

A NEW HYALURONIC ACID POLYMER IN THE AUGMENTATION AND RESTORATION OF LABIA MAJORA

N. ZERBINATI¹, R.G. HADDAD², A. BADER³, R. RAUSO⁴, E. D'ESTE⁵,
G. CIPOLLA⁵, A. CALLIGARO⁶, P. GONZALEZ⁷, S. SALVATORE⁸
and D. SERAFIN⁹

¹*Department of Surgical and Morphological Sciences, University of Insubria (Varese), Italy;*
²*Consultant Obstetric Gynecology Faculty of the American Academy Aesthetic Gynecology Dubai - Paris - Riyadh;* ³*Consultant OB&GYN. Founder of the European Society of Aesthetic Gynecology "ESAG" London - Dubai - Athens;* ⁴*Department of Dentistry-University of Foggia, Foggia, Italy;*
⁵*Department of Dermatology, Multispecialised Medical Center, Pavia, Pavia, Italy;* ⁶*Department of Public Health, Experimental and Forensic Medicine, Unit of Histology and Embryology, University of Pavia, Pavia, Italy;* ⁷*Department of Obstetrics and Gynecology, Urogynecology Unit, Head Chief Hospital Universitario San Jorge.-Pereira, Colombia;* ⁸*Department of Gynecology, IRCCS San Raffaele Hospital, Milano, Italy;* ⁹*Department of Aesthetic and Reconstructive Gynecology, Multispecialised Medical Center Gliwice, Poland*

Aesthetic surgery of female external genitalia has gained increasing popularity over the past decade, with reduction of the labia minora (labiaplasty) being the procedure most commonly requested and performed. Female external genitalia lose elasticity and volume with age, but few studies describe the techniques for labia majora augmentation. Currently, very few studies have investigated the effectiveness and safety of labia majora augmentation with hyaluronic acid (HA) injection. This study aims to evaluate the effectiveness and safety of labia majora augmentation with hyaluronic acid filler injection. We retrospectively analyzed 37 patients affected by hypotrophy of the labia majora, treated with HA dermal filler 28mg/ml PEG crosslinked (Neauvia® Intense Rose, Matex Lab, Switzerland) between May 2015 and July 2016. Global evaluation of the aesthetics of the intimate area and clinical data were investigated with VAS (Visual Analogic Scale) *ad hoc*. Adverse events and complications were recorded. A total of 37 women affected by labia majora hypotrophy were treated with 28mg/ml HA dermal filler. A significant clinical improvement was observed in the score provided by both patients and doctor. Only mild adverse events and complications were recorded. HA hydrogel with a novel crosslinking agent is able to provide a considerable rejuvenation with a simple outpatient procedure and to bring a significant clinical improvement. HA-based filler infiltration treatment in labia majora is repeatable, has virtually no complications, and is reversible.

Lately, there has been an increasing demand of patients requesting improvements in body contouring with safe and minimally invasive procedures (1).

Aesthetic surgery of female external genitalia has

gained increasing popularity over the past decade, with reduction of the labia minora (labiaplasty) being the procedure most commonly requested and performed (2). The surgical procedure, labiaplasty, is

Key words: hyaluronic acid, dermal filler, augmentation, restoration, labia majora, labiaplasty, PEG, poly-ethylene-glycol

Mailing address:

Dr. Nicola Zerbinati,
Department of Surgical and Morphological Sciences,
University of Insubria, Varese, Italy
Tel.: +39 0382 556680
e-mail: nzerbinati@centro-medico.it

153(S)

0393-974X (2016)

Copyright © by BIOLIFE, s.a.s.

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.

Unauthorized reproduction may result in financial and other penalties
DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

