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A Randomized, Double-Blind, Placebo-Controlled Pilot Study to Evaluate the Efficacy and Safety of Latanoprost for Eyelash Growth in Aesthetic Medicine

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Abstract: Eyelashes, in addition to fulfilling eye-protective functions, generate an aesthetic impact. Latanoprost is a prostaglandin analog, commonly used for the treatment of glaucoma and intraocular hypertension. The side effect reported most often is the stimulation of eyelash growth. The aim of this study was to evaluate the efficacy and safety of latanoprost in inducing eyelash growth and darkening. Thirty healthy volunteers were recruited in a 3-month, randomized, double-blind, placebo-controlled pilot study. A transparent eyelash mascara was used as a vehicle. The placebo group (n = 15) received only the vehicle, and the latanoprost group (n = 15) received the vehicle + 0.005% latanoprost. The participants were asked to apply the latanoprost topically to the eyelashes while avoiding entry into the eye, daily at night. The latanoprost group showed a significant increase in eyelash length and color change, while the placebo group maintained eyelash length and color throughout the study. The intraocular pressures of all the participants were maintained in normal ranges (10–20 mmHg). Topical application of both the placebo and the latanoprost medications was well tolerated; none of the participants withdrew, generated side effects, or developed any ophthalmic pathology. In conclusion, latanoprost is effective and safe to stimulate the growth of eyelashes.

Keywords: latanoprost; eyelash growth; aesthetic medicine; prostaglandin

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1. Introduction

The eyelashes, in addition to having an aesthetic impact, provide a protective function by maintaining the integrity of the eyeball [1]. In addition, they help to spread lacrimal fluid over the surface of the eye to transfer nutrients and oxygen to the cornea, thus contributing to achieving homeostasis [2]. Furthermore, eyelashes reduce ultraviolet light reaching the cornea by up to 24% and prevent particles from entering [3]. To preserve the integrity of the eye, the length of the eyelashes is key to avoid and reduce the number of particles that reach the eye; the eyelashes trap the particles, preventing them from reaching the eye or reducing the number that do reach the eye [2]. On the upper eyelid, the eyelashes are distributed between five and six rows, holding approximately between 90 and 160 eyelashes, while on the lower eyelid there are approximately 80 eyelashes, distributed in approximately three to four rows [1,4].

The follicles of the eyelashes are terminal specialized hair. Their structure is similar to that of curly hair from the scalp hair follicle; however, the eyelash follicle has shorter hair shafts and does not have an arrector pili muscle, which serves to straighten the hair as a reaction to cold or emotions. Lastly, the follicles are not dependent on sexual hormones, and their growth cycle is shorter [5].

The life cycle of eyelashes lasts from four to eleven months and consists of three phases: (i) growth, (ii) degradation, and (iii) rest. These phases are known as the anagen, catagen, and telogen phase, respectively. In each new life cycle, the old follicle falls out and a new hair shaft is formed, caused by the activation of specialized epithelial stem cells. The first signal to initiate the cycle in the anagen phase occurs in the stem cells of the follicular germ located between the lower bulge and the dermal papilla. These cells are stimulated by active growth signals through the release of hair germ activating factors, such as fibroblast growth factor-10 (FGF-10), fibroblast growth factor-7 (FGF-7), transforming growth factor β2 (TGF-β2) from dermal papillae, platelet-derived growth factor α (PDGF-α) from adipocyte precursor cells, and the bone morphogenic protein (BMP) inhibitor Noggin. Together, these signals achieve the initiation of follicle growth, along with Shh and the canonical Wnt/β-catenin signaling pathway [6–10]. There are several studies that focus mainly on this pathway, including that of L. Zhou et al., who showed that this pathway accelerates the growth of matrix cells and the dermal papilla cells [11]. During this stage, hair pigmentation is observed due to the migration, proliferation, and increased activity of melanocyte progenitor cells. There are factors that influence the maintenance of this phase when receiving external stimuli, including hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), and insulin-like growth factor 1 (IGF-1) [12]. When the stem cells exhaust their regenerative capacity, the growth phase ends, and the follicle begins the catagen phase [13] in which the area of the pons remains intact and the lower two thirds of the follicle degrade [14]. Finally, the telogen or resting phase is maintained by K6+ cells, which are quiescent stem cells of the lower protuberance, along with adipocytes and fibroblasts, releasing follicle-growth-inhibitory factors such as BMP6, FGF-18, BMP4, and BMP2 [15].

