Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

# Open, Randomized, Parallel Group Study Assessing Efficacy, Safety, and Tolerability of 8 weeks of Vaginal Hyaluronic (V-HYAL) Gel in Peri- and Post-Menopausal Female

**Rajis Santhirasegar**
University Malaya
**Sarah Samsudin**
Hospital Seberang Jaya
**Meyyammai Swaminathan**
University Malaya
**Sutha Rajakumar**
University Malaya
**Purnima Devi Suppiah**
Hospital Seberang Jaya
**Juliana Mohd Noor**
Hospital Seberang Jaya
**Irene Looi**
Hospital Seberang Jaya
**Yuen Kah Hay**
University Sains Malaysia
**Hasniza Zaman Huri**
hasnizazh@um.edu.my
University Malaya
**Baharudin Ibrahim**
University Malaya

**Research Article**

**Keywords:**

**Posted Date:** November 26th, 2025

**DOI:** https://doi.org/10.21203/rs.3.rs-7406152/v1

**License:** This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

**Additional Declarations:** No competing interests reported.

## Abstract

Introduction

Vulvovaginal atrophy (VVA) affects nearly 50% of peri- and postmenopausal women, causing dryness, irritation, and discomfort due to lower estrogen levels. It can also lead to sexual dysfunction and significantly reduce quality of life, akin to serious conditions like arthritis and COPD. Treatments like low-dose local estrogen therapies are effective and safe, while hyaluronic acid offers a non-hormonal alternative with similar results. This study evaluates the efficacy, safety, and tolerability of vaginal Hyal (V-HYAL) gel for treating VVA symptoms in these women.

Method

The study is an open, randomized, parallel-group design involving 70 peri- and post-menopausal women, evenly divided into two groups: one receiving V-HYAL Gel and the other serving as the control. Participants were randomized in a 1:1 ratio, beginning with a screening visit, followed by assessments at Baseline (Day 0), Week 4 (via phone contact), and Week 8 (concluding the study). Efficacy was evaluated using various measures, including the Visual Analog Scale (VAS), High Vaginal Swab (HVS) based on Nugent score, vaginal pH, Vaginal Health Index (VHI), Female Sexual Functioning Index (FSFI), DIVA questionnaire, and monitoring for adverse events. Clinical Trial ID NCT06964646 (Registered 5/6/2025)

Results

By week 8, the intervention group showed a significant reduction in vaginal dryness scores ($p = 0.042$) compared to the control, though there were no significant differences in VAS scores for itching, irritation, or urination issues. Vaginal Health Index (VHI) scores improved significantly in the intervention group, suggesting better outcomes in alleviating vaginal atrophy. While there was no significant difference in Nugent scores or vaginal pH between groups, the intervention group showed higher pH levels. FSFI results indicated notable improvements by day 56 in satisfaction with arousal ($p = 0.017$), orgasm ($p = 0.033$), emotional closeness ($p = 0.042$), sexual relationship satisfaction ($p = 0.015$), and overall sexual life ($p = 0.008$). The DIVA questionnaire reported increased spontaneity in sexual activity ($p = 0.038$) and interest in sexual relationships ($p = 0.023$). Three mild adverse events occurred, with only one possibly related to the product (vaginal itchiness).

Conclusion

This study provides evidence of HA as a viable non-hormonal alternative to estrogen-based treatments, highlighting its comparable efficacy, safety, and tolerability. Minimal adverse effects were reported, with only one subject experiencing mild vaginal itching likely related to the investigational product.

Registry: ClinicalTrials.gov, TRN: NCT06964646, Registration date: 05 June 2025.


## Background

Vulvovaginal atrophy (VVA) is a common condition caused by decreased estrogenization of vaginal tissue, causing genitourinary symptoms such as dryness, irritation, and discomfort. VVA has a prevalence of close to 50% in postmenopause women and increases with breast cancer treatment [1]. A study conducted by the European Vulvovaginal Epidemiological Survey (EVES) reported 90% of postmenopause women experienced VVA[2].Vulvovaginal atrophy is strongly associated with female sexual dysfunction in sexually active peri- and post-menopausal women, suggesting that therapies reducing one condition may also relieve symptoms of the other[3]. Postmenopausal vulvovaginal atrophy (VVA) can be accurately diagnosed and managed by a specialist, relying on history, questionnaires, physical exam, and objective vaginal assessment [4]. Vulvovaginal atrophy symptoms significantly impact the quality of life(QOL) in postmenopausal women, with severity levels comparable to those of serious conditions like arthritis, asthma, and irritable bowel syndrome [5]. Current low-dose local estrogen formulations are effective and safe for treating vulvovaginal atrophy, while potential new treatments show promise for reducing side effects [6]. Non-oestrogen based vaginal gel contains Hyaluronic acid (HA), which is a glycosaminoglycan produced by fibroblasts. HA is found naturally in the vaginal epithelium and other bodily tissues, where it is responsible for maintaining proper hydration levels in moisture-sensitive environments[7, 8]. HA promotes neoangiogenesis and tissue regeneration through its ability to bind to water [9]. HA is a valid alternative to oestrogen treatment [10, 11], as it was found to be as effective, with some studies showing greater efficacy in HA-based treatments [12, 13]. There are multiple systematic reviews

covering the efficacy and safety of HA. There is a very low chance of adverse effects; out of 17 studies, one reported slight bleeding, and another study reported fungal diseases [14].

There are multiple HA administration routes, such as cream, gel, ovules and suppositories, but they were reported to be of similar efficacy[14]. Vaginal moisturisers can have a long-term effect with frequent use, up to several times a week, depending on the severity of the symptoms. Recent clinical studies on HA-based treatments for vaginal dryness were conducted [12, 15, 16]. All studies report improved VVA symptoms and sexual function. It was also noted that there were no negative impacts on the microenvironment of the vagina or any irritation. L-arginine, which is a component of Vaginal HYAL gel, is commonly used to treat hypoactive sexual disorders in women. Being a naturally occurring precursor to nitric oxide, it can increase vasodilation and increase blood flow to the genitals [17]. Therefore, L-arginine is a key mediator involved in circulation and sexual function. Multiple clinical studies have well-supported L-arginine's benefits on sexual-related conditions [18–20]. Products containing liposomes are capable of penetrating deep vaginal tissues and releasing water molecules to hydrate the deepest level of the vaginal wall[21].

The current study is conducted to assess the efficacy, safety and tolerability of repeated doses of V-HYAL Gel among peri- and post-menopausal women on vaginal dryness, vaginal pH, vaginal lactobacillus flora, vaginal thrush, bacterial vaginosis, and trichomonas vaginalis as well as adverse events. The Female Sexual Functioning Index (FSFI) scoring and Day-to-day Impact of Vaginal Aging Questionnaire (DIVA) was also carried out among the peri- and post-menopausal women in this trial.

# Methods

# Trial Design and participants

Open, randomised study in 70 female subjects; 35 subjects per treatment regimen with two parallel groups. Selected subjects were randomised into the Intervention Group (with V-HYAL Gel (formerly Vaginal Hyal Gel)) and Control Group (without V-HYAL Gel (formerly Vaginal Hyal Gel)) in a ratio of 1:1. The study was conducted in Hospital Seberang Jaya, Malaysia from 8 th January 2024 till 27th June 2024 (Clinical Trial ID NCT06964646). Seventy peri- and post-menopausal women (aged > 45 years) were randomized (1:1) into intervention (V-HYAL Gel) and control groups using an online randomization tool. The study adhered to CONSORT 2010 guidelines and was approved by the Medical Research Ethics Committee (NMRR ID-23-02085-IH7).The consultant reviewed the obstetrics and gynaecology clinic patients, provided referrals, and notified independent researchers for eligibility assessment and group assignments.

## Intervention

The intervention group received repeat doses of 2.5g of V-HYAL Gel® (formerly Vaginal Hyal Gel) every three days, while the control group did not receive the gel. The research officer contacts the subjects to ensure compliance and provide reminders. Three visits during the study period: a Baseline Visit, a Final Visit in Week 8, and one intermediate phone contact visit in Week 4, respectively (Refer to Fig. 1). The study was designed in adherence to Consort Statement.

## Investigational Device Description

The investigational product classified as a medical device Class 1 (EU Directive 93/42/EEC), V-HYAL Gel, formerly known as Vaginal Hyal gel is manufactured by Crab Sinergy Srl, Costa Volpino, Italy [22]. V-HYAL comprises of an aqueous base vaginal hydrating agent; High-Molecular-Weight Hyaluronic Acid (HMWHA), which absorbs moisture up to 300 times its weight along with 5% liposomes and L-arginine [23].

## Participants

Female adult patients more than 45 years of age who fulfil the following inclusion and exclusion criteria and are determined to be peri- or post-menopause by a gynaecologist at Seberang Jaya Hospital, Penang, were recruited for the study. Patients not meeting the inclusion or exclusion criteria were classified as screening failures. These individuals were recorded in a Screening Log, documenting their subject number, initials, demographics, and reasons for exclusion. The log, maintained in the Investigator's Study File, was reviewed to identify and assess any potential systematic bias in patient selection for the study.

#### Inclusion criteria

1. Females age > 45 years old
2. Peri or post-menopausal.
3. BMI between 18 kg/$m^2$ and 30 kg/$m^2$.
4. Able to give written informed consent before starting any study-related procedure.
5. Medical history and physical examination normal or abnormal but not clinically relevant to the investigator's judgment.
6. ECG tracing, blood pressure, and laboratory tests are within normal range or abnormal but not clinically relevant as per the investigator's judgment.
7. Not under any administrative or legal supervision.
8. Sexually active in the last three months

**Exclusion criteria**

1. Inability or unwillingness to comply with the investigator's protocol requirements.
2. Inability or unwillingness to provide written consent.
3. Pregnancy, breastfeeding, or use of nonreliable methods of contraception.
4. Other medical conditions which, in the investigator's judgment, may be associated with increased risk to the subject or may interfere with study assessments or outcomes.
5. History or evidence of allergy, idiosyncrasy or hypersensitivity to hyaluronic acid.
6. A history or clinical evidence of significant or uncontrolled cardiovascular, respiratory, renal, hepatic, endocrine, metabolic, gastrointestinal, haematological, neurological, or psychiatric pathology may affect the study outcomes.
7. Chronic diseases such as HIV I and II, HBV and HCV surgery within the previous 6 months, or blood loss > 400 mL within the previous 3 months.
8. History of alcohol or drug abuse.
9. Took hormone therapy or tibolone in the 3 months before the first dosing occasion and during the study.
10. Took any pharmacological treatment which may affect the vaginal dryness or study outcomes, such as Menopausal Hormone Therapy (except for symptomatic short-term paracetamol use)
11. Received concomitant treatment with other investigational drugs or had participated in another clinical trial within the previous 6 months prior to dosing / 4 weeks prior to the screening visit.
12. Had any surgery or medical treatment which may interfere with study outcomes (i.e. hysterectomy).
13. Any subject who cannot be contacted in case of emergency.

Subjects were free to withdraw from the study for any reason. Subjects may also be withdrawn from the study at any time at the investigator's discretion. Only those who gave their informed consent were included in the research project.

## Objective and main outcome measures

The primary objective of this study was to assess the efficacy of repeat doses of V-HYAL Gel using Visual Analog Score (VAS). The secondary objectives was to assess the effect of V-HYAL Gel on High Vaginal Swab (HVS) based on Nugent score, vaginal pH, Vaginal Health Index (VHI), Female Sexual Functioning Index (FSFI), DIVA questionnaire, and monitoring for safety and tolerability. The choice of outcomes was performed following international recommendations for the clinical evaluation of products to treat VVA [10, 22], using the experience of previous observational and clinical trials on the execution, as well as the prevalence and management of the symptoms [10, 12, 16, 23, 24]This study collected baseline demographic data, including the race, usage of tobacco and alcohol,age of participants, body mass index (BMI), systolic and diastolic blood pressure and pulse rate.

# Primary outcome:

The primary outcome was the subjective performance evaluation in terms of the Visual Analogic Scale (VAS) for vaginal dryness was used as the primary criteria for determining the results of the trial. VAS was assessed at baseline (Day 0), during a telephone contact after the fifth administration, and at the final visit (week 8) after 19 administrations. VAS assessed are vaginal dryness, vaginal itching, vaginal irritation, and painful urination (0–10, 0 = absent, 10 = intolerable). Between-group differences in the proportion of patients with a reduction in VAS score at week 8, at p-value < 0.05 significant. The categorisation was done as follows: 0–2: not bothersome, 3–5: mildly bothersome, 6–8: moderately bothersome and 9–10: most bothersome

## Secondary outcome:

Criteria used as secondary outcome measures are as follows:

- High vaginal swab (HVS) to measure vagina lactobacillus flora, vaginal thrush, bacterial vaginosis and trichomonas vaginalis from the posterior fornix observing. These measurements were based on the Nugent Score of the subjects assessed as well as the intensity and variety of microflora on baseline (Day 1) and Day 56. Nugent score of 0 to 3 was interpreted as negative for bacterial vaginosis, a Nugent score of 4 to 6 was interpreted as intermediate for bacterial vaginosis, and a Nugent score of 7 to 10 as positive for bacterial vaginosis.
- Vaginal pH measurements were taken using pH strips on baseline (Day 1) and Day 56
- Vaginal Health Index (VHI) (cut-off < 15 index of atrophic vagina) and between-group difference in mean change from baseline to end of study (Day 56)
- Female Sexual Function Index Scoring (FSFI) on Day 1 and Day 56.The Female Sexual Function Index (FSFI) is a comprehensive questionnaire used to assess key aspects of sexual function in women. It covers six domains; desire, arousal, lubrication, orgasm, satisfaction and pain.
- Day-to-day Impact of Vaginal Aging Questionnaire (DIVA) on Day 1 and 56.The day-to-Day Impact of Vaginal Aging (DIVA) Questionnaire is a specialized tool used to measure the impact of vaginal ageing symptoms on women's daily lives. It assesses four key domains; emotional well-being,physical well-being, sexual well-being and lifestyle Impact
- An adverse event is any untoward medical occurrence in a subject administered an investigational product that does not necessarily have a causal relationship with the treatment. An AE can, therefore, be any unfavourable and unintended sign, symptom, laboratory observation, or disease temporally associated with using the investigational product, whether or not related to the investigational product.Adverse events include any form of vaginal bleeding, bothersome vaginal irritations, bacterial vaginosis, vaginal yeast infection and hypersensitivity reactions.

## Sample size

To detect a clinically significant difference of 35% for the primary endpoint, Visual Analogic Scale (VAS) for vaginal dryness, using a two-tailed z-test of proportions between two groups with 80% power and a 5% level of significance, 27 subjects are needed. 20% dropout is added, thus rounding the total sample size per arm to 35 subjects. The total sample size required for the study is 70.

# Ethical approval

This study was conducted according to standard ethics principles for scientific research in Good Clinical Practice (GCP) 4th edition guidelines and the Declaration of Helsinki. It was approved by the Medical Research Ethics Committee (MREC) at the National Medical Research Registry(NMRR ID-23-02085-IH7) under protocol number VDMEN-V4.0-2023, and all participants provided informed consent before their inclusion in the study.

## Results

**Demographic and Other Baseline characteristics**

Table 1: Baseline characteristics of study participants

| Baseline Characteristics | Control (n=35) | Intervention (n=35) | p-value |
|---|---|---|---|
| Age | 50.74 ± 5.15 | 50.86 ± 4.17 | 0.919 |
| Pre pH | 5.05 ± 0.34 | 5.02 ± 0.37 | 0.712 |
| Pre-Vaginal Health Index | 18.91 ± 2.86 | 18.97 ± 2.37 | 0.928 |
| Pre Nugent Score | | | |
| Negative for bacterial vaginosis | 17 | 16 | 0.834 |
| Intermediate for bacterial vaginosis | 17 | 17 | |
| Positive for bacterial vaginosis | 1 | 2 | |
| Body mass index ($kg/m^2$) | 26.25 ± 3.17 | 26.45 ± 2.74 | 0.785 |
| Systolic blood pressure (mmHg) | 122.51 ± 13.87 | 119.00 ± 13.70 | 0.290 |
| Diastolic blood pressure (mmHg) | 74.63 ± 9.65 | 73.09 ± 10.68 | 0.528 |
| Pulse rate (beats per minute) | 75.49 ± 11.60 | 75.74 ± 10.14 | 0.922 |

* p-value <0.05 considered significant, reported in Mean (Standard Deviation) for continuous variable and frequency for categorical variables

All participants in the study were of Malay ethnicity, except for one individual of Indian ethnicity and another with Indonesian descent. Except for one participant who occasionally smoked, none of the participants consumed tobacco or alcohol. The total sample size consisted of 70 participants, evenly split between the control and intervention groups. One participant dropped out on day 56 due to the Haj pilgrimage, resulting in assessments for this subject being limited to day 14. The baseline characteristics indicated no significant differences between the control and intervention groups (Table 1).

Table 2: Efficacy of the investigational product in reducing the severity of specific adverse effects using Visual Analog Scale (VAS) scores

| VAS score | | Not Bothersome | | Most Bothersome | | Mild Bothersome | | Moderate Bothersome | | p-value[a] |
|---|---|---|---|---|---|---|---|---|---|---|
| VAS categories | | Control (n=35) | Intervention (n=35) | Control (n=35) | Intervention (n=35) | Control (n=35) | Intervention (n=35) | Control (n=35) | Intervention (n=35) | |
| **Vaginal Dryness** | Day 1 | 25 (52.1) | 23 (47.9) | 2 (100.0) | 0 (0.0) | 7 (38.9) | 11(61.1) | 1 (50.0) | 1 (50.0) | 0.396 |
| | Day 14 | 27 (46.6) | 31 (53.4) | 2 (100.0) | 0 (0.0) | 5 (62.5) | 3 (37.5) | 1 (50.0) | 1 (50.5) | 0.427 |
| | Day 56 [b] | 24 (42.1) | 33 (57.9) | 3 (100.0) | 0 (0.0) | 7 (77.8) | 2 (22.2) | 0 (0.0) | 0 (0.0) | 0.042* |
| **Vaginal Itching** | Day 1 | 26 (48.1) | 28 (51.9) | 0 (0.0) | 0 (0.0) | 8 (61.5) | 5 (38.5) | 1 (33.3) | 2 (66.7) | 0.577 |
| | Day 14 | 31 (48.4) | 33 (51.6) | 0 (0.0) | 0 (0.0) | 4 (66.7) | 2 (33.3) | 0 (0.0) | 0 (0.0) | 0.673 |
| | Day 56 [b] | 28 (48.3) | 30 (51.7) | 1 (0.0) | 0 (0.0) | 6 (60.0) | 4 (40.0) | 0 (0.0) | 1 (100.0) | 0.481 |
| **Vaginal Irritation** | Day 1 | 27 (48.6) | 31 (53.4) | 1 (100.0) | 0 (0.0) | 7 (63.6) | 4 (36.4) | 0 (0.0) | 0 (0.0) | 0.351 |
| | Day 14 | 30 (46.9) | 34 (53.1) | 1 (100.0) | 0 (0.0) | 3 (75.0) | 1 (25.0) | 1 (100.0) | 0 (0.0) | 0.355 |
| | Day 56 [b] | 28 (45.2) | 34 (54.8) | 1 (100.0) | 0 (0.0) | 5 (100.0) | 0 (0.0) | 0 (0.0) | 1 (100.0) | 0.072 |
| **Painful urination** | Day 1 | 31 (49.2) | 32 (50.8) | 1 (100.0) | 0 (0.0) | 3 (60.0) | 2 (40.0) | 0 (0.0) | 1 (100.0) | 0.529 |
| | Day 14 | 34 (49.3) | 35 (50.7) | 1 (100.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1.000 |
| | Day 56 [b] | 31 (47.7) | 34 (52.3) | 1 (100.0) | 0 (0.0) | 2 (100.0) | 0 (0.0) | 0 (0.0) | 1 (100.0) | 0.273 |

[a] Chi-square test and Fischer exact test (more than 50% cell count less than 5)

[b] Day 56 showed drop-out of 1 patient in control arm

* p-value <0.05 considered significant

Table 2 indicates that there were no significant differences between the two groups in the reduction of VAS scores for itching, irritation, and urination issues from baseline to week 8. However, at week 8 (Day 56), the intervention group demonstrated a significantly higher proportion of patients with improved vaginal dryness scores compared to the control group (p=0.042). These results suggest that the investigational product is effective in alleviating vaginal dryness, as reflected in the significant reduction of VAS scores observed at week 8.

Table 3 : Clinical outcomes before and after the use of the investigational product

| Clinical Outcomes | Control (n=35) | Intervention (n=35) | Mean difference | p-value |
|---|---|---|---|---|
| **Nugent score** [a] | | | | |
| Pre | 3.29 (1.759) | 3.31 (2.069) | -0.029 | 0.951 |
| Post [b] | 2.76 (1.776) | 2.29 (1.775) | 0.479 | 0.267 |
| **Nugent categories**[c] | | | | |
| **Pre** | | | | |
| Negative for bacterial vaginosis | 17 (51.5) | 16 (48.5) | | 0.834 |
| Intermediate for bacterial vaginosis | 17 (50.0) | 17 (50.0) | | |
| Positive for bacterial vaginosis | 1 (33.3) | 2 (66.7) | | |
| **Post** [b] | | | | |
| Negative for bacterial vaginosis | 22 (46.8) | 25 (53.2) | | 0.542 |
| Intermediate for bacterial vaginosis | 11 (52.4) | 10 (47.6) | | |
| Positive for bacterial vaginosis | 1 (100.0) | 0 (0) | | |
| **Vaginal pH** | | | | |
| Pre | 5.05 (0.343) | 5.02 (0.367) | | 0.712 |
| Post | 5.09 (0.260) | 5.24 (0.547) | | 0.148 |
| **Vaginal Health Index** | | | | |
| Pre | 18.91 (2.863) | 18.97 (2.370) | 0.057 | 0.928 |
| Post | 19.59 (1.971) | 20.80 (2.286) | -1.212 | 0.021* |
| **Vaginal Health Index (<15 VA)** | | | | |
| **Pre** | | | | |
| **Non-VA** | 32 (48.5) | 34 (51.5) | | 0.303 |
| **VA** | 3 (75.0) | 1 (25.0) | | |
| **Post** | | | | |
| **Non-VA** | 32 (48.5) | 34 (51.5) | | 0.321 |
| **VA** | 3 (75.0) | 1 (25.0) | | |

[a] Independent sample T-test conducted

[b] Assessment showed drop-out of 1 patient in the control arm

[c] Chi square test conducted for categorical variable

* Continous variable reported in Mean (Standard Deviation) and categorical variable reported in frequency( percentage), VA : Vaginal atrophy

The intervention group demonstrated a greater improvement in Nugent Scores compared to the control group; however, this difference was not statistically significant (p=0.267). Both the pre- and post-Nugent categories showed no statistically significant differences for either the control or intervention arms. Additionally, there was no significant variation in vaginal pH between the control and intervention groups both before and after the intervention. Nevertheless, the intervention group exhibited a higher, although non-significant, increase in pH relative to the control group. Regarding the Vaginal Health Index (VHI) (with a cut-off of <15 indicating an atrophic vagina), the mean change from baseline to the conclusion of the study (week 8) revealed significant improvements in post-VHI

scores. The intervention group experienced a substantial improvement in vaginal atrophy compared to the control group at week 8. When comparing VHI scores between individuals with vaginal atrophy (VA) and those without, there was no significant difference observed in pre- and post-usage of the investigational product, nor was there a significant distinction in VHI scores between those with VA and those without (Table 3).

### Adverse Events (AEs)

There were three cases of AE, which were mild and transient. In the first (rashes on hands, trunk and thigh) and third cases (folliculitis), the symptoms presented were unrelated to investigational products and resolved without treatment. However, AE was possibly related to the investigational product for the second case whereby the patient experienced itchiness in the vaginal area, and the condition was treated with a single dose of Cetirizine 10mg and subsequently resolved without the need for further follow-up. No cases of deaths or other SAE occurred. Overall, the usage of investigational product was deemed to be safe as there were only three cases of adverse events which were in mild severity.

Table 4: Female Sexual Function Index Scoring (FSFI) on day 1 and day 56 (n=70)

| Questions | Options | Day 1 (n=70) | | | Day 56(n=69) | | |
|---|---|---|---|---|---|---|---|
| | | Control (n=35) | Intervention (n=35) | p-value | Control [a] (n=34) | Intervention (n=35) | p-value |
| Q1- Over the past 4 weeks, how often did you feel sexual desire or interest? | Almost always or always | 0 (0.0) | 0(0.0) | 0.330 | 1(100.0) | 0(0.0) | 0.535 |
| | Most times (more than half the time) | 7(70.0) | 3(30.0) | | 4(66.7) | 2(33.3) | |
| | Sometimes (about half the time) | 17(50.0) | 17(50.0) | | 19(45.2) | 23(54.8) | |
| | A few times (less than half the time) | 11(42.3) | 15(57.7) | | 10(52.6) | 9(47.4) | |
| | Almost never or never | 0(0.0) | 0(0.0) | | 0(0.0) | 1(100.0) | |
| Q2- Over the past 4 weeks, how would you rate your level (degree) of sexual desire/interest? | Very high | 0(0.0) | 1(100.0) | 0.044 | 0(0.0) | 0(0.0) | 0.308 |
| | High | 6(85.7) | 1(14.3) | | 2(25.0) | 6(75.0) | |
| | Moderate | 24(53.3) | 21(46.7) | | 27(50.0) | 27(50.0) | |
| | Low | 4(25.0) | 12(75.0) | | 4(80.0) | 1(20.0) | |
| | Very low or none at all | 1(100.0) | 0(0.0) | | 1(50.0) | 1(50.0) | |
| Q3- Over the past 4 weeks, how often did you feel sexually aroused ('turned on") during sexual activity or intercourse? | No sexual activity | 0(0.0) | 1(100.0) | 0.769 | 0(0.0) | 0(0.0) | 0.421 |
| | Almost always or always | 2(33.3) | 4(66.7) | | 4(36.4) | 7(63.6) | |
| | Most times (more than half the time) | 8(53.3) | 7(46.7) | | 9(50.0) | 9(50.0) | |
| | Sometimes (about half the time) | 14(51.9) | 13(48.1) | | 12(50.0) | 12(50.0) | |
| | A few times (less than half the time) | 11(52.4) | 10(47.6) | | 9(64.3) | 5(35.7) | |
| | Almost never or never | 0(0.0) | 0(0.0) | | 0(0.0) | 2(100.0) | |
| Q4- Over the past 4 weeks, how could you rate your level of sexual arousal ('turn on") during sexual activity or intercourse? | No sexual activity | 0(0.0) | 0(0.0) | 0.696 | 0(0.0) | 0(0.0) | 0.872 |
| | Very high | 1(50.0) | 1(50.0) | | 1(50.0) | 1(50.0) | |
| | High | 9(64.3) | 5(35.7) | | 8(42.1) | 11(57.9) | |
| | Moderate | 18(46.2) | 21(53.8) | | 22(51.2) | 21(48.8) | |
| | Low | 7(46.7) | 8(53.3) | | 2(66.7) | 1(33.3) | |
| | Very low or none at all | 0(0.0) | 0(0.0) | | 1(50.0) | 1(50.0) | |
| Q5- Over the past 4 weeks, how confident were you about becoming sexually aroused during sexual activity or intercourse? | No sexual activity | 0(0.0) | 0(0.0) | 0.761 | 0(0.0) | 0(0.0) | 0.121 |
| | Very high confidence | 3(75.0) | 1(25.0) | | 4(40.0) | 6(60.0) | |

| Questions | Options | Day 1 (n=70) | | | Day 56(n=69) | | |
|---|---|---|---|---|---|---|---|
| | | Control (n=35) | Intervention (n=35) | p-value | Control [a] (n=34) | Intervention (n=35) | p-value |
| | High confidence | 19(50.0) | 19(50.0) | | 15(40.5) | 22(59.5) | |
| | Moderate Confidence | 11(45.8) | 13(54.2) | | 12(66.7) | 6(33.3) | |
| | Low confidence | 2(50.0) | 2(50.0) | | 3(100.0) | 0(0.0) | |
| | Very low or no confidence | 0(0.0) | 0(0.0) | | 0(0.0) | 1(100.0) | |
| Q6- Over the past 4 weeks, how often have you been satisfied with your arousal (excitement) during sexual activity or intercourse? | No sexual activity | 0(0.0) | 0(0.0) | 0.524 | 0(0.0) | 0(0.0) | 0.017* |
| | Almost always or always | 9(60.0) | 6(40.0) | | 6(31.6) | 13(68.4) | |
| | Most times (more than half the time) | 10(43.5) | 13(56.5) | | 8(34.8) | 15(65.2) | |
| | Sometimes (about half the time) | 12(57.1) | 9(42.9) | | 16(72.7) | 6(27.3) | |
| | A few times (less than half the time) | 4(36.4) | 7(63.6) | | 4(80.0) | 1(20.0) | |
| | Almost never or never | 0(0.0) | 0(0.0) | | 0(0.0) | 0(0.0) | |
| Q7- Over the past 4 weeks, how often did you become lubricated ("wet") during sexual activity or intercourse? | No sexual activity | 0(0.0) | 0(0.0) | 0.649 | 0(0.0) | 0(0.0) | 0.421 |
| | Almost always or always | 14(50.0) | 14(50.0) | | 11(42.3) | 15(57.7) | |
| | Most times (more than half the time) | 7(63.6) | 4(36.4) | | 7(46.7) | 8(53.3) | |
| | Sometimes (about half the time) | 9(52.9) | 8(47.1) | | 11(57.9) | 8(42.1) | |
| | A few times (less than half the time) | 3(30.0) | 7(70.0) | | 4(80.0) | 1(20.0) | |
| | Almost never or never | 2(50.0) | 2(50.0) | | 1(25.0) | 3(75.0) | |
| Q8- Over the past 4 weeks, how difficult was it to become lubricated ('wet") during sexual activity or intercourse? | No sexual activity | 0(0.0) | 1(100.0) | 0.308 | 0(0.0) | 1(100.0) | 0.169 |
| | Extremely difficult or impossible | 0(0.0) | 1(100.0) | | 2(66.7) | 1(33.3) | |
| | Very difficult | 3(100.0) | 0(0.0) | | 1(100.0) | 0(0.0) | |
| | Difficult slightly | 5(38.5) | 8(61.5) | | 8(80.0) | 2(20.0) | |
| | Difficult | 19(50.0) | 19(50.0) | | 15(38.5) | 24(61.5) | |
| | Not difficult | 8(57.1) | 6(42.9) | | 8(53.3) | 7(46.7) | |
| Q9- Over the past 4 weeks, how often did you maintain your | No sexual | 0(0.0) | 0(0.0) | 0.155 | 0(0.0) | 1(100.0) | 0.319 |

| Questions | Options | Day 1 (n=70) | | | Day 56(n=69) | | |
|---|---|---|---|---|---|---|---|
| | | Control (n=35) | Intervention (n=35) | p-value | Control [a] (n=34) | Intervention (n=35) | p-value |
| lubrication (“wetness”) until completion of sexual activity or intercourse? | activity | | | | | | |
| | Almost always or always | 11(47.8) | 12(52.2) | | 11(40.7) | 16(59.3) | |
| | Most times (more than half the time) | 9(64.3) | 5(35.7) | | 7(43.8) | 9(56.3) | |
| | Sometimes (about half the time) | 9(69.2) | 4(30.8) | | 8(53.3) | 7(46.7) | |
| | A few times (less than half the time) | 5(27.8) | 13(72.2) | | 7(77.8) | 2(22.2) | |
| | Almost never or never | 1(50.0) | 1(50.0) | | 1(100.0) | 0(0.0) | |
| Q10- Over the past 4 weeks, how difficult was it to maintain your lubrication (“wetness”) until the completion of sexual activity or intercourse? | No sexual activity | 0(0.0) | 0(0.0) | 0.862 | 0(0.0) | 1(100.0) | 0.104 |
| | Extremely difficult or impossible | 1(100.0) | 0(0.0) | | 1(100.0) | 0(0.0) | |
| | Very difficult | 1(50.0) | 1(50.0) | | 1(100.0) | 0(0.0) | |
| | Difficult | 12(46.2) | 14(53.8) | | 11(78.6) | 3(21.4) | |
| | Slightly difficult | 17(50.0) | 17(50.0) | | 18(40.9) | 26(59.1) | |
| | Not difficult | 4(57.1) | 3(42.9) | | 3(37.5) | 5(62.5) | |
| Q11- Over the past 4 weeks, when you had sexual stimulation or intercourse, how often did you reach orgasm (climax)? | No sexual activity | 0(0.0) | 0(0.0) | 0.759 | 0(0.0) | 1(100.0) | 0.362 |
| | Almost always or always | 9(50.0) | 9(50.0) | | 10(41.7) | 14(58.3) | |
| | Most times (more than half the time) | 11(57.9) | 8(42.1) | | 6(40.0) | 9(60.0) | |
| | Sometimes (about half the time) | 11(50.0) | 11(50.0) | | 14(63.6) | 8(36.4) | |
| | A few times (less than half the time) | 4(40.0) | 6(60.0) | | 4(66.7) | 2(33.3) | |
| | Almost never or never | 0(0.0) | 1(100.0) | | 0(0.0) | 1(100.0) | |
| Q12- Over the past 4 weeks, when you had sexual stimulation or intercourse, how difficult was it for you to reach orgasm (climax)? | No sexual activity | 0(0.0) | 0(0.0) | 0.595 | 0(0.0) | 1(100.0) | 0.330 |
| | Extremely difficult or impossible | 1(100.0) | 0(0.0) | | 0(0.0) | 1(100.0) | |
| | Very difficult | 1(100.0) | 0(0.0) | | 1(100.0) | 0(0.0) | |
| | Difficult | 8(50.0) | 8(50.0) | | 9(69.2) | 4(30.8) | |
| | Slightly difficult | 19(45.2) | 23(54.8) | | 17(42.5) | 23(57.5) | |

| Questions | Options | Day 1 (n=70) | | | Day 56(n=69) | | |
|---|---|---|---|---|---|---|---|
| | | Control (n=35) | Intervention (n=35) | p-value | Control [a] (n=34) | Intervention (n=35) | p-value |
| | Not difficult | 6(60.0) | 4(40.0) | | 7(53.8) | 6(46.2) | |
| Q13- Over the past 4 weeks, how satisfied were you with your ability to reach orgasm (climax) during sexual activity or intercourse? | No sexual activity | 0(0.0) | 0(0.0) | 0.787 | 0(0.0) | 1(100.0) | 0.033* |
| | Very satisfied | 7(50.0) | 7(50.0) | | 11(57.9) | 8(42.1) | |
| | Moderately satisfied | 19(50.0) | 19(50.0) | | 13(35.1) | 24(64.9) | |
| | About equally satisfied and dissatisfied | 8(47.1) | 9(52.9) | | 9(90.0) | 1(10.0) | |
| | Moderately dissatisfied | 0(0.0) | 0(0.0) | | 1(50.0) | 1(50.0) | |
| | Very dissatisfied | 1(100.0) | 0(0.0) | | 0(0.0) | 0(0.0) | |
| Q14- Over the past 4 weeks, how satisfied have you been with the amount of emotional closeness during sexual activity between you and your partner? | No sexual activity | 0(0.0) | 0(0.0) | 0.745 | 0(0.0) | 1(100.0) | 0.042* |
| | Very satisfied | 8(53.3) | 7((46.7) | | 9(50.0) | 9(50.0) | |
| | Moderately satisfied | 24(51.1) | 23(48.9) | | 15(38.5) | 24(61.5) | |
| | About equally satisfied and dissatisfied | 3(37.5) | 5(62.5) | | 7(100.0) | 0(0.0) | |
| | Moderately dissatisfied | 0(0.0) | 0(0.0) | | 2(100.0) | 0(0.0) | |
| | Very dissatisfied | 0(0.0) | 0(0.0) | | 1(50.0) | 1(50.0) | |
| Q15- Over the past 4 weeks, how satisfied have you been with your sexual relationship with your partner? | No sexual activity | 0(0.0) | 0(0.0) | 0.379 | 0(0.0) | 1(100.0) | 0.015* |
| | Very satisfied | 0(0.0) | 0(0.0) | | 10(43.5) | 13(56.5) | |
| | Moderately satisfied | 11(64.7) | 6(35.3) | | 15(41.7) | 21(58.3) | |
| | About equally satisfied and dissatisfied | 19(45.2) | 23(54.8) | | 9(100.0) | 0(0.0) | |
| | Moderately dissatisfied | 5(45.5) | 6(54.5) | | 0(0.0) | 0(0.0) | |
| | Very dissatisfied | 0(0.0) | 0(0.0) | | 0(0.0) | 0(0.0) | |
| Q16- Over the past 4 weeks, how satisfied have you been with your overall sexual life? | No sexual activity | 0(0.0) | 0(0.0) | 0.322 | 0(0.0) | 1(100.0) | 0.008* |
| | Very satisfied | 12(66.7) | 6(33.3) | | 8(44.4) | 10(55.6) | |
| | Moderately satisfied | 19(45.2) | 23(54.8) | | 16(40.0) | 24(60.0) | |
| | About equally satisfied and dissatisfied | 4(44.4) | 5(55.6) | | 10(100.0) | 0(0.0) | |

| Questions | Options | Day 1 (n=70) | | | Day 56(n=69) | | |
|---|---|---|---|---|---|---|---|
| | | Control (n=35) | Intervention (n=35) | p-value | Control [a] (n=34) | Intervention (n=35) | p-value |
| | Moderately dissatisfied | 0(0.0) | 1(100.0) | | 0(0.0) | 0(0.0) | |
| | Very dissatisfied | 0(0.0) | 0(0.0) | | 0(0.0) | 0(0.0) | |
| Q17- Over the past 4 weeks, how often did you experience discomfort or pain during vaginal penetration? | Did not attempt intercourse | 0(0.0) | 0(0.0) | 0.836 | 0(0.0) | 1(100.0) | 0.095 |
| | Almost always or always | 2(50.0) | 2(50.0) | | 1(100.0) | 0(0.0) | |
| | Most times (more than half the time) | 1 (33.3) | 2(66.7) | | 2(50.0) | 2(50.0) | |
| | Sometimes (about half the time) | 11(61.1) | 7(38.9) | | 10(71.4) | 4(28.6) | |
| | A few times (less than half the time) | 11(47.8) | 12(52.2) | | 13(59.1) | 9(40.9) | |
| | Almost never or never | 10(45.5) | 12(54.5) | | 8(29.6) | 19(70.4) | |
| Q18- Over the past 4 weeks, how often did your experience discomfort or pain following vaginal penetration? | Did not attempt intercourse | 0(0.0) | 0(0.0) | 0.570 | 0(0.0) | 1(100.0) | 0.089 |
| | Almost always or always | 1(100.0) | 0(0.0) | | 0(0.0) | 0(0.0) | |
| | Most times (more than half the time) | 2(66.7) | 1(33.3) | | 2(66.7) | 1(33.3) | |
| | Sometimes (about half the time) | 13(59.1) | 9(40.9) | | 9(60.0) | 6(40.0) | |
| | A few times (less than half the time) | 10(41.7) | 14(58.3) | | 15(65.2) | 8(34.8) | |
| | Almost never or never | 9(45.0) | 11(55.0) | | 8(29.6) | 19(70.4) | |
| Q19- Over the past 4 weeks, how would you rate your level (degree) of discomfort or pain during or following vaginal penetration? | Did not attempt intercourse | 0(0.0) | 0(0.0) | 0.937 | 0(0.0) | 1(100.0) | 0.120 |
| | Very high | 0(0.0) | 0(0.0) | | 1(100.0) | 0(0.0) | |
| | High | 3(60.0) | 2(40.0) | | 2(66.7) | 1(33.3) | |
| | Moderate | 11(52.4) | 10(47.6) | | 14(70.0) | 6(30.0) | |
| | Low | 11(45.8) | 13(54.2) | | 8(50.0) | 8(50.0) | |
| | Very low or none at all | 10(50.0) | 10(50.0) | | 9(32.1) | 19(67.9) | |

[a] Control group showed drop-out of 1 patient on Day 56, *Statistically significant p-value ( $p<0.05$)

Table 5: Day-to-day Impact of Vaginal Aging Questionnaire (DIVA) for baseline and day 56 (n=70)

| Day-to-day Impact of Vaginal Aging Questionnaire (DIVA) | | Day 1 (n=70) | | | Day 56 [a] (n=69) | | |
|---|---|---|---|---|---|---|---|
| | Options | Control (n=35) | Intervention (n=35) | p-value | Control (n=34) | Intervention (n=35) | p-value |
| **Part A** | | | | | | | |
| Q1- Walk at your usual speed? | Not at all | 26 (53.1) | 23 (46.9) | 0.632 | 24 (43.6) | 31 (56.4) | 0.225 |
| | A little bit | 7 (46.7) | 8 (53.3) | | 7 (77.8) | 2 (22.2) | |
| | Moderately | 2 (33.3) | 4 (66.7) | | 2 (50.0) | 2 (50.0) | |
| | Quite a bit | 0 (0.0) | 0 (0.0) | | 1 (100.0) | 0 (0.0) | |
| | Extremely | 0 (0.0) | 0 (0.0) | | 0 (0.0) | 0 (0.0) | |
| Q2- Wear the clothing or underwear you want? | Not at all | 30 (52.6) | 27 (47.4) | 0.512 | 26 (44.8) | 32 (55.2) | 0.328 |
| | A little bit | 3 (42.9) | 4 (57.1) | | 6 (66.7) | 3 (33.3) | |
| | Moderately | 0 (0.0) | 2 (100.0) | | 1 (100.0) | 0 (0.0) | |
| | Quite a bit | 2 (50.0) | 2 (50.0) | | 1 (100.0) | 0 (0.0) | |
| | Extremely | 0 (0.0) | 0 (0.0) | | 0 (0.0) | 0 (0.0) | |
| Q3- Use the toilet or wipe yourself after using the toilet? | Not at all | 28 (53.8) | 24 (46.2) | 0.485 | 24 (42.1) | 33 (57.9) | 0.146 |
| | A little bit | 5 (35.7) | 9 (64.3) | | 7 (77.8) | 2 (22.2) | |
| | Moderately | 1 (50.0) | 1 (50.0) | | 1 (100.0) | 0 (0.0) | |
| | Quite a bit | 0 (0.0) | 1 (100.0) | | 1 (100.0) | 0 (0.0) | |
| | Extremely | 1 (100.0) | 0 (0.0) | | 1 (100.0) | 0 (0.0) | |
| Q4- Sit for more than an hour | Not at all | 26 (51.0) | 25 (49.0) | 0.797 | 1 (100.0) | 0 (0.0) | 0.222 |
| | A little bit | 7 (50.0) | 7 (50.0) | | 26 (44.8) | 32 (55.2) | |
| | Moderately | 2 (50.0) | 2 (50.0) | | 7 (77.8) | 2 (55.2) | |
| | Quite a bit | 0 (0.0) | 1 (100.0) | | 0 (0.0) | 0 (0.0) | |
| | Extremely | 0 (0.0) | 0 (0.0) | | 0 (0.0) | 0 (0.0) | |
| Q5- Get a good night's sleep? | Not at all | 25 (52.1) | 23 (47.9) | 0.407 | 19 (41.3) | 27 (58.7) | 0.200 |
| | A little bit | 5 (55.6) | 4 (44.4) | | 11 (78.6) | 3 (21.4) | |
| | Moderately | 1 (20.0) | 4 (80.0) | | 2 (50.0) | 2 (50.0) | |
| | Quite a bit | 1 (25.0) | 3 (75.0) | | 1 (50.0) | 1 (50.0) | |
| | Extremely | 3 (75.0) | 1 (25.0) | | 1 (33.3) | 2 (66.7) | |
| **Part B** | | | | | | | |
| Q6- Depressed or down? | Never | 21 (48.8) | 22 (51.2) | 0.960 | 21 (40.4) | 31 (59.6) | 0.075 |
| | Rarely | 9 (52.9) | 8 (47.1) | | 11 (78.6) | 3 (21.4) | |
| | Sometimes | 5 (50.0) | 5 (50.0) | | 1 (50.0) | 1 (50.0) | |
| | Fairly often | 0 (0.0) | 0 (0.0) | | 1 (100.0) | 1 (100.0) | |

| Day-to-day Impact of Vaginal Aging Questionnaire (DIVA) | | Day 1 (n=70) | | | Day 56 [a] (n=69) | | |
|---|---|---|---|---|---|---|---|
| | Options | Control (n=35) | Intervention (n=35) | p-value | Control (n=34) | Intervention (n=35) | p-value |
| | Very often | 0 (0.0) | 0 (0.0) | | 0 (0.0) | 0 (0.0) | |
| Q7- Embarrassed? | Never | 23 (51.1) | 22 (48.9) | 0.352 | 20 (43.5) | 26 (56.5) | 0.354 |
| | Rarely | 9 (56.3) | 7 (43.8) | | 11 (64.7) | 6 (35.3) | |
| | Sometimes | 2 (25.0) | 6 (75.0) | | 3 (50.0) | 3 (50.0) | |
| | Fairly often | 1 (100.0) | 0 (0.0) | | 0 (0.0) | 0 (0.0) | |
| | Very often | 0 (0.0) | 0 (0.0) | | 0 (0.0) | 0 (0.0) | |
| Q8- Frustrated or resentful? | Never | 24 (53.3) | 21 (46.7) | 0.519 | 23 (46.0) | 27 (54.0) | 0.614 |
| | Rarely | 6 (37.5) | 10 (62.5) | | 8 (57.1) | 6 (42.9) | |
| | Sometimes | 5 (55.6) | 4 (44.4) | | 3 (60.0) | 2 (40.0) | |
| | Fairly often | 0 (0.0) | 0 (0.0) | | 0 (0.0) | 0 (0.0) | |
| | Very often | 0 (0.0) | 0 (0.0) | | 0 (0.0) | 0 (0.0) | |
| Q9- Bad about yourself? | Never | 27 (54.0) | 23 (46.0) | 0.456 | 24 (47.1) | 27 (52.9) | 0.277 |
| | Rarely | 6 (50.0) | 6 (50.0) | | 9 (69.2) | 4 (30.8) | |
| | Sometimes | 2 (28.6) | 5 (71.4) | | 1 (25.0) | 3 (75.0) | |
| | Fairly often | 0 (0.0) | 1 (100.0) | | 0 (0.0) | 1 (100.0) | |
| **Part C** | | | | | | | |
| Q10- Your desire or interest in having sexual intercourse or other types of sexual activity (including self-stimulation or masturbation)? | Not at all | 25 (52.1) | 23 (47.9) | 0.410 | 21 (42.0) | 29 (58.0) | 0.168 |
| | A little bit | 8 (50.0) | 8 (50.0) | | 10 (71.4) | 4 (28.6) | |
| | Moderately | 1 (20.0) | 4 (80.0) | | 3 (60.0)2 | (40.0) | |
| | Quite a bit | 1 (100.0) | 0 (0.0) | | 0 (0.0) | 0 (0.0) | |
| | Extremely | 0 (0.0) | 0 (0.0) | | 0 (0.0) | 0 (0.0) | |
| Q11- How frequently you had sexual intercourse or other types of sexual activity (including self-stimulation or masturbation)? | Not at all | 25 (59.5) | 17 (40.5) | 0.095 | 21 (44.7) | 26 (55.3) | 0.525 |
| | A little bit | 6 (35.3) | 11 (64.7) | | 10 (62.5) | 6 (37.5) | |
| | Moderately | 1 (14.3) | 6 (85.7) | | 1 (33.3) | 2 (66.7) | |
| | Quite a bit | 2 (66.7) | 1 (33.3) | | 2 (66.7) | 1 (33.3) | |
| | Extremely | 1 (100.0) | 0 (0.0) | | 0 (0.0) | 0 (0.0) | |
| Q12- Your ability to become aroused during sexual activity (including self-stimulation or masturbation)? | Not at all | 21 (55.3) | 17 (44.7) | 0.519 | 21 (42.9) | 28 (57.1) | 0.217 |
| | A little bit | 11 (47.8) | 12 (52.2) | | 10 (71.4) | 4 (28.6) | |
| | Moderately | 1 (20.0) | 4 (80.0) | | 3 (60.0) | 2 (40.0) | |
| | Quite a bit | 2 (50.0) | 2 (50.0) | | 0 (0.0) | 0 (0.0) | |

| Day-to-day Impact of Vaginal Aging Questionnaire (DIVA) | | Day 1 (n=70) | | | Day 56 [a] (n=69) | | |
|---|---|---|---|---|---|---|---|
| | Options | Control (n=35) | Intervention (n=35) | p-value | Control (n=34) | Intervention (n=35) | p-value |
| | Extremely | 0 (0.0) | 0 (0.0) | | 0 (0.0) | 1 (100.0) | |
| Q13- Your ability to be spontaneous about sexual activity (including self-stimulation or masturbation)? | Not at all | 28 (60.9) | 18 (39.1) | 0.038* | 20 (45.5) | 24 (54.5) | 0.331 |
| | A little bit | 5 (26.3) | 14 (73.7) | | 11(68.8) | 5(31.3) | |
| | Moderately | 1 (25.0) | 3 (75.0) | | 3(42.9) | 4(57.1) | |
| | Quite a bit | 0 (0.0) | 0 (0.0) | | 0 (0.0) | 0(0.0) | |
| | Extremely | 0 (0.0) | 0 (0.0) | | 0(0.0) | 1(100.0) | |
| Q14- The amount of pleasure you experienced during sexual activity (including self-stimulation or masturbation)? | Not at all | 23 (59.0) | 16 (41.0) | 0.175 | 17 (39.5) | 26 (60.5) | 0.140 |
| | A little bit | 7 (35.0) | 13 (65.0) | | 13 (76.5) | 4 (23.5) | |
| | Moderately | 2 (28.6) | 5 (71.4) | | 1 (25.0) | 3 (75.0) | |
| | Quite a bit | 1 (50.0) | 1 (50.0) | | 1 (100.0) | 0 (0.0) | |
| | Extremely | 1 (100.0) | 0 (0.0) | | 1 (50.0) | 1 (50.0) | |
| Q15- Your desire or interest in being in a sexual relationship? | Not at all | 21 (53.8) | 18 (46.2) | 0.522 | 17 (37.8) | 28 (62.2) | 0.023* |
| | A little bit | 8 (53.3) | 7 (46.7) | | 14 (82.4) | 3 (17.6) | |
| | Moderately | 4 (30.8) | 9 (69.2) | | 2 (40.0) | 3 (60.0) | |
| | Quite a bit | 1 (50.0) | 1 (50.0) | | 1 (100.0) | 0 (0.0) | |
| | Extremely | 1 (100.0) | 0 (0.0) | | 0 (0.0) | 1 (100.0) | |
| Q16- Your confidence that you could sexually satisfy a partner? | Not at all | 23 (59.0) | 16 (41.0) | 0.304 | 19 (39.6) | 29 (60.4) | 0.036 |
| | A little bit | 7 (43.8) | 9 (56.3) | | 12 (85.7) | 2 (14.3) | |
| | Moderately | 2 (25.0) | 6 (75.0) | | 2 (66.7) | 1 (33.3) | |
| | Quite a bit | 1 (25.0) | 3 (75.0) | | 1 (33.3) | 2(66.7) | |
| | Extremely | 2 (66.7) | 1 (33.3) | | 0 (0.0) | 1(100.0) | |
| Q17- Your overall satisfaction with your sex life? | Not at all | 21 (58.3) | 15 (41.7) | 0.421 | 20(40.8) | 29(59.2) | 0.076 |
| | A little bit | 8 (44.4) | 10 (55.6) | | 10(83.3) | 2(16.7) | |
| | Moderately | 4 (33.3) | 8 (66.7) | | 3(50.0) | 3(50.0) | |
| | Quite a bit | 1 (100.0) | 0 (0.0) | | 1(100.0) | 0(0.0) | |
| | Extremely | 1 (33.3) | 2 (66.7) | | 0(0.0) | 1(100.0) | |
| **Part D** | | | | | | | |
| Q18 – My vaginal symptoms make me feel like I'm getting old | Not at all true | 10 (52.6) | 9 (47.4) | 0.638 | 14 (53.8) | 12 (46.2) | 0.295 |
| | A little true | 15 (57.7) | 11 (42.3) | | 9 (36.0) | 16(64.0) | |
| | Somewhat true | 7 (41.2) | 10 (58.8) | | 10(62.5) | 6(37.5) | |

| Day-to-day Impact of Vaginal Aging Questionnaire (DIVA) | | Day 1 (n=70) | | | Day 56 [a] (n=69) | | |
|---|---|---|---|---|---|---|---|
| | Options | Control (n=35) | Intervention (n=35) | p-value | Control (n=34) | Intervention (n=35) | p-value |
| | Mostly true | 3 (37.5) | 5 (62.5) | | 1(100.0) | 0(0.0) | |
| | Definitely true | 0 (0.0) | 0 (0.0) | | 0(0.0) | 1(100.0) | |
| Q19- I feel undesirable because of my vaginal symptoms | Not at all true | 15 (57.7) | 11 (42.3) | 0.405 | 13(52.2) | 12(48.0) | 0.271 |
| | A little true | 14 (53.8) | 12 (46.2) | | 12(40.0) | 18(60.0) | |
| | Somewhat true | 5 (35.7) | 9 (64.3) | | 9(69.2) | 4(30.8) | |
| | Mostly true | 1 (25.0) | 3 (75.0) | | 0(0.0) | 0(0.0) | |
| | Definitely true | 0 (0.0) | 0 (0.0) | | 0(0.0) | 1(100.0) | |
| Q20- When I think about vaginal symptoms, I feel like I have lost something | Not at all true | 13 (54.2) | 11 (45.8) | 0.361 | 12 (46.2) | 14 (53.8) | 0.495 |
| | A little true | 15 (51.7) | 14 (48.3) | | 15 (46.9) | 17(53.1) | |
| | Somewhat true | 5 (35.7) | 9 (64.3) | | 5(55.6) | 4(44.4) | |
| | Mostly true | 2 (100.0) | 0 (0.0) | | 2(100.0) | 0(0.0) | |
| | Definitely true | 0 (0.0) | 1 (100.0) | | 0(0.0) | 0(0.0) | |
| Q21- My vaginal symptoms make me feel like my body is deteriorating | Not at all true | 10 (52.6) | 9 (47.4) | 0.735 | 12(50.0) | 12(50.0) | 0.585 |
| | A little true | 15 (53.6) | 13 (46.4) | | 11(42.3) | 15(57.7) | |
| | Somewhat true | 8 (42.1) | 11 (57.9) | | 10(55.6) | 8(44.4) | |
| | Mostly true | 2 (66.7) | 1 (33.3) | | 1(100.0) | 0(0.0) | |
| | Definitely true | 0 (0.0) | 1 (100.0) | | 0(0.0) | 0(0.0) | |
| Q22- I feel less sexy because of my vaginal symptoms | Not all true | 14 (60.9) | 9 (39.1) | 0.390 | 11(47.8) | 12(52.2) | 0.690 |
| | A little true | 15 (50.0) | 15 (50.0) | | 16(47.1) | 18(52.9) | |
| | Somewhat true | 4 (30.8) | 9 (69.2) | | 6(54.5) | 5(45.5) | |
| | Mostly true | 2 (50.0) | 2 (50.0) | | 1(100.0) | 0(0.0) | |
| | Definitely true | 0 (0.0) | 0 (0.0) | | 0(0.0) | 0(0.0) | |

[a] Control group showed drop-out of 1 patient on Day 56

*Statistically significant p-value ( $p<0.05$)

Table 4 displays the Female Sexual Function Index (FSFI) scores at baseline and on day 56 for both the control and intervention groups. Notable improvements were observed on day 56 across five questions in the scoring system. A significant finding emerged regarding

satisfaction with arousal (excitement) during sexual activity or intercourse (p=0.017). Over the four weeks, several questions yielded significant results, including the ability to reach orgasm (p=0.033), satisfaction with emotional closeness during sexual activity (p=0.042), satisfaction with the sexual relationship (p=0.015), and satisfaction with overall sexual life (p=0.008). Table 5 illustrates the significance of the Day-to-day Impact of Vaginal Aging Questionnaire (DIVA) results for baseline and day 56. On day 1, patients reported improvements in their ability to be spontaneous regarding sexual activity (p=0.038) after using the investigational product. By day 56, the subjects exhibited a significant interest in engaging in a sexual relationship (p=0.023).

## Discussion

The current trial of Vaginal HYAL gel control group's mean age was 50.74 years (SD = 5.153), while the intervention group's mean age was 50.86 years (SD = 4.167). The mean ages of the two groups are remarkably similar, with only a 0.12-year difference representing the peri and post-menopausal women age range. This close alignment in mean age indicates that the two groups are well-matched in age distribution, which is essential for minimising age-related confounding variables. Such similarity helps ensure that any observed intervention effects are less likely to be attributed to age differences between the groups, thereby enhancing the validity of the current study's findings.

Vaginal HYAL gel contains the active ingredient Hyaluronic acid and was formulated as a vaginal gel. HA vaginal gel is increasingly being studied as a treatment for vaginal dryness and atrophy, particularly in postmenopausal women and those who cannot use hormonal treatments. This current trial provides the opportunity to review the efficacy, safety, and potential benefits of HA vaginal gel. The current trial revealed that for peri- and postmenopausal women in the intervention group, there was significant improvement in vaginal dryness and atrophy. This study demonstrated that HA used as vaginal gel effectively alleviated vaginal dryness, itching, burning, and dyspareunia, making it a valid alternative to estrogen-based treatments with comparable efficacy, safety, and tolerability. Numerous studies using HA revealed consistent findings with this trial [11, 25, 26]. Studies have also shown that non-hormonal intravaginal moisturisers significantly improve vaginal moisture, fluid volume, elasticity, and epithelial integrity in postmenopausal women, with a notable increase in vaginal moisture levels and overall sexual function[27, 28].

This investigational product could potentially act as an effective moisturizer for women with vulvovaginal atrophy and sexual well-being issues and benefit endometrial cancer survivors and postpartum women [16]. An animal study by Liu S. et al. (2015) suggested that hyaluronic acid vaginal gel effectively reverses vaginal atrophy and improves vaginal microecosystem in postmenopausal rats, while not increasing uterine weight. This implied potential benefit for use in human subjects [23]. Studies suggests HA used in the form of vaginal gel is a viable non-hormonal alternative for women who cannot or prefer not to use estrogen-based treatments, such as those with a history of hormone receptor-positive cancers [29, 30].

The investigational product exhibited minimal adverse effects in the test subjects during this trial. Only one subject experienced an adverse effect, which was mild in severity and likely linked to the investigational product, presenting as vaginal itching. However, a study carried out by Stute et al. (2013) showed that HA used as vaginal gel resulted in an improvement of vaginal itching, burning, and dyspareunia [26]. HA vaginal gel is well-tolerated with a safety profile comparable to that of vaginal estrogens and does not significantly alter the vaginal micro ecosystem or increase uterine weight [11, 26, 30].

There was no significant difference in vaginal pH between the control and intervention groups both before and after the intervention in this trial. However, the intervention group showed a higher, albeit non-significant, increase in pH compared to the control group. Vaginal pH is influenced by multiple factors, including bacterial pathogens, estrogen levels, and certain sexual practices. High estrogen levels support a low vaginal pH by promoting Lactobacilli growth, while decreased estrogen levels in menopausal women lead to higher pH. Practices that transmit vaginal fluids, such as the use of lubricants and douching, can disrupt the vaginal microbiota and affect pH [31, 32]. Increased vaginal pH for these trial subjects could be due to vaginal pH is inversely correlated with serum estradiol levels; lower estradiol levels are associated with higher vaginal pH, particularly in menopausal women which are the target of this trial [31, 33]. Hemalatha et al. (2013) suggested that even when vaginal pH is a useful measure of infection, however, it is not highly specific [33].

In the perimenopausal years, vaginal atrophy increases sexual dysfunction by 30%, with vaginal dryness being the symptom most closely related to all female sexuality domains [10]. In this trial, Vaginal Health Index among subjects at the end of the study (week 8) revealed a significant improvement in scores compared to the control group. However, there was no significance of VHI score improvements between those who have VA versus those who did not. The Vaginal Health Index (VHI) Score is a clinical tool used to assess the degree of atrophy in the genitourinary tract. It evaluates five parameters are evaluated such as vaginal elasticity, secretions,

pH, epithelial mucous membrane, and hydration that reflects the overall condition. According to a few studies, Topical application of hyaluronic acid significantly improves VHI scores and alleviates symptoms of VA in postmenopausal women [13, 34, 35].

Female Sexual Functioning Index (FSFI) scores in the current study revealed that the use of investigational products improved the subject's satisfaction with arousal (excitement) during sexual activity or intercourse. After 4 weeks of use, subject's ability to reach orgasm improved as well as satisfaction with the amount of emotional closeness during sexual activity. There was also improved satisfaction with sexual relationship and overall sexual life. Similarly, Gustavino et al. (2021) suggested that hyaluronic acid use as vaginal gel significantly improved sexual function, including desire, arousal, and lubrication in puerperal women [16].

Day-to-day Impact of Vaginal Aging Questionnaire (DIVA) showed improvement in ability to be spontaneous about sexual activity after using investigational product. This is supported by Carter et al. (2020) where it was found that hyaluronic acid vaginal gel improved vulvovaginal health and sexual function in endometrial cancer survivors, including increased confidence in future sexual activity[30].

## Conclusion

The clinical trial showed that Vaginal HYAL gel led to significant improvements in vaginal health and sexual function over 8 weeks. By week 8, participants reported reduced vaginal dryness and improved vaginal atrophy. FSFI scores on day 56 indicated notable improvements in arousal, orgasm, emotional closeness, and satisfaction with sexual activity. After 4 weeks, participants were more satisfied with their sexual relationships and overall sexual life. The DIVA questionnaire revealed enhanced spontaneity in sexual activity from day 1, and by day 56, participants had increased interest in being in a sexual relationship.

## Abbreviations

Vaginal Hyal gel, Peri and post menopausal , vaginal dryness, vaginal health index, Day-to-day Impact of Vaginal Aging Questionnaire , Female Sexual Functioning Index

## Declarations

# Ethics approval and consent to participate

This study was conducted according to standard ethics principles for scientific research in Good Clinical Practice (GCP) 4th edition guidelines and the Declaration of Helsinki. It was approved by the Medical Research Ethics Committee (MREC) at the National Medical Research Registry(NMRR ID-23-02085-IH7) under protocol number VDMEN-V4.0-2023, and all participants provided informed consent before their inclusion in the study.

### Consent for publication

Obtained from Crab Sinergy

# Competing Interests

Nil

# Funding

This study was funded by Crab Sinergy Srl, which provided financial support for the research, and publication of this manuscript. Crab Sinergy Srl had no role in the design of the study, data collection and analysis, or preparation of the manuscript. The authors independently analyzed the data and prepared this manuscript, ensuring the integrity of the findings and conclusions presented.

# Author Contribution

R.S wrote the main manuscript text and all authors reviewed the manuscript. Y.K.H, H.Z.H and B.I supervised the project. S.R conducted data cleaning and statistical analysis. M.S, S.S, P.D.S, J.M.N and I.L conducted data collection and data entry.

# Acknowledgements

Nil


# Data Availability

Can be obtained from the principal investigator

## References


1. Mac Bride MB, Rhodes DJ, Shuster LT. *Vulvovaginal Atrophy.* Mayo Clinic Proceedings, 2010. 85(1): pp. 87–94.
2. Palacios S, et al. The European Vulvovaginal Epidemiological Survey (EVES): prevalence, symptoms and impact of vulvovaginal atrophy of menopause. Climacteric. 2018;21(3):286–91.
3. Levine KB, Williams RE, Hartmann KE. Vulvovaginal atrophy is strongly associated with female sexual dysfunction among sexually active postmenopausal women. Menopause, 2008. 15(4).
4. Pérez-López FR, et al. Clinical manifestations and evaluation of postmenopausal vulvovaginal atrophy. Gynecol Endocrinol. 2021;37(8):740–5.
5. DiBonaventura M, et al. The Association Between Vulvovaginal Atrophy Symptoms and Quality of Life Among Postmenopausal Women in the United States and Western Europe. J Women's Health. 2015;24(9):713–22.
6. Ibe C, Simon JA. Continuing Medical Education: Vulvovaginal Atrophy: Current and Future Therapies (CME). J Sex Med. 2010;7(3):1042–50.
7. Benini V et al. New Innovations for the Treatment of Vulvovaginal Atrophy: An Up-to-Date Review. Med (Kaunas), 2022. 58(6).
8. Lev-Sagie A. Vulvar and Vaginal Atrophy: Physiology, Clinical Presentation, and Treatment Considerations. Clin Obstet Gynecol. 2015;58(3):476–91.
9. Stabile G et al. Induced Dryness Stress on Human Vaginal Epithelium: The Efficacy of a New Vaginal Gel. Gels, 2021. 7(4).
10. Cagnacci A, et al. Female sexuality and vaginal health across the menopausal age. Menopause. 2020;27(1):14–9.
11. Chen J, et al. Evaluation of the efficacy and safety of hyaluronic acid vaginal gel to ease vaginal dryness: a multicenter, randomized, controlled, open-label, parallel-group, clinical trial. J Sex Med. 2013;10(6):1575–84.
12. de Garcia S, et al. Vaginal hormone-free moisturising cream is not inferior to an estriol cream for treating symptoms of vulvovaginal atrophy: Prospective, randomised study. PLoS ONE. 2022;17(5):e0266633.
13. Jokar A, et al. Comparison of the Hyaluronic Acid Vaginal Cream and Conjugated Estrogen Used in Treatment of Vaginal Atrophy of Menopause Women: A Randomized Controlled Clinical Trial. Int J Community Based Nurs Midwifery. 2016;4(1):69–78.
14. Buzzaccarini G, et al. Hyaluronic acid in vulvar and vaginal administration: evidence from a literature systematic review. Climacteric. 2021;24(6):560–71.
15. De Seta F, et al. Efficacy and safety of a new vaginal gel for the treatment of symptoms associated with vulvovaginal atrophy in postmenopausal women: A double-blind randomized placebo-controlled study. Maturitas. 2021;147:34–40.
16. Gustavino C, et al. Efficacy and safety of prolonged-release hyaluronic acid derivative vaginal application in the postpartum period: a prospective randomised clinical trial. Ann Med. 2021;53(1):1589–97.
17. Cieri-Hutcherson NE et al. Systematic Review of l-Arginine for the Treatment of Hypoactive Sexual Desire Disorder and Related Conditions in Women. Pharm (Basel), 2021. 9(2).
18. Cesarone MR, et al. Prevention of vaginal dryness in perimenopausal women. Supplementation with Lady Prelox®. Minerva Ginecol. 2019;71(6):434–41.
19. Ito TY, Trant AS, Polan ML. A double-blind placebo-controlled study of ArginMax, a nutritional supplement for enhancement of female sexual function. J Sex Marital Ther. 2001;27(5):541–9.
20. Meston CM, Worcel M. The Effects of Yohimbine Plus L-arginine Glutamate on Sexual Arousal in Postmenopausal Women with Sexual Arousal Disorder. Arch Sex Behav. 2002;31(4):323–32.
21. Sinergy C. Evaluation of Efficacy and Safety of V-HYAL for Improvement of Vaginal Dryness. Italy; 2014.

22. FDA. *Guidance for Industry Guidance for Industry Estrogen and Estrogen / Progestin Drug. In Center for Drug Evaluation and Research (Issue January).*
23. Liu JH, Bernick B, Mirkin S. Estradiol softgel inserts for the treatment of VVA symptoms: an expert opinion. Expert Opin Drug Deliv. 2020;17(11):1573–81.
24. Palma F, et al. Vaginal atrophy of women in postmenopause. Results from a multicentric observational study: The AGATA study. Maturitas. 2016;83:40–4.
25. dos Santos CCM, et al. Hyaluronic Acid in Postmenopause Vaginal Atrophy: A Systematic Review. J Sex Med. 2021;18(1):156–66.
26. Stute P. Is vaginal hyaluronic acid as effective as vaginal estriol for vaginal dryness relief? Arch Gynecol Obstet. 2013;288(6):1199–201.
27. Sparavigna A, et al. A controlled, randomized, open label study in postmenopausal women to assess the safety and the efficacy of a vaginal moisturizer: An instrumental approach. Open J Obstet Gynecol. 2013;3:395–9.
28. Vale F, et al. Efficacy and safety of a non-hormonal intravaginal moisturizer for the treatment of vaginal dryness in postmenopausal women with sexual dysfunction. Eur J Obstet Gynecol Reproductive Biology. 2019;234:92–5.
29. dos Santos CCM, et al. Hyaluronic Acid in Postmenopause Vaginal Atrophy: A Systematic Review. J Sex Med. 2020;18(1):156–66.
30. Carter J, et al. A single-arm clinical trial investigating the effectiveness of a non-hormonal, hyaluronic acid-based vaginal moisturizer in endometrial cancer survivors. Gynecol Oncol. 2020;158(2):366–74.
31. Caillouette JC et al. *Vaginal pH as a marker for bacterial pathogens and menopausal status.* Am J Obstet Gynecol, 1997. 176(6): pp. 1270-5; discussion 1275-7.
32. Amabebe E, Anumba DOC. The Vaginal Microenvironment: The Physiologic Role of Lactobacilli. Front Med (Lausanne). 2018;5:181.
33. Hemalatha R, et al. Evaluation of vaginal pH for detection of bacterial vaginosis. Indian J Med Res. 2013;138(3):354–9.
34. Origoni M, et al. Postmenopausal vulvovaginal atrