Aesthetic Medicine 2023; Vol. 9, N. 1: e2023005 DOI: 10.57662/am.v9i1.14731

Original article

Hyaluronic acid used in the female genital area: technique proposal

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Abstract. *Background:* external female genitalia lose elasticity and volume with age due to a deficiency of oestrogen leading to hormonal and anatomical changes in the genitourinary tract, with vaginal dryness, dyspareunia, and reduced lubrication. Most of these symptoms can be attributed to a syndrome called genitourinary syndrome (GS) which affects approximately 27% to 84% of postmenopausal women; GS is associated to vulvo-vaginal atrophy (VVA) impairing health, sexual function, and quality of life. The primary goal of the treatment of VVA is to relive all these symptoms. The First-line treatment consists of non-hormonal therapies such as lubricants, while hormonal therapy is generally considered the "gold standard", although some women may experience side effects. Newer therapeutic approaches with hyaluronic acid can be employed as alternative options to achieve faster results and without any side effects, but further research is required to standardize techniques and to investigate the scope of their implementation in a daily clinical practice. *Aim:* to report the description of two specific techniques regarding: 1) the labia majora augmentation, using the cross-linked hyaluronic acid with an aesthetic goal and 2) the vulvo-vaginal bio-stimulation, using an injection of high and low molecular weight HA (HCC), as a treatment of the genito-urinary syndrome pre- and post-menopause. *Materials and methods:* cross-linked hyaluronic acid injections have been performed into the fibrous tunic using a 25 G (70 mm) nano-cannula with 2 ml of product for each side. HCC injections, however, have been administered around the vulvar vestibule, following a linear retrograde technique: one injection at the anterior (0.3 mL), one at the posterior entries (0.5 mL), and two injections into each of the lateral sides (0.3 mL) using a 29 G 13 mm long needle (Picture 7). The outcome assessment included genital examination images and histological biopsies. *Results:* cross-linked hyaluronic acid is an effective treatment for women who wish to seek a youthful genital appearance; in addition, genital examination images and histological biopsies show the ability of HCC to increase thickness in the epithelial layer and dermo-epidermal junctions, decreasing the infiltration of T lymphocytes. *Conclusions:* HCC injections in the vaginal-vestibular area are associated with tissue regeneration of atrophic tissues in pre and post-menopausal women, with moderate-to-severe symptoms associated with vulvo-vaginal atrophy (VVA).

Key words: chyaluronic acid, Genitourinary Syndrome, Vulvovaginal Atrophy, labia majora augmentation, genital bio-stimulation.

Introduction

Hyaluronic acid (HA) is a polysaccharide and an essential component of the extracellular matrix, together with elastin and collagen. Thanks to its hydrophilic carboxyl groups, HA attracts water and hydrates tissues, stimulates fibroblasts to produce a new matrix, and shows a powerful antioxidant action which promotes tissue protection. [1] Recent studies on thermally stabilized hybrid cooperative complexes of HA showed that

this molecule stimulates keratinocytes, boosts the production of mesenchymal stem cells in the adipose tissue, proving to be a promising compound in regenerative medicine and wound healing as well [2-3]. Furthermore, being present in all living beings, HA shows a universal antigenic profile, and does not cause any immunological reaction. [3-5] In its cross-linked form (i.e. filler), HA has been used to fill wrinkles and volumize some face areas, whereas in its hybrid cooperative complex form it shows a dermic bio-stimulating action, which results in improved skin elasticity and texture. [2, 6-9]

Due to hypoestrogenic conditions, female genital tissues can become atrophic, and this might sometimes be the sign of a more complex syndrome called genito-urinary syndrome (GS) which more frequently occurs in post-menopausal subjects, and whose incidence is expected to increase together with the worldwide survival age. [10] However, GS can also affect women during their fertile period, and in this case, it is usually linked to hormone and oncological treatments in general (i.e. radiotherapy, chemotherapy), recurrent vulvovaginitis, puerperium, and oophorectomy. Vulvo-vaginal atrophy (VVA) mostly occurs in postmenopausal women because of the physiological drop in oestrogens. During menopause several changes take place: pubis and labia majora fat decreases, labia minora become hypertrophic, vulvar skin shrivels, vaginal mucosa appears dry and flat, while vestibular skin, especially in the posterior introitus, is dry, pale, inelastic, sclero-atrophic, and thin (picture 1 and 2). On the other hand, in fertile patients affected by VVA, an aseptic inflammation reaction is described (picture 3 and 4). Regardless of patients' age, the skin is fragile, and fissures may appear after coitus or spontaneously, causing pain, dyspareunia, burning, itching and dryness, responsible for impaired sexual functions, and urinary problems, such as urge incontinence, dysuria and infections.