The hairs of the eyelashes are the last to fall, and, in general, have the characteristic of being darker than those of the rest of the human body. They usually do not gray with aging, which could be explained by the existence of different populations of melanocytes in the dermal papilla: (a) progenitors of melanocytes and (b) tyrosinase-2 associated melanocytes. If tyrosinase-2 associated melanocytes are activated, they can inhibit melanocytes and lead to the appearance of gray hair [16–18]. The mechanism that regulates the turnover of melanocytes and melanogenesis in the eyelash hair follicles is so far unknown [19,20]. However, it is known that the eyelash hair follicles are sensitive to prostaglandins (PG) [21,22].

Latanoprost (or, to use its IUPAC name, propan-2-yl (Z)-7-[(1R,2R,3R,5S)-3,5-dihydroxy-2-[(3R)-3-hydroxy-5-phenylpentyl]cyclopentyl]hept-5-enoate ($C_{26}H_{40}O_5$)) is a prostaglandin analogue drug which is used to lower intraocular pressure; it is commonly used for the treatment of glaucoma or ocular hypertension and serves as an intraocular hypotensive agent. Its first clinical use was reported in 1995 [23]; in 1997, the first case of the side effect of eyelash growth stimulation was reported, as was an increase in the number of eyelashes and a change in pigmentation which made the eyelashes darker [24]. Since then and to date, the same adverse effect has been reported in people using latanoprost and/or PG analogues [22,24–30]. However, other studies have reported its use with possible limitations or drawbacks associated with the use of latanoprost for this purpose; these include possible unwanted side effects such as iridial or lid pigmentation [30,31].

The objective of this protocol was to evaluate the efficacy and safety of latanoprost applied topically to eyelashes to stimulate their growth in aesthetic medicine.

2. Materials and Methods

2.1. Materials

A commercially available transparent mascara for eyelashes ("Pro Gel Máscara de Kiko Milano") composed of deionized water, carbomer, copolymer, and methacrylate was used as the vehicle for the study. Latanoprost 0.005%—2.5 mL without preservatives (Latof, Saval pharmaceutical, Renca, Chile) was purchased from a pharmacy and added to the bottle of the mascara, shaking gently until homogenized.

2.2. Study Design

The presented study was a three-month randomized, double-blind, placebo-controlled clinical trial in healthy women, to evaluate the efficacy and safety of latanoprost used topically on the eyelashes to induce the growth of the eyelashes.

Our clinical trial was exploratory, since there is nothing reported in the literature on the use of mascara as a vehicle. In order to obtain preliminary information on the efficacy of this drug for aesthetic purposes, a pilot study must always be started before a phase I study, in order to take care of the safety and integrity of the participants; in cases such as this where a pilot study take place, the number of the sample is by convenience (30 participants).

This study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of State Investigation, Health Services of the State of Querétaro Sub-directorate of Education, Training and Research with an ethics code of 1490/09-12-2022; the date of approval was 21 December 2022. Each participant received the descriptive information for the study protocol. Each participant was described, and all their doubts about informed consent were resolved. Informed consent was received from each participant.

2.3. Participants

During the selection visit, the principal investigator (PI) explained and clarified any doubts regarding the informed consent. In addition, the PI evaluated the following parameters of each volunteer patient: clinical status, medical history, pregnancy test, and medication use. The participants underwent an ophthalmological evaluation to determine intraocular pressure and rule out ophthalmic pathologies. The exclusion and inclusion criteria are shown in Table 1. The participants' data were treated confidentially.

Table 1. Inclusion and exclusion criteria.