well described with some very interesting pre-surgical classifications and new techniques (3, 4), such as the effect of the laser energy device in the vulvar, and vaginal tissue modification (5, 6). External female genitalia lose elasticity and volume with age. The thickness of the vulvar epithelium reaches its peak during the reproductive years and decreases with age. Indeed, the thickness of the whole epithelium is influenced by estrogens, which induce nuclear modifications among the superficial epithelial cells during each menstrual cycle. Thus, macroscopically, labia majora decrease their volume and tone, and change their coloration because vascularization rapidly decreases after menopause. Following the loss of follicular activity, the external genitalia lose subcutaneous fat, while the connective tissue relaxes and becomes less elastic; this is associated with pain during sexual intercourse (7-10). Also for these reasons, an increasing interest in the aesthetic appearance of female genitalia has also been observed in the field of cosmetic medicine. Hyaluronic acid (HA) can be injected during a simple outpatient procedure to augment labia majora, providing youthful and natural results (11). In the last years, the industry has developed new dermal fillers for facial and body treatments. HA has no antigenicity for species or tissue and thus shows low potential risk for allergies or immunogenic reactions (12). There are various HA fillers presenting different rheological properties, cross-links, concentrations, viscosity and consistency. Currently, there are very few studies which have investigated the effectiveness and safety of labia majora augmentation with hyaluronic acid injection (13).

In this article we report our observational clinical study on the effectiveness and safety of soft-tissue augmentation of labia majora with an HA dermal filler 28mg/ml PEG crosslinked.

MATERIALS AND METHODS

Materials

A new HA dermal filler of bacterial origin was used for the volumetric correction of labia majora. In this case, the monophasic hydropolymer was created with 28 mg/ml of HA extract by *Bacillus subtilis* and crosslinked with

polyethylene-glycol (PEG) (Neuvia® Intense Rose, Matex Lab, Switzerland).

PEGylation (14, 15, 17), seems to offer considerable advantages also in the field of fillers for aesthetic use, both in terms of safety and of gel performance.

Both PEG and hyaluronic acid are polymers, and their union allows to create matrices with scaffold architecture (16, 18, 19), that is a three-dimensional weft consisting of large meshes which offer high assimilation of the gel into the tissue, and the possibility to include and gradually release molecules which are useful for skin rejuvenation.

Methods

Indications for tissue augmentation of external female genitalia are limited to the labia majora and mons pubis.

Labia majora are two prominent longitudinal cutaneous folds that extend from the mons pubis to the perineum. Anteriorly, they are joined forming the anterior labial commissure, located just above the clitoral hood, and posteriorly they form the posterior labial commissure, located approximately 2 cm from the anus. Labia are thicker anteriorly. They have connective tissue and subcutaneous adipose tissue intermixed with smooth muscles resembling the scrotal dartos muscle (13).

The infiltrations of HA fillers in this region should be placed between the lip dartos and the fibrous tunic containing the adipose body of labia majora. The vulva is supplied by the anterior labial arteries, branches of external pudendal arteries, posterior labial arteries and branches of the internal pudendal arteries. An adequate knowledge of their localization is fundamental to avoid intraluminal infiltration with potential severe complications, such as vascular occlusion or embolization.

The procedure involves administering HA filler to the subcutaneous tissue of labia majora through an injection using a small-diameter cannula. Correct instruments are essential, as only a blunt-tip cannula reduces the risk of inadvertently injuring the tissue or blood vessels and helps prevent further complications. It is also essential to determine the location of blood vessels and nerves supplying the vulva prior to treatment. They are located within the lower pole of labia majora, therefore caution is advised when the first injection is carried out at the lower pole.

The first step involves a thorough decontamination and anaesthetising of the area to be injected. Local anaesthesia is used with approximately 5 ml of 2% lidocaine

administered along the labia in a single injection. It is repeated in the same manner on both labia. Firstly, a 16G needle is used to access the labia. Then, the 18G cannula is used to administer the filler to the upper pole of the labia. A linear, downward application mode should be followed. It is important to administrate the product in the whole length of the labia majora, but it is more important to create a natural shape by dividing this as one fourth of the syringe at the top pole, two fourths in the middle and one fourth at the lower pole. In order to achieve tissue augmentation or labial contour enhancement, a very superficial subcutaneous injection is performed. In order to correct tissue volume, the filler should be administered slightly deeper. The depth of injection plays an important role, as accessing too deep structures may result in administering the filler to the adipose tissue, thus losing its effect (Fig. 1).

No more than 1.5 ml of Hydrogel 28 mg/ml PEG-crosslinked of cross-linked HA was injected on each side for all the patients treated.