In peri-menopausal conditions, cross-linked HA is used to volumize labia majora, while non-cross-linked HA can promote healing and the regeneration in vulvo-vaginal tissues both in fertile and menopausal women, resolving symptoms that are not completely overcome using estrogen-based treatments. [10 15]. Due to its stereometric features, HA is not able to penetrate the skin barrier, therefore it is introduced in the dermal layer in the non-cross-linked form, or in the deeper tissue in the cross-linked form through injections. Although some HA formulations are currently available for this use, the literature is limited. [16]

We present our technique on labia majora augmentation by means of a cross-linked HA filler, and the first experience with hybrid cooperative complexes (HCC) of high and low molecular weight of HA injections in the vaginal-vestibular area to bio-stimulate atrophic tissues in pre- and postmenopausal women with moderate-to-severe symptoms associated with GS. We also describe methods to clinically evaluate the safety of this treatment and its effectiveness over time.

Materials and methods

Aesthetic gynecological procedure: labia majora augmentation with cross-linked HA

Eligible subjects

Patients affected by labia majora hypotrophy.

Products

Cross-linked Hyaluronic Acid with medium rigidity (G'= 168+/-28) suitable for genital volume increase was used (1 ml syringe: 25mg/ml cross-linked HA, phosphate buffer; IBSA Farmaceutici Italia Srl)

Methods

Labia majora are two large longitudinal skin folds which span antero-posteriorly from the mons pubis to the perineum. From the most superficial to the deepest, different layers can be identified (picture 5) [17]:

- The skin composed by the epidermidis and the derma, with pilosebaceous units and sweat glands;
- The thin labialis dartos muscle;
- Superficial adipose tissue with vessels stretching from the external and internal pudendal arteries and veins, and nerves (i.e. pudendal nerve);
- Fibrous tunic which is described as a cylindric, elastic channel closed at the posterior extremity, open at the anterior tip, which contains the subcutaneous opening of the inguinal channel;
- The deepest adipose tissue with the fan-shape end of the round ligament, whose superficial layer is formed by Camper's fascia, while the extension of

Scarpa's fascia in the abdominal wall is localized below: the thicker Colles fascia that forms the deeper layer. The Colles fascia is inferiorly attached to the ischiopubic rami and posteriorly to the urogenital diaphragm, but lacks anterior attachments.

Previous antibiotic therapy is not necessary, however a prophylactic antiviral therapy is suggested in women affected by simplex genital herpes.

As the blood supply comes predominantly from a posterior direction and the fibrous tunic opens anteriorly and closes posteriorly, both sides of the mons pubis are approached superiorly without any product dispersion. Following a topic disinfection, local anaesthesia with 1 ml lidocaine 2% in each entry side is performed. A 23 G needle is used to create access to a 25 G (70 mm) nano-cannula with 2 ml of product, which is passed through the skin, dartos muscle, adipose tissue, and finally pushed through the fibrous tunic up until the posterior extremity. The correct penetration of said tissue is indicated by a slight resistance of the tunic, like the procedure of deep injection of a cross HA under the fascia of face, accompanied by a typical sound. An aspiration test is performed to rule out the possibility of having hit a vessel. The cannula is then slowly retrieved, releasing the filler, along all the space delimited by the fibrous tunic, following a posterior to anterior direction. Even though this vulvar elastic sac can receive a large amount of product, only 1-2 ml for each side is injected to avoid the rare complications reported (thromboembolism), and the procedure is eventually repeated after 1 month, if the patient desires more augmentation.

We have treated labia majora hypotrophy in 19 patients and no haematoma or ecchymosis was reported, only a slight, transient discomfort during the fibrous tunic passage. The Results are evaluated based on the patients' satisfaction, and by the physician who observes a visible vulvar enlargement. In these cases, labia minora are less visible and shrank (picture 6).