Criteria	Description
Inclusion	Healthy women between 18–50 years Any amount of eyelash prominence Any iris color Intraocular pressure between 10–20 mmHg
Exclusion	Diseases such as hypertension, diabetes mellitus, obesity, cardiovascular and respiratory disease, cancer, psychiatric, neurological disorders, mental illness, abuse of substances Eye diseases such as cataracts, active keratitis, history of herpetic keratitis, iritis, uveitis, glaucoma, intraocular hypertension. Dermatological conditions such as alopecia, atopic or contact dermatitis, vitiligo No eyelash growth treatment in the last 6 months No eyelash extension No history of tattoos on the upper and/or lower eyelids Patients allergic to analogues of prostaglandins

2.4. Experimental Procedure

Latanoprost 0.005% without preservatives was added to the bottle of mascara (3.4 mL), shaking gently until homogenized. The final concentration of the drug was 0.00367%.

The secondary investigator (SI), by randomization, established the list of participants for group 1 without latanoprost (placebo; n = 15) and group 2 with latanoprost (n = 15). The placebo group received only the mascara (vehicle), and the latanoprost group received the mascara + latanoprost. The SI was in charge of labeling each product with the name and date of birth of each participant. Only the SI had access to the information throughout the protocol; this information remained secret and stored in a safe place until the statistical analysis was performed.

To avoid increasing errors in the measurements, the PI was the only person in charge of measuring the length of the participants' eyelashes and recording adverse effects. The first measurement was at month 0, which was prior to the start of treatment, followed by three more measurements every month, until the conclusion of the protocol month 3 (Figure 1). Furthermore, the participants went to the same ophthalmologist to measure their intraocular pressure monthly. The PI was in charge of getting in daily contact with the participants to remind them of the daily application of the product at night. The instructions given to the participants were as follows: Carry out the usual routine to remove makeup; clean the face with foaming cleanser (CeraVe, Bridgewater, NJ, USA); apply and distribute the product evenly with the help of the applicator brush, starting from the edge of the upper eyelid at the base of the eyelashes; do not use the product inside the eye; leave the product to act at least 7 h at night.

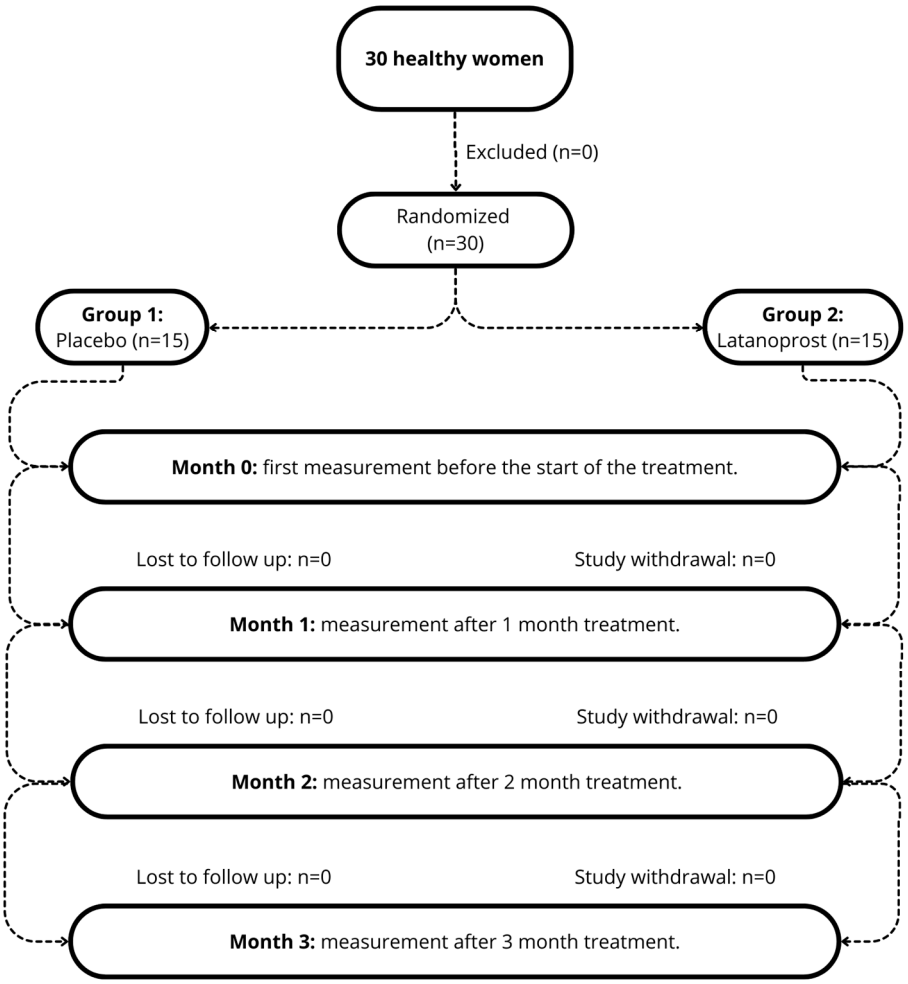

Figure 1. Flow chart of the study protocol.