We retrospectively analyzed 37 patients affected by hypotrophy of labia majora, treated with hyaluronic acid filler between May 2015 and July 2016. All patients included in the study provided written informed consent

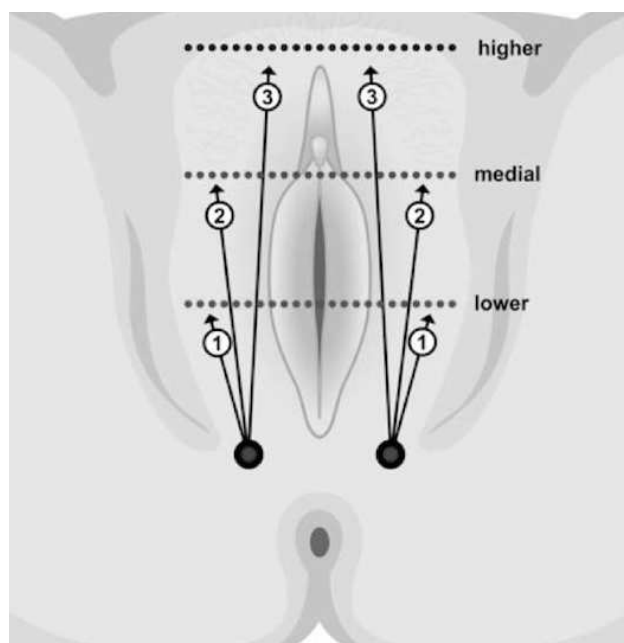


Fig. 1. The injection in the three different layers was performed with a cannula of 18G with the first injection point in the lowest edge of the labia. The maximum quantity of 1.5 ml of Hydrogel was distributed with a fan technique in the medial and lower part of labia.

and the procedure was compliant with the ethical guidelines Declaration of Helsinki. Demographic data, menopause, and pharmacologic treatment were recorded.

Inclusion criteria were hypotrophy of labia majora and indication to treatment with dermal fillers. Exclusion criteria were active local herpes simplex or herpes zoster infection, active herpes zoster infection in other sites, local dermatitis, mycosis, bacterial infections, previous surgery on external genitalia, history of vulvar cancer, vulvar squamous papilloma, previous regional radiotherapy, autoimmune diseases, history of adverse reactions to hyaluronic acid fillers, treatment with anticoagulants, pregnancy or breastfeeding.

The effectiveness of the treatment was investigated by firstly evaluating the change in elasticity and volume of the labia majora before and after treatment by using the Visual Analogue Scale (VAS) with a range from 1 (lack of elasticity and volume) to 10 (sufficient elasticity and volume). Secondly, the intensity of pain before and after treatment both during sexual intercourse (the patient) and during the medical examination (the doctor) using the VAS scale of pain assessment with range from 1 (no pain) to 10 (severe pain). Thirdly, the global aesthetic evaluation of intimate parts before and after treatment using a VAS scale of satisfaction with the range scale from 1 (very dissatisfied) to 10 (very satisfied).

Patients and medical observers wrote their score on a form. The forms were then collected and analyzed once all evaluations had been completed. Adverse reactions and complications were recorded at the periodic follow-up visits after 1, 3, and 6 months. Pre-treatment and post-treatment results were analyzed with the descriptive statistics.

RESULTS

Thirty-seven women affected by hypotrophy of labia majora, average age 45-years (range 36-68 years) were enrolled in this study. The analysis carried out on our sample shows an improvement of the clinical data after treatment, noticed both by patients and the investigator (one patient had only the doctor's evaluation).

53% (19/36) of the patients reported moderate elasticity and 47% (17/36) sufficient elasticity of labia majora after treatment than they did before treatment, when 64% (23/36) reported low elasticity,

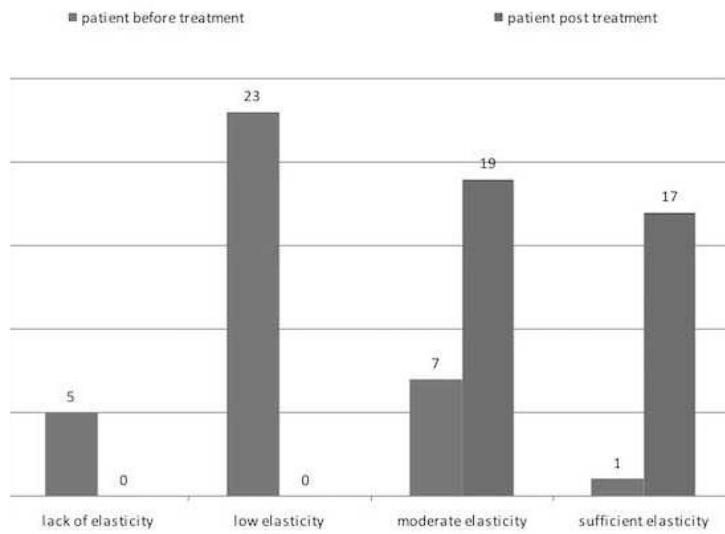


Fig. 2. Patient evaluation of elasticity before and after treatment.