Functional gynecological procedure: vaginal bio-stimulation with Hybrid Cooperative Complexes of High and Low molecular weight HA (HCC)

Eligible Subjects

The patients affected by VVA, with symptoms predominantly localized at the posterior vaginal introitus (vaginal atrophy) are suitable for treatment. Subjects with vascular, connective tissue and skin diseases, genital infections (e.g. herpes), pregnancy and paediatric age, previous permanent genital treatments, drug allergy, and use of immunosuppressive drugs, are excluded.

Products

Hybrid Cooperative Complexes of High and Low molecular weight HA (HCC) (2 ml syringe: 32 mg/mL of 1,100-1,400 kD MW HA plus 32 mg/mL of 80–100 kD MW HA, IBSA Farmaceutici Italia Srl)

Methods

Deep intradermal injections are performed with a prefilled syringe containing 2 mL of Hybrid Cooperative Complexes of High and Low molecular weight HA (HCC), a thermally stabilized hyaluronan hybrid cooperative complex based on patented NAHYCO™ technology (32 mg/mL of HA of 1,100-1,400 kD MW plus 32 mg/mL of HA of 80–100 kD) (IBSA Farmaceutici Italia Srl) in two treatments at a 1-month interval, following the same protocol. First, the patient applies an anaesthetic cream (2.5% lidocaine plus 2.5% prilocaine) on the vaginal-vestibular area 30 minutes before the procedure. Afterwards the area is locally disinfected using 0.1% of benzoxonium chloride, and local anaesthesia is performed with 2–3 mL of a lidocaine hydrochloride 2% w/v solution for dermic injections, using a 30 G, 13 mm long needle. Starting from a first anesthetized circle area, the successive injections are performed inside the previous circle edge, to avoid patient discomfort, and relate to the needle passage. Every step needs of few drops of anaesthetic, and all the vestibular area is treated. Once the anaesthetic procedure is completed and effective, 6 HCC injections are administered in the derma around the whole vulvar vestibule, following a linear retrograde technique: one at the anterior (0.3 mL) and one at the posterior entries (0.5 mL), and two per each of the lateral sides (0.3 mL), using 29 G 13 mm long needles (picture 7). The product distributes quickly and after about 15 minutes, it cannot be observed in accumulation. Patients are advised to perform a local manual massage during the following 3 days.

Our personal experience is reported on a recent article on 26 patients, followed for 1 year and treated with these techniques [13]: some light, transient (24-48 h) side-effects often occur: erythema or ecchymosis. They do not cause pain, and all of these may be easily managed by the patients at home following the physicians' prescription. We did not observe major complications such as persistent palpable nodules, hematomas or haemorrhages or strong pain.

Clinical results can be evaluated by means of 3 internationally validated questionnaires [18-20] which are administered before the first and second treatment, and after 6 and 12 months, to estimate improvement over time. These questionnaires are:

- *Visual Analogic Scale test (VAS)* to assess burning, itching, dryness, superficial and deep dyspareunia, and urinary symptoms (urge incontinence, dysuria, pollakiuria). For each item, scores range from 0 to 10 (with 10 indicating the worst condition).
- *Health-related quality of life test (SF12)* to assess both physical and mental domains, that are combined, weighted and standardized. The highest score indicates the healthiest status.
- *Female Sexual Function Index (FSFI)* to assess quality of sexual life in terms of desire, arousal, lubrification, orgasm and pain.

Results and conclusions

HA is currently the most-used molecule in aesthetic medicine. [6] Its cross-linked form is widely used to treat wrinkles and volumize certain areas of the body, especially facial districts. In its HCC form, HA shows multiple key functions, not only at the level of the extracellular matrix, but also at the different cells of skin, thanks to its molecular features and the chemical peculiarities of the different weight of its components [2]: Wherease the cross-linked ones promote tissue hydration, show anti-oxidant effects and provide protection against mechanical stress, the HCC one boosts HA, collagen and elastin production by fibroblasts, regulates the metabolism of keratinocyte and enhances the production of mesenchymal stem cells in the adipose tissue. [1-9] Through specific receptors, HA modulates inflammation, fibroblast migration, cell growth and angiogenesis in response to overall stress. Moreover, HA demonstrates an immunological action, as it coordinates an early host defence against bacteria and viruses. [21]

Multiple properties of HA can be leveraged in aesthetic genital medicine and its cross-linked form can successfully be used in the augmentation of the labia majora. The procedure is quick and is appreciated by women who are looking for genital rejuvenation (pictures 6). Our techniques (table 1) avail the use of HA injections, in the elastic sacks delimited by fibrous tunics, because the cosmetic result will appear more natural avoiding abnormal accumulations of HA in the extracellular matrix; the approach is inspired by plastic surgeons who insert the breast implants in deep tissue, under the pectoral muscles.