2.5. Measurement of Eyelash Growth

The measurement of eyelash growth was carried out by the same person (PI), in order to avoid bias. Eyelash growth was measured with a ruler, and an average of each patient's eyelash length was considered, based on other studies that measure change in eyelash length [32].

2.6. Measurement of Eyelash Color Change

The measurement of eyelash color change was carried out by the same person (the PI). Color change was measured using a color palette. Six different shades of black were coded, with 1 being the lightest black and 6 being the darkest black.

2.7. Measurement of Intraocular Pressure

Intraocular pressure was measured by an ophthalmologist using a Goldmann applanation tonometer, Haag-Streit brand, AT 900 model (Koeniz, Switzerland). The measurements were performed in triplicate.

2.8. Statistical Analysis

The statistical analysis was carried out using Minitab v19.2020 software (State College, PA, USA). The comparative analysis was carried out with one-way ANOVA for the comparison between the groups (latanoprost vs. placebo). This was followed by a Student's *t* test, in which the variables measured were eyelash growth and color change, making comparisons between all the measurements (month 0, month 1, month 2, and month 3). Values of $p < 0.05$ were considered to denote a significant difference between the control (placebo) and test (latanoprost) groups.

3. Results

A total of thirty healthy female volunteers between the ages of 18 and 42 years were enrolled in the trial. Among them, fifteen received latanoprost and fifteen received the placebo. All 30 participants completed the study (i.e., none withdrew from the study). All the participants had dark brown irises. None of the participants reported any adverse effects or developed any ophthalmic pathology.

3.1. Eyelash Growth

The results of the growth of the left and the right eyelash are shown in Table 2 and Table S1 (Supplementary Information). In addition, in Figure 2 we show the eyelash growth during the three-month treatment (Figure 2A—left eyelash; Figure 2B—right eyelash). The blue line indicates the placebo group, and the orange line indicates the latanoprost group. The placebo group did not present significant eyelash growth. All the participants maintained the same eyelash length during the three months of treatment, while the group treated with latanoprost presented significant growth starting with month 1. This indicated that treatment with latanoprost is time-dependent. Thus, comparisons were made between all the months as shown in Table 2; the only month that was not statistically significant was month 2 vs. month 3, for both eyelashes (left and right). Finally, comparison of the placebo group and the group treated with latanoprost showed a significant difference.

Figure 3 shows the eyelash growth of a participant in the group treated with latanoprost. The measurement was made using a ruler, and the participant was asked to close the eye so that the PI could measure and take photographs. The results outline the significant growth that is presented in Table 2. Figure 4 demonstrates no eyelash growth of a participant in the placebo group when applying only the vehicle (mascara for eyelashes).

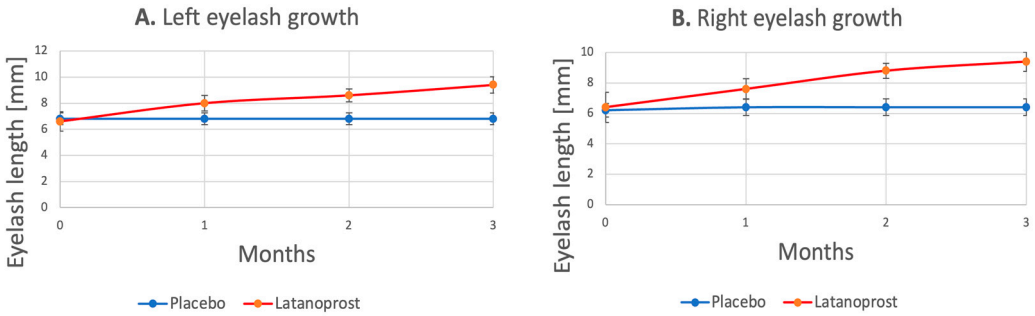

Figure 2. Eyelash growth between placebo (blue line) and latanoprost (orange line) treatments; (**A**). Left eyelash growth; (**B**). Right eyelash growth. The data are presented as average, and the error bars indicate standard deviation.