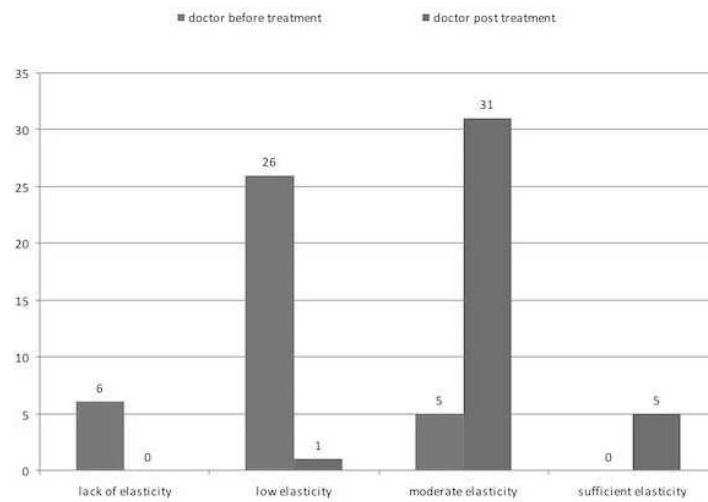


Fig. 3. Physician evaluation of elasticity before and after treatment.

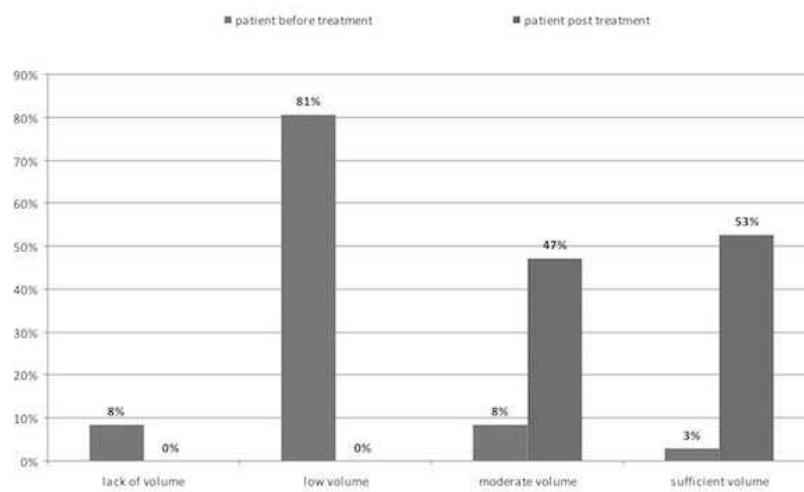


Fig. 4. Patient evaluation of volume before and after treatment.

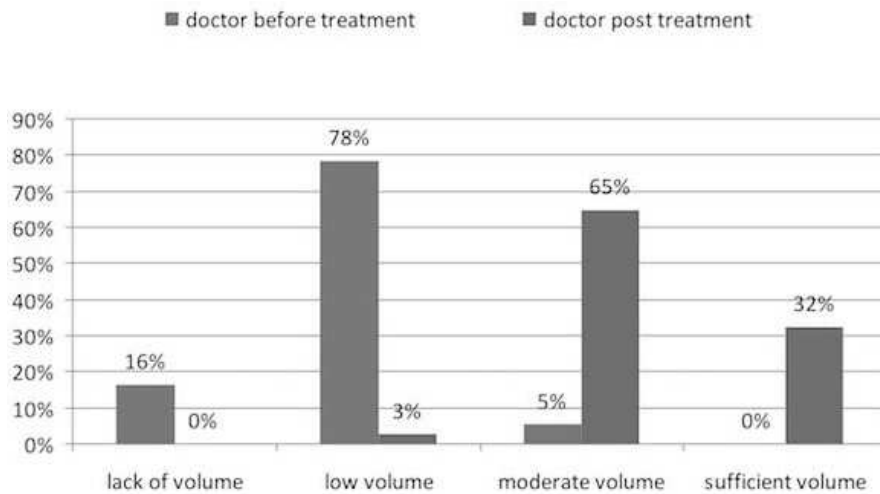


Fig. 5. Physician evaluation of volume before and after treatment.