Despite the usage of a cross linked HA being generally safe and overall well-tolerated, thromboembolism may represent a severe complication: following an accidental intravascular injection, HA, which shows an affinity to vascular endothelial cells, may reach the lungs. HA thromboembolism can also be due to a dysregulation of haemostasis since intravascular HA interacts with fibrinogen and accelerates the thrombin-induced formation of fibrin clots. The quantity, and not the site of injection, as well as an inexperienced practitioner, can both represent risk factors which can lead to these events. [22,23]

VVA diagnosis is based on a gynaecological examination, and on the typical symptoms the patient presents. Vaginal-vestibular alterations may present at all ages, but with different characteristics. In fertile women symptoms often occur after recurrent candida vaginitis which makes vestibular skin fragile, thin, and dry (pictures 3 and 4), chronic inflammation is mainly localized at the level of the posterior introitus (pictures 1, 2). In these conditions, HA can act as a powerful bio-stimulator with an important anti-inflammatory action. [21] Since vulvo-vaginal atrophic alterations are prevalent during menopause, the injection of high and low molecular weight of HA (HCC) in genital introitus instead of the cross-linked form has been considered the most appropriate choice. In vulvo-vaginal atrophy, the vaginal vestibule appears pale, thin and fragile (pictures 1 and 2). With aging, skin metabolism slows down; therefore, it requires activators such as HCC of HA, which have

multiple crucial properties: hydration, mechanical protection, anti-ageing and anti-inflammatory effects on keratinocytes, fibroblasts, and adipocytes, via specific receptors. [2] Moreover, HA shows a micro-angiogenetic effect, increasing tissue vascularization. These features lead to an overall tissue regeneration (pictures 9 and 10). Genital injections demand a high level of skills and particular attention at the level of the anterior vestibule area, where the presence of the peri-urethral venous plexus represents a risk for the occurrence of post-procedure hematoma or bleeding.

Non-cross-linked HA is currently and widely used in genital areas despite the scarcity of scientific literature. [11-16]

The benefits from HCC of HA treatment on VVA can be observed both during a normal gynaecological examination (pictures 8 and 9), but they are also evident in histological biopsies (pictures 10 and 11), which show an increase in thickness of the epithelial layer and dermo-epidermal junctions, dermal activation, and a reduction of lymphocyte inflammatory infiltration.

HCC of HA does not react with any hormonal receptors, and it can be administered to oncological patients who might suffer from GS after any possible surgery, chemotherapy, radiotherapy, and hormonal therapies. The efficacy of HCC of HA can be assessed by three international, validated questionnaires, which evaluate the improvements of patients' gynaecological symptoms (VAS: burning, itching, dryness, superficial and deep dyspareunia, and urinary symptoms), sexual activity (FSFI), and their general quality of life (SF12). [18-20] Vulvovaginal atrophy is only one of the multiple symptoms of the complex GS that can be characterized by other disorders: urinary, pelvic floor hyper-tone, vaginism and psychological sexual disfunction. Consequently, the genital injection of HCC of HA is part of the multistep treatment of this condition, together with other therapies (oestrogens, DHEA, ospemifene), as well as other specialist interventions (i.e. those of urogynecologists, psychotherapists and/or a pelvic midwife).

In conclusion, HA presents great field of application also in female genital areas: the cross-linked form for aesthetic labia majora augmentation, the HCC of HA form for vulvo-vaginal atrophy bio-stimulation. Both procedures are fast, effective, and safe and can be performed in the practitioner's office.