Table 2. Eyelash growth measurements.

Eyelash	Groups	Growth Measurements	Group Comparison
Left eyelash	Placebo (n = 15)	Month 0 vs. Month 1 Month 0 vs. Month 2 Month 0 vs. Month 3 Month 1 vs. Month 2 Month 1 vs. Month 3 Month 2 vs. Month 3	Placebo vs. latanoprost *
	Latanoprost (n = 15)	Month 0 vs. Month 1 * (+24%) Month 0 vs. Month 2 * (+34%) Month 0 vs. Month 3 * (+43%) Month 1 vs. Month 2 * (+10%) Month 1 vs. Month 3 * (+16%) Month 2 vs. Month 3 (+6%)	
Right eyelash	Placebo (n = 15)	Month 0 vs. Month 1 Month 0 vs. Month 2 Month 0 vs. Month 3 Month 1 vs. Month 2 Month 1 vs. Month 3 Month 2 vs. Month 3	Placebo vs. latanoprost *
	Latanoprost (n = 15)	Month 0 vs. Month 1 * (+23%) Month 0 vs. Month 2 * (+38%) Month 0 vs. Month 3 * (+46%) Month 1 vs. Month 2 * (+12%) Month 1 vs. Month 3 * (+18%) Month 2 vs. Month 3 (+5%)	

* Indicates significant difference, p-value < 0.05. The growth measurements were counted in millimeters. Each group had a sample size of 15. The percentage expressed is the average value of the entire group. No percentages values are given to the placebo groups because there were no significant differences.

3.2. Eyelash Color Change

For the change in the color of the eyelashes (Table 3) and Table S1 (Supplementary Information), the participants in the placebo group are not showing a significant change because they maintained the same eyelash color throughout the treatment. By contrast, the latanoprost group presented a significant change in color until the second month (month 0 vs. month 2) but did not present a significant difference in the third month (month 0 vs. month 3); the only values that showed a significant difference were "month 0 vs. month 2" and "month 1 vs. month 3".

3.3. Intraocular Pressure and Ophthalmic Pathologies

The thirty participants were evaluated (month 0, month 1, month 2, month 3) by an ophthalmologist. The latanoprost concentrations used were the same as those used in the treatment of glaucoma. In all the check-ups, the participants presented intraocular pressure within the normal ranges (10–20 mmHg), and in none of the measurements were ophthalmic pathologies present.

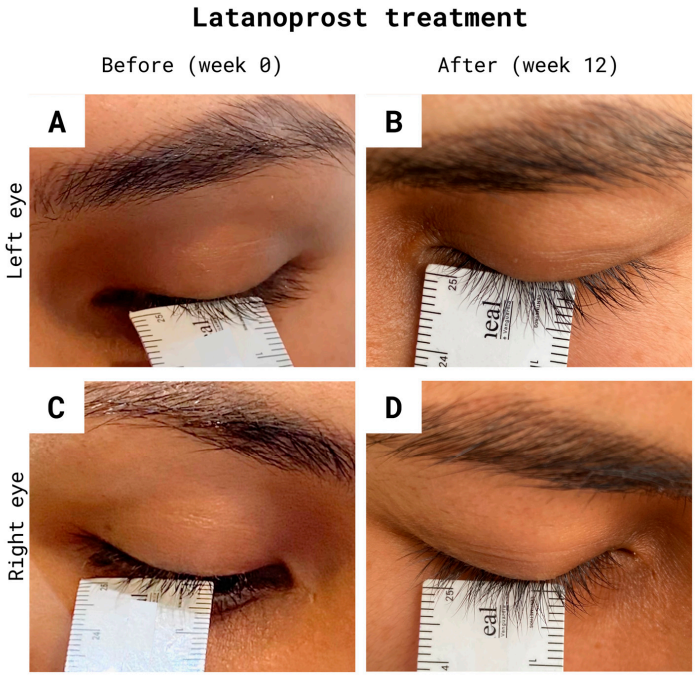

Figure 3. Growth of both eyelashes (left and right) from a latanoprost-treated participant; (**A**) baseline measurement (week 0) of the participant's left eye prior to treatment; (**B**) final measurement (week 12) of the participant's left eye at the end of treatment; (**C**) baseline measurement (week 0) of the participant's right eye prior to treatment; (**D**) final measurement (week 12) of the participant's right eye at the end of treatment. Measurements and photos were performed by the PI in triplicate.