14% (5/36) lack of elasticity, 19% (7/36), moderate elasticity and only 1 woman out of 36 reported sufficient elasticity (Fig. 2).

The investigator observed moderate elasticity of labia majora in 84% (31/37) of patients after treatment, sufficient elasticity in 14% (5/37) and only in 1 patient low elasticity was reported, whereas the doctor detected little elasticity in 70% (26/37) of patients, and lack of elasticity in 16% (6/37) before treatment (Fig. 3).

Clinical improvement was also determined by evaluating the volume of labia majora before and after treatment: 47% (17/36) of the patients reported moderate volume and 53% (19/36) sufficient volume after treatment, whereas 81% (29/36) of patients reported little volume, and 8% (3/36) lack of volume before treatment (Fig. 4). The doctor observed moderate volume of labia majora in 65% (24/37) of patients, and sufficient volume in 32% (12/37) of patients after treatment, whereas in 78% (29/37) of patients scarce volume, and in 16% (6/37) lack of volume was detected before treatment (Fig. 5).

After treatment, 89% (32/36) of patients reported no pain during sexual intercourse; before treatment, 44% (16/36) had reported mild pain, 17% (6/36) moderate pain, and 6% (2/36) intense pain (Fig. 6). The doctor reported no pain during the gynecologic

examination in 41% (15/37) of patients before and after treatment, while in 59% (22/37) of patients mild pain after treatment was detected (Fig. 7).

The satisfaction with the aesthetics of the private parts was evaluated before and after treatment: before, 72% (26/36) of the patients was rather dissatisfied and 8 (3/36) very dissatisfied; after, 81% (29/36) of patients reported to be very satisfied, and 19% (7/36) rather satisfied (Figs. 8, 9). The doctor was quite satisfied with 86% (32/37) of patients and very satisfied with 14% (5/37) after treatment, whereas he was fairly dissatisfied for 78% (29/37) and very dissatisfied for 11% (4/37) of patients before treatment (Fig. 10, Fig. 11).

Only mild adverse events and complications were observed. In 17% of the patients we observed temporary pain of labias and minimal swelling for about 3 to 5 days after treatment.

DISCUSSION

Volume loss of labia majora is a new and promising indication for HA-based fillers, potentially giving patients satisfactory results, with few adverse events. The demand for cosmetic procedures of female genitalia is increasing. In particular, labiaplasty demonstrates excellent results in 96.6% of patients

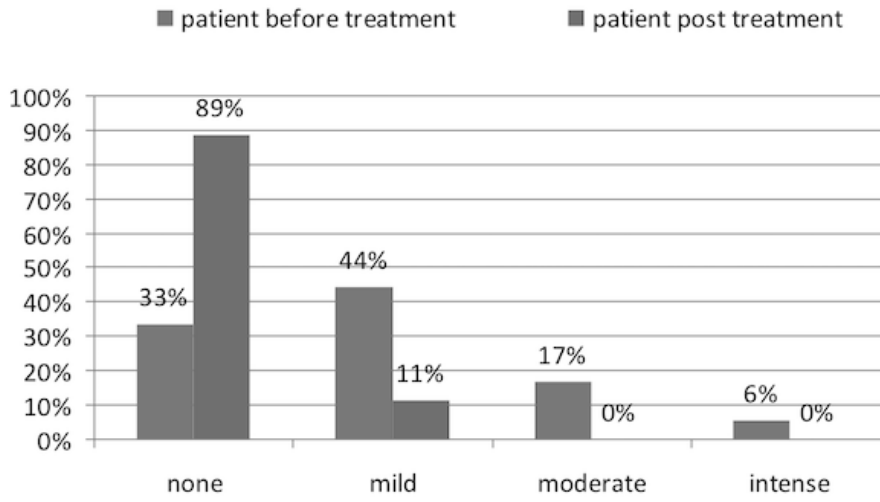


Fig. 6. Patient evaluation of pain during sexual intercourse.

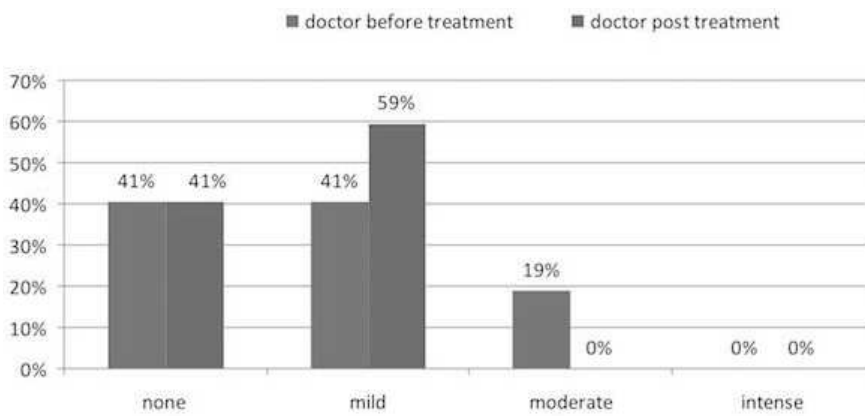


Fig. 7. Physician evaluation of pain during the examination.

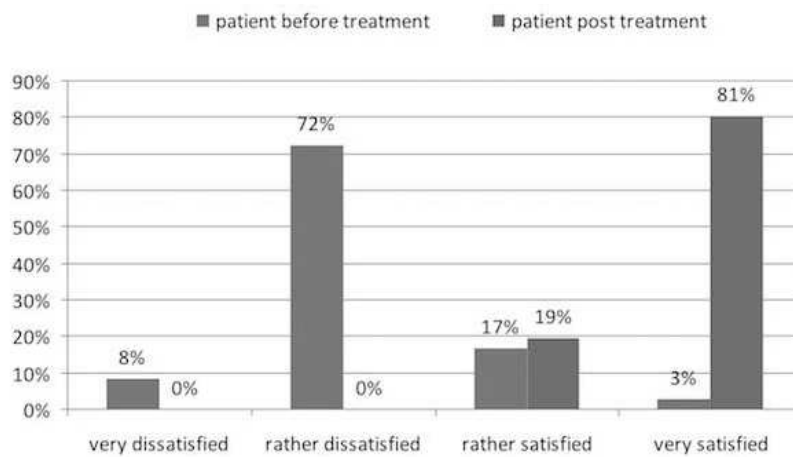


Fig. 8. Patient aesthetic evaluation of intimate area.

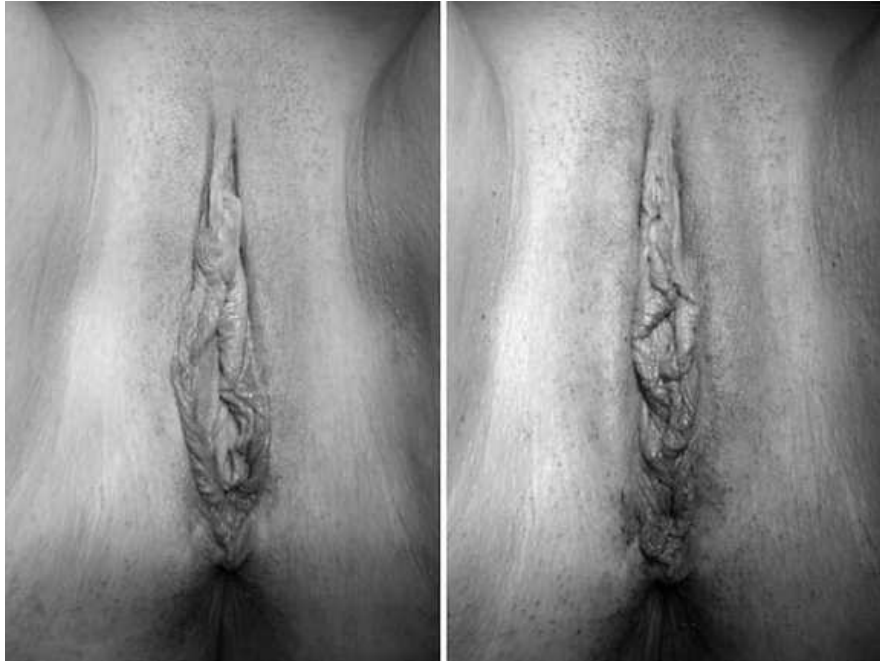


Fig. 9. Forty-one-year-old patient with an atrophy of labia majora. It is possible to appreciate the reshaping of the anatomic area 3 months after injection. Left) Before. Right) After.

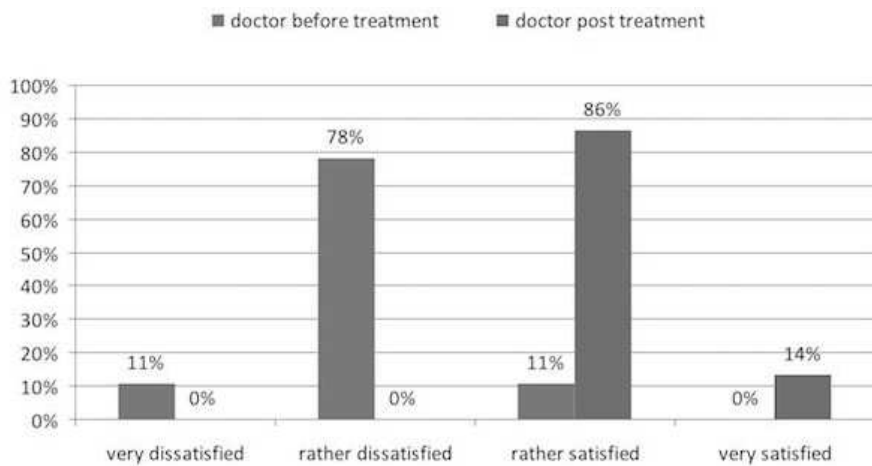


Fig. 10. Physician aesthetic evaluation of intimate area.

(11). There are very few studies in the literature concerning the effectiveness and safety of HA-based filler infiltration in labia majora, although hyaluronic acid fillers allow a significant rejuvenation with a simple outpatient procedure and few and theoretical complications. Otherwise, a surgical approach on external genitalia could have complications in 7.3% of cases (11).

In this study for the soft tissue augmentation of the intimate area (labia majora) an HA hydrogel was used with a novel crosslinking agent with a higher safety profile and a lower toxicity compared with other crosslinking used to date. PEG has a peculiar and extremely interesting characteristic, namely that of concealing the agent to which it binds by the host immune system, with consequent reduction of



Fig. 11. Fifty-one-year-old patient with an atrophy of labia majora. It is possible to appreciate the volume restoring of the anatomical area 4 months after injection. Left) Before. Right) After.

immunogenicity and antigenicity.

From a clinical point of view, biomimetic properties, which allow a physiologic integration of the gel into the host tissue and high biomechanical compatibility of the new hydrogel matrix with a large mesh structures, offer a higher yield in terms of aesthetic correction, the quantity being equal to that of other fillers, as well as a higher mechanical stress resistance, thereby extending the half-life of the HA filler.

Another important development is the use of a new hyaluronic acid no longer obtained from pathogenic bacteria, but rather from the fermentation of a bacterial strain of the *Bacillus subtilis*, a non-pathogenic bacterium belonging to the class of probiotics.

The treatment with HA-based filler infiltration in labia majora is repeatable, has virtually no complications and is reversible; we recommend labia majora rejuvenation even in severe hypotrophies.

The main limitations of our study were the sample size and the method of evaluation, as it is a subjective evaluation.

CONCLUSIONS

HA-based filler infiltration in labia majora can bring considerable rejuvenation with a simple outpatient procedure.

A total of 37 women affected by labia majora hypotrophy underwent this procedure. The analysis of the clinical data distribution before and after treatment, the sensation of pain, the satisfaction of aesthetic change of intimate parts and of the overall satisfaction in all measured parameters showed that there is a rightward shift in the post-treatment, which demonstrates a clinically significant improvement in all the parameters considered in patients with hypotrophy of labia majora. In our study, no serious adverse events and complications were observed.