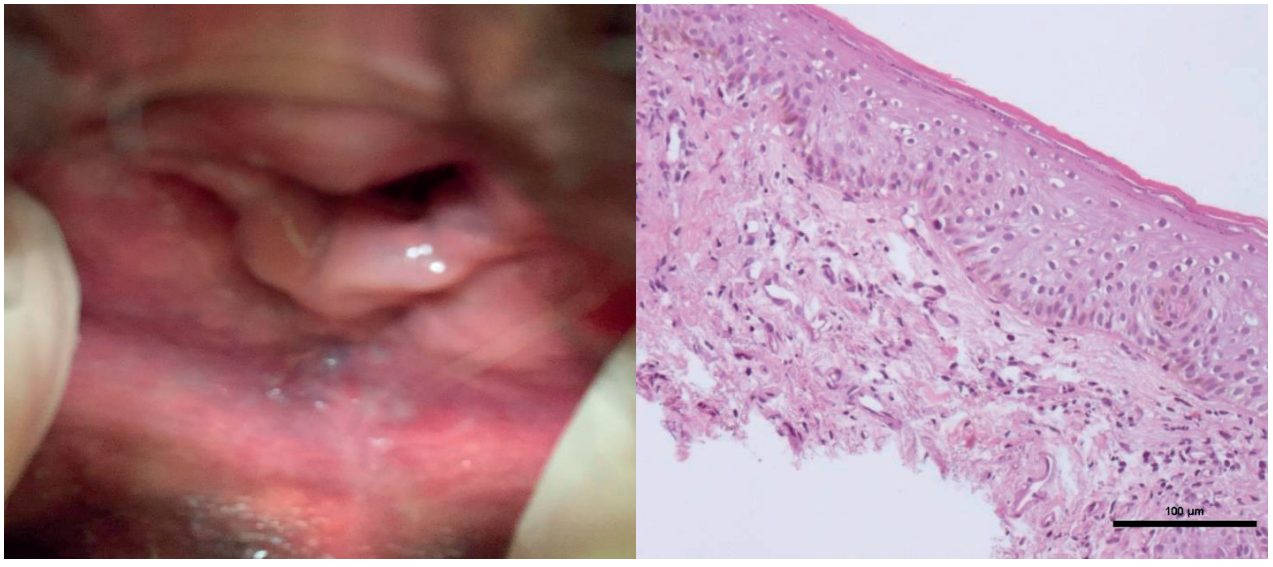


Figure 1 - Example of an atrophic vaginal vestibule in menopause, you can observe, between thetwo lines, the most suffering posterior introitus.
Figure 2 - Histology shows an irregular, focally flat epidermal layer, less expressed dermo-epidermic papillae, dilated vessels and edematous stroma.