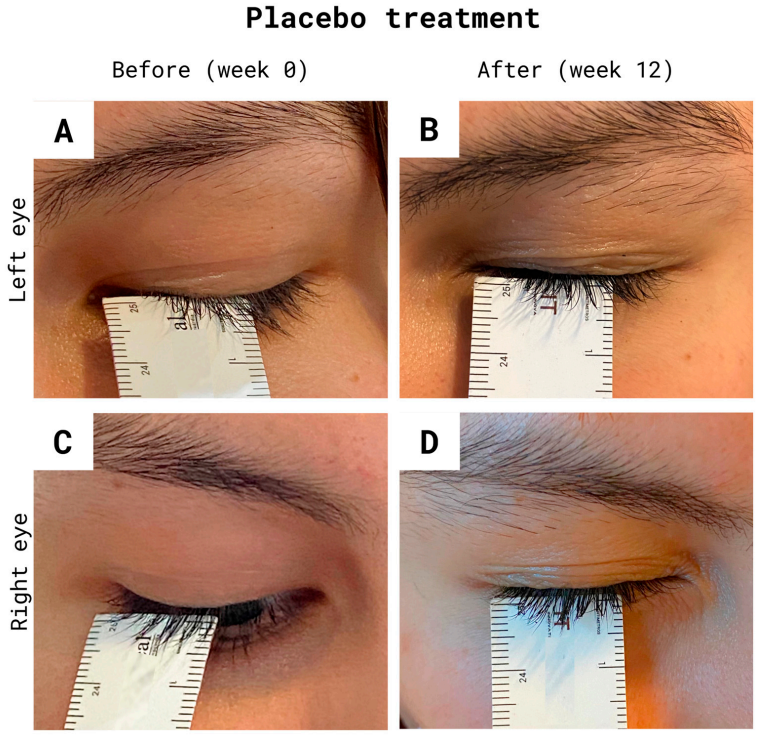

Figure 4. Eyelashes (left and right) from a placebo-treated participant; (**A**) baseline measurement (week 0) of the participant's left eye prior to treatment; (**B**) final measurements (week 12) of the participant's left eye at the end of treatment; (**C**) baseline measurement (week 0) of the participant's right eye prior to treatment; (**D**) final measurement (week 12) of the participant's right eye at the end of treatment. Measurements and photos were performed by the principal investigator in triplicate.

Table 3. Eyelash color change.

Eyelash	Groups	Color Change	Group Comparison
Left eyelash	Placebo (n = 15)	Month 0 vs. Month 1 Month 0 vs. Month 2 Month 0 vs. Month 3 Month 1 vs. Month 2 Month 1 vs. Month 3 Month 2 vs. Month 3	Placebo vs. latanoprost *
	Latanoprost (n = 15)	Month 0 vs. Month 1 (+10%) Month 0 vs. Month 2 * (+23%) Month 0 vs. Month 3 (+15%) Month 1 vs. Month 2 (+5%) Month 1 vs. Month 3 * (+15%) Month 2 vs. Month 3 (+7%)	
Right eyelash	Placebo (n = 15)	Month 0 vs. Month 1 Month 0 vs. Month 2 Month 0 vs. Month 3 Month 1 vs. Month 2 Month 1 vs. Month 3 Month 2 vs. Month 3	Placebo vs. latanoprost *
	Latanoprost (n = 15)	Month 0 vs. Month 1 (+10%) Month 0 vs. Month 2 * (+23%) Month 0 vs. Month 3 (+15%) Month 1 vs. Month 2 (+5%) Month 1 vs. Month 3 * (+15%) Month 2 vs. Month 3 (+7%)	

* Indicates significant difference, p-value < 0.05. Each group had a sample size of 15. The percentage expressed is the average value of the entire group. No percentage values are given to the placebo group because there were no significant differences.