Certainly, further studies with a larger sample size and less subjective evaluation methodology are needed to find the most accurate data to confirm the potential of injectable fillers with hyaluronic acid in the treatment of disorders of the female intimate body areas.

REFERENCES

1. Hexsel D, Dal'Forno T, Caspary P, Hexcel CL. Soft-tissue augmentation with hyaluronic filler for labia majora and mons pubis. *Dermatol Surg* 2016; 42(7):911-14.
2. Cosmetic Surgery National Data Bank Statistics. *Aesthet Surg J* 2016; 36(S1):1-29.
3. Gonzalez PI. Classification of hypertrophy of labia minora: consideration of a multiple component approach. *Surg Technol Int* 2015; 27:1919-4.
4. Rauso R, Tartaro G, Salti G, Zerbinati N, Colella

- G. Utilization of needles in the surgical reduction of labia minora: a simple and cost-effective way to reduce operating time. *Aesthet Surg J* 2016; 36(10):NP310-2.
5. Zerbinati N, Serati M, Origoni M, et al. Microscopic and ultrastructural modifications of postmenopausal atrophic vaginal mucosa after fractional carbon dioxide laser treatment. *Lasers Med Sci* 2015; 30:429-36.
 6. Salvatore S, Nappi RE, Zerbinati N, et al. A 12-week treatment with fractional CO2 laser for vulvovaginal atrophy: a pilot study. *Climacteric* 2014; 17:363-69.
 7. Farage M, Maibach H. Lifetime changes in the vulva and vagina. *Arch Gynecol Obstet* 2006; 273:195-202.
 8. Salgado CJ, Tang JC, Desrosiers AE 3rd. Use of dermal fat graft for augmentation of the labia majora. *J Plast Reconstr Aesthet Surg* 2012; 65:267-70.
 9. Erickson KL, Montagna W. New observations on the anatomical features of the female genitalia. *J Am Med Womens Assoc* 1972; 27:573-81.
 10. Elsner P, Wilhelm D, Maibach HI. Frictional properties of human forearm and vulvar skin: influence of age and correlation with transepidermal water loss and capacitance. *Dermatologica* 1990; 181:88-91.
 11. Fasola E, Gazzola R. Labia majora augmentation with hyaluronic acid filler: technique and results. *Aesthet Surg J* 2016; 36:1155-63.
 12. Monheit GD, Coleman KL. Hyaluronic acid fillers. Review. *Dermatol Ther* 2006; 19(3):141-50.
 13. Roberts MJ, Bentley MD, Harris JM. Chemistry for peptide and protein PEGylation. *Adv Drug Deliv Rev* 2002; 54:459-76.
 14. Park YD, Tirelli N, Hubbell JA. Photopolymerized Ha-based hydrogels and interpenetrating networks. *Biomaterials* 2003; 24:893-900.
 15. Leach JB, Schmidt CE. Characterization of protein release from photocrosslinkable hyaluronic acid-polyethylene glycol hydrogel tissue engineering scaffolds. *Biomaterials* 2005; 26:125-35.
 16. Jin R, Moreira Teixeira LS, Krouwels A, Dijkstra PJ, van Bitterswijk CA, Karperien M, Feijen J. Synthesis and characterization of HA-poly (ethylene glycol) hydrogels via Michael addition: an injectable biomaterial for cartilage repair. *Acta Biomater* 2010; 6(6):1968-77.
 17. Hassan W, Dong Y, Wang W. Encapsulation and 3D culture of human adipose-derived stem cells in an in-situ crosslinked hybrid hydrogel composed of PEG-based hyperbranched copolymer and HA. *Stem Cell Res Ther* 2013; 4(2):32.
 18. Aurand ER. The characterization of HA and polyethylene glycol hydrogels for neural tissue engineering. University of Colorado Denver, Anschutz Medical Campus 2014; 3622545.
 19. Skaalure SC, Dimson SO, Pennington AM, Bryant SJ. Semi-interpenetrating networks of hyaluronic acid in degradable PEG hydrogels for cartilage tissue engineering. *Acta Biomater* 2014; 10(8):3409-20.
 20. Goodman MP, Placik OJ, Benson RH 3rd, et al. A large multicenter outcome study of female genital plastic surgery. *J Sex Med* 2010; 7:1565-77.