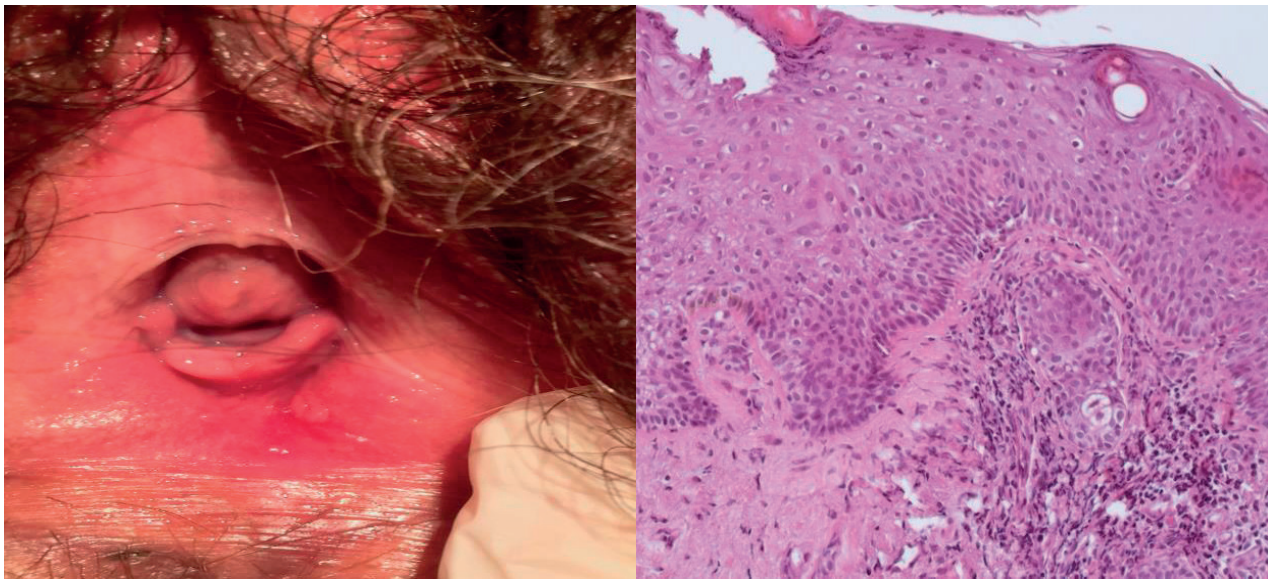


Figure 3 - An example of a not-infected inflammatory posterior introitus in a fertile woman withGS and **Figure 4** the corresponding histology. Epithelium is hyperplastic with basal lymphocyteexocytosis, stroma with focal fibrosis and inflammatory infiltration

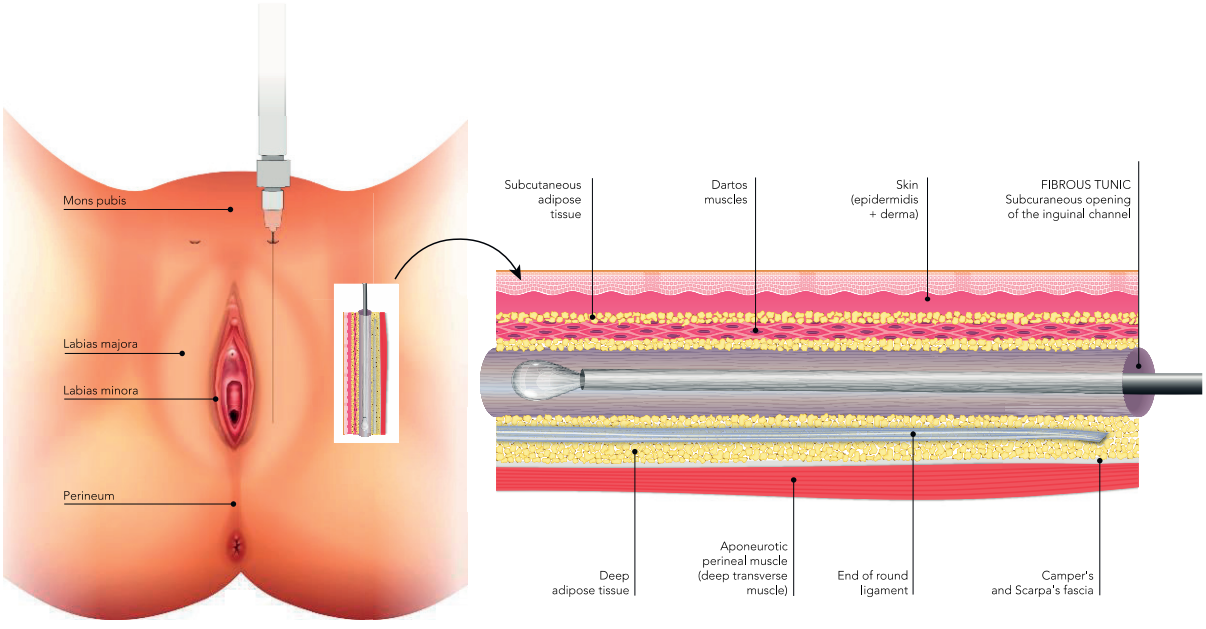


Figure 5 - On the left, the anatomical site of labia majora augmentation technique, on the right the magnification and the description of the topographical anatomy of the labia majora with the exact site of filler deposition: inside the fibrous tunic.

Figure 6 - Labia majora augmentation with cross-linked HA. Photographs of patient 1 (A and B), patient 2 (C and D), before labia majora augmentation (A-C) and after (B-E).