4. Discussion

In order to analyze the efficacy of the use of the eyelash-growth-stimulating agent latanoprost, without generating adverse effects in patients for aesthetic purposes, we conducted a randomized, double-blind, placebo-controlled pilot study. The relevant information provided by our study is the use of a mascara-based vehicle for eyelashes with a lower concentration of latanoprost 0.00367% than that commonly used in other studies. In addition, previous studies have shown that the use of latanoprost generates iridial or lid pigmentation [30,31]. To avoid this with our patients, we asked them to use latanoprost topically on the eyelashes with the help of a mascara to prevent droplets from flowing into the eye, but other studies have either used cotton or have asked the patient to place the latanoprost around the edge of their eyelids with their own finger [24,33]. Our patients were asked to apply the latanoprost at night as previously reported [29,34].

To measure the growth of the eyelashes, some studies have used qualitative scales that are also controlled by the patient; that is, the patients define whether they consider that their eyelashes have grown or not; the scales used included the following: "no regrowth", "slight", "moderate", and "total" [33]. We consider that using this type of scale managed by the patient could lead to biasing the data and provide inaccurate statistical results. Based on studies in which eyelash growth was measured with a ruler [32], we opted to use quantitative scales for accurate statistical data analysis. In our case, we asked the patient to close the eye, and the PI carefully placed a ruler under the eye to measure the length of the eyelash. However, since other studies have reported that the eyelash was extracted with tweezers, even with its root, prior to measurement [29,31], we consider our method to be less invasive. The time of the study was determined at 3 months by taking references from studies that have observed eyelash growth at 10 weeks [31] or 3 months [24,33].

Our results for both eyelashes (left and right) show that the placebo group did not present eyelash growth; the length of their eyelashes was maintained during the

three months of the study, while the group treated with latanoprost presented a statistically significant increase in length from the first month 1. This is consistent with other authors who reported an increase in eyelash length of 6.3% at 1 month [30] and 12.1% at six weeks [31]. In order to determine that our treatment was time-dependent—i.e., that there was an increase with respect to the time of use—comparisons were made between all months. Our results were favorable, as reported in other studies that presented statistically significant results at the second month [35]. The only month that was not statistically significant was "month 2 vs. month 3"; this indicates that from month 2 to month 3 there was no longer a significant increase. This goes against what was previously reported; other studies even report significant statistics in later months, such as 19.5% at 10 weeks [24] and 0.5–0.7 cm at six months, comparing to the initial measurement [29]. It must be mentioned that latanoprost has a reversible effect; that is, if latanoprost is stopped, the new eyelashes will grow in their original shape (prior to treatment) [33]. However, this effect persists for several months [36]. Latanoprost can lengthen the anagen phase [12], which is the growth phase of the eyelash's life cycle [37]. Nesher et al. demonstrated that F2α receptors are expressed only in the anagen phase. These receptors are located mainly in the inner sheath of the root of the bulb and the stalk of the eyelashes [38].

The molecular target of latanoprost is the prostaglandin F receptor, PTGFR [36]. Several cell types express the gene-encoding PTGFR. Among these are endothelium and smooth muscle cells of blood vessels of the iris, ciliary muscle, ovarian, and uterine myometrium [39,40]. PTGFR is also expressed in the outer root sheath and in the bulb of the eyelash hair follicle [31,41]. Examples for natural agonists of this well-studied receptor are the hormones prostaglandin F2α (PGF2α), PGD2 and PGE2, with PGF2α displaying the highest binding affinity to PTGFR. The binding affinity of latanoprost to PTGFR is similar to PGF2α [42,43]. When stimulated, a conformational change occurs in PTGFR that activates an associated G protein by exchanging bound GFP to GTP (i.e., stimulated PTGFR is acting as a guanine nucleotide exchange factor). The G protein consists of three subunits, α, β and γ, respectively. The α subunit, in its GTP-bound form, dissociates from the β and γ subunits. More specifically, PTGFR is associated with a G protein containing a Gαq/11-type α subunit which activates beta-type phospholipase C (PLC-β). PLC-β increases the levels of the signaling molecules IP3 (inositol trisphosphate) and DAG (diacylglycerol) by hydrolysis of the precursor molecule PIP2 (phosphatidylinositol 4,5-bisphosphate). IP3 acts as a soluble second messenger to release stored calcium from the endoplasmic reticulum into the cytoplasm, while DAG constitutes a membranous second messenger that activates protein kinase C (PKC) [44]. This chain of events stimulates the release of activating factors from the hair germs, which causes changes in the gene expression of the resting stem cells located in the germ of the follicle, stimulating them for the subsequent activation of the Shh and Wnt signaling pathways; this ultimately prolongs the anagen or growth phase [6–9,31]. Furthermore, these mechanisms elicit a plethora of cellular responses which ultimately lead to an increase in uveoscleral outflow of aqueous humor with a concomitant reduction in intraocular pressure. For the present study, it is relevant to note that latanoprost has documented positive effects on the length, pigmentation, curvature, thickness, and number of eyelashes [26,44].

Our research group decided to use latanoprost because latanoprost and travoprost are more selective than bimatoprost for PTGFR receptors; latanoprost and travoprost are structural analogues of the natural PGF2α molecule [45]. Furthermore, when comparing the three PG analogues (latanoprost, bimatoprost, and travoprost), it has been shown that latanoprost is the best tolerated with the least adverse effects [34]. None of our participants presented any side effects; however, side effects such as lid pigmentation and blurred vision have been reported in other studies [31].

The participants presented a change in the color of their eyelashes; the results with a statistically significant difference were "month 0 vs. month 2" and "month 1 vs. month 3". This indicates that at least two months of treatment with latanoprost are necessary to result in a change in eyelash color. Fagien et al. reported subjectively based on the patient's

assessment; in their study they used bimatoprost (another PG analogue), which generated a change in the color of the patients' eyelashes. The authors commented that the color change was due to putative pathways in melanin production [46]. The change could also be explained by the fact that during the anagen stage, hair pigmentation is observed due to the migration, proliferation, and increased activity of the melanocyte progenitor cells [12].

Our results showed that latanoprost applied topically is effective in inducing eyelash growth. In future experiments we want to increase our sample number to yield better statistical results and include more male participants.

5. Conclusions

Eyelashes serve several important functions in maintaining the integrity of the eye and play a significant role a person's aesthetic perception, especially in women. Here we evaluated the commercially available prostaglandin analog latanoprost in its capacity to stimulate growth and pigmentation of eyelashes. Participants who applied latanoprost showed a significant increase in their eyelash length and a change in color towards darker hues. This was in contrast to the placebo group already after one and two months of treatment, respectively. Overall, topical applications of latanoprost and the vehicle alone were well tolerated. Importantly, intraocular pressure was not influenced in any way and remained in a physiological range throughout the study. Therefore, latanoprost, when topically applied, is both effective and safe to increase the length of eyelashes and their pigmentation within a relatively short time frame.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cosmetics10050136/s1.

Author Contributions: Conceptualization, J.I.E.-S., E.M.-N. and M.A.T.-V.; data curation, C.Q.S.; formal analysis, C.Q.S. and M.A.T.-V.; funding acquisition, C.Q.S.; investigation, J.I.E.-S. and M.A.T.-V.; methodology, J.I.E.-S. and M.A.T.-V.; project administration, C.Q.S. and M.A.T.-V.; resources, J.I.E.-S. and M.A.T.-V.; supervision, C.Q.S. and M.A.T.-V.; validation, C.Q.S. and M.A.T.-V.; visualization, J.I.E.-S., E.M.-N., C.Q.S. and M.A.T.-V.; writing—original draft, J.I.E.-S., C.Q.S. and M.A.T.-V.; writing—review & editing, J.I.E.-S., C.Q.S. and M.A.T.-V. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of State Investigation, Health Services of the State of Querétaro Sub-directorate of Education, Training and Research (protocol code 1490/09-12-2022 and date of approval 21 December 2022).

Informed Consent Statement: Informed consent was obtained from all the subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

Data Availability Statement: Not applicable.

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Conflicts of Interest: The authors declare no conflict of interest.

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