination controls.

In order to have a correct evaluation of the patients' tolerability, they were warned about a possible temporary scalp redness, due to the presence of a local vasodilator in both products in order to enhance the penetration of the active principles.

No side effects were reported by any subject in both groups, and the patch tests were negative for both substances.

The above results let us state that the tolerability of both substances was excellent.

3.5) ACCEPTABILITY AND COMPLIANCE

The cosmetic acceptability reported by the 97 subjects is the following :

	COSMETIC ACCEPTABILITY	
	ANAGEN	ANAGEN 3
Excellent	30	24
Good	17	20
Neutral	2	3
Unpleasant	0	1

These are the judgements directly reported by the patients; for a direct comparison between the test and the control substance, it should be noted that the quantity of product for each application is different (5 ml of Anagen and 7 ml of Anagen 3).

43 subjects treated with Anagen felt the product as pleasant and " effective ", while 35 subjects expressed a similar opinion of Anagen 3.
Almost all the patients of the control group(91.8 %) reported to have much shiner and fuller hair, while a similar judgement was given by 77.5 % of the patients in the Anagen 3 group.

A considerable percentage of both groups reported to have a decreased hair oiliness (40 subjects in the Anagen group and 38 in the Anagen 3 group).

5 patients gave a neutral judgement, while only one patient defined Anagen 3 unpleasant basically because he thought to have an increased hair stickiness.

4 – DISCUSSION

As we have previously seen in the background, there are only two theories internationally accepted as playing a predominant role in the field of androgenetic alopecia .

1. **The role of the dermal papilla in hair cycle control** : Westgate and colleagues *(5)* suggested that proteoglycans in the dermal sheath and dermal papilla form a protective barrier, preventing the putative immune attack.
2. **Androgens** : androgens are the most important systemic modulators of human hair growth. They are necessary for the conversion of vellus hair to terminal hair, a process that starts at puberty and continues for several decades.

Androgens circulate in the blood either free or bound to proteins, especially sex hormone binding globulins. It is believed that free steroids diffuse into the cell where it binds to a specific nuclear receptor. Interaction between the receptor hormone complex and nuclear DNA activates the relevant genes to regulate the appropriate RNA. *(5)*

The metabolism of testosterone to the more potent 5α-dihydrotestosterone (DHT) by the enzyme 5α-reductase appears to be an important step in many androgen-responsive tissues. *(5)*

According to the formulation of the test product Anagen that we used in this trial, it is clear that :

- ✓ Serenoa repens possesses a well established activity in inhibiting 5α-reductase *(9)* and consequently the transformation of testosterone in 5α DHT, which is the main responsible of the alopecic androgenetic process
- ✓ The presence of proteoglycans (sodium chondroitin sulfate) is able to tackle the decrease of the proteoglycans themselves in the dermal papilla during the telogen phase.

As we have seen in the background, other factors are involved in the alopecic process. In the Anagen formulation a component named Capigen® which contains glycosaminoglycans of marine origin; homotaurine, a synthetic homologue of the aminoacids taurine, and a bacterial filtrate of vegetal origin rich in peptides. It was chosen mainly for three reasons :

- ✓ It slows down excessive hair loss
- ✓ It stimulates hair renewal and regeneration
- ✓ It regulates seborrhea, often associated to the alopecic process.

A clinical study, which proves these activities, is available. *(10)*

5 – CONCLUSION

The results obtained during the investigation of the effects of the test product ANAGEN have demonstrated the efficacy of its active principles.
In fact it was presented as a very interesting product because it possesses a topical anti-androgen action, a characteristic which avoids the usual side effects of the products used for this purpose. The advantages of a pleasant and safe formulation for a preparation indicated for the hairloss treatment certainly increases the chances of compliance, which is essential in order to achieve clinically significant results.

The results obtained seem to confirm these facts. In fact there was a significant change in the registered values, in particular the percentage of anagen hairs and the anagen/telogen ratio of the frontal vertex region. It should be underlined that these values were obtained through reliable technique such as the trichogram.

Another interesting result is the increased diameter of the hair shaft obtained after the treatment, one of the most indicative parameters of the activity on the trichokeratinocyte stimulation. *(6)*

The major efficacy of the test product can probably be attributed to the anti-androgenic effect of the Serenoa Repens extract, in addition to the activity of the components named Capigen® and the already ascertained capacity of GAG's, panthenol and biotin in stimulating the energetic metabolism of the bulb matrix cells.

We can say that our investigation has confirmed the efficacy of the test product ANAGEN. Comparison between before- and after- treatment values, recorded through clinical and instrumental observation, indicates positive results obtained in 91.84 % patients treated with ANAGEN. Such results were achieved by a lower percentage (70.83 %) in the reference group of ANAGEN 3, even if we can consider good also this result.

These facts should be considered in light of the fact that the reference product ANAGEN has been on the market for some years, with clinically demonstrated results, as reported by a clinical trial effected some years ago versus a product that is widely used still today. *(11)*

Finally, we can conclude that the obtained results allow us to state that the test product ANAGEN is effective in the treatment of male and female pattern of androgenetic alopecia, and it is safe to use because it has no components with systemic activity.

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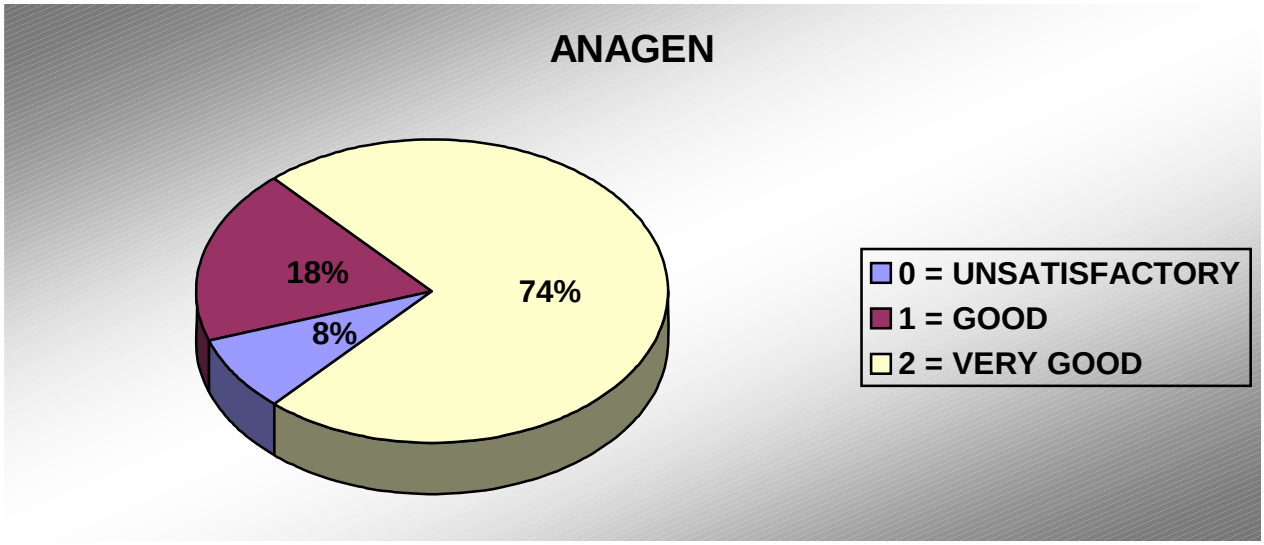
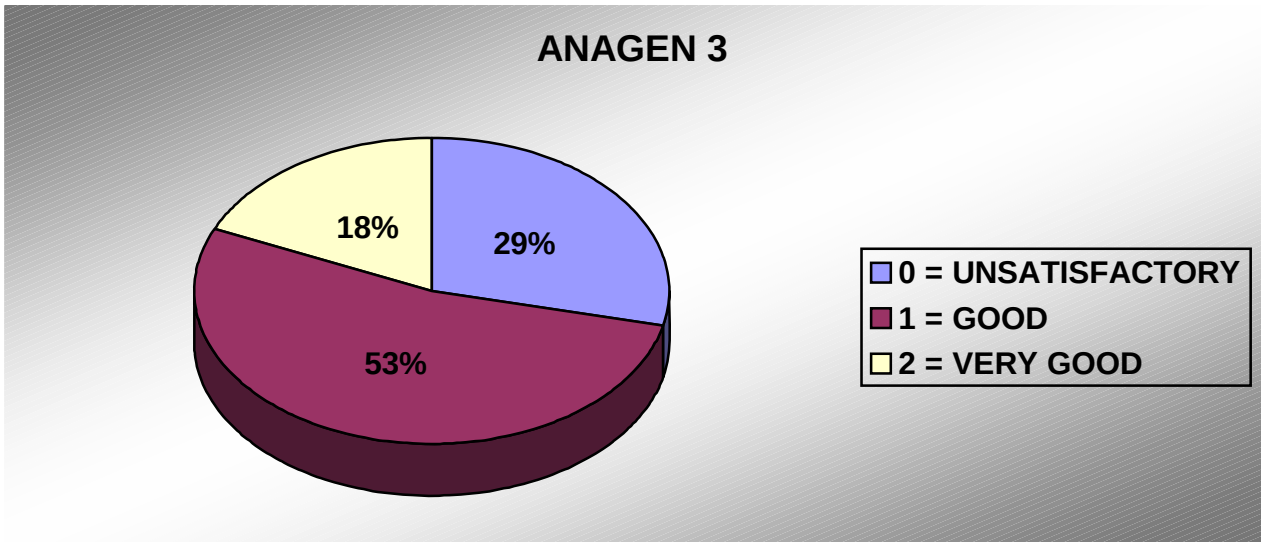
GENERAL CLINICAL JUDGEMENT ON EFFICACY





ANAGEN / NON ANAGEN RATIO