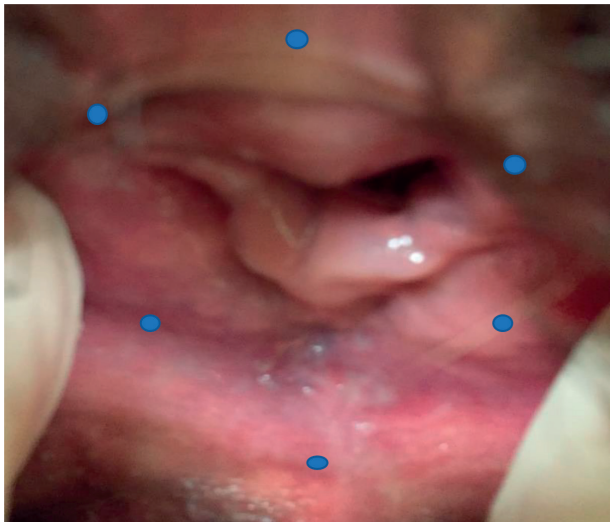
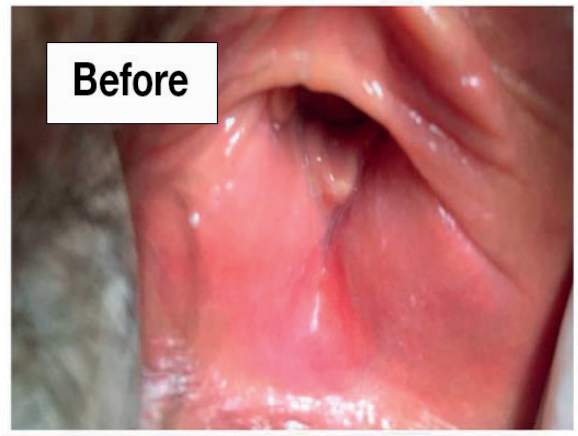
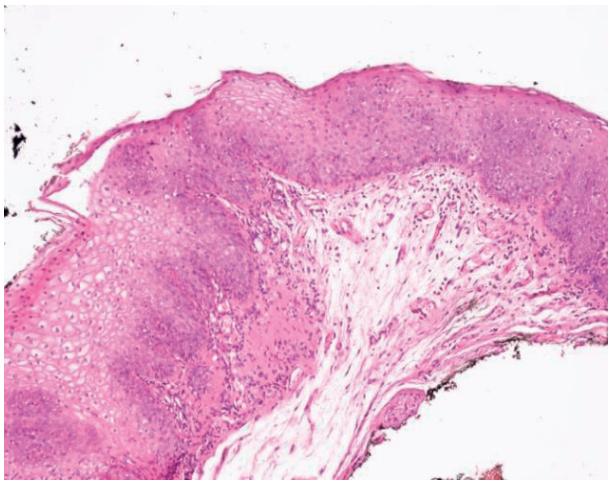
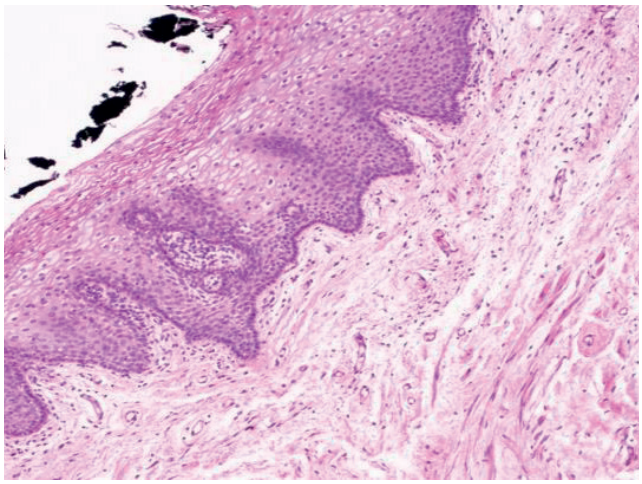


Figure 7 - Free HA vaginal vestibular bio-stimulation. The 6 blue points represent the HA deep dermal injection sites.



Figures 8 and 9 - A vulvo-vaginal atrophy before and after the first HA biostimolation.





Figures 10 and 11 - The corresponding histology 10) Focal squamous acanthosis and stroma with focal fibrosis, edema and superficial inflammatory infiltration 11) Mild acanthosis and papillomatosis without stromal inflammation

Ethics and consent: All patients were provided an informed signed consent for the publication of their cases and images. No Approval from the institutional review board is needed.

Conflict of interest: None to declare

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Received: 22 January 2022
Accepted: 10 January 2023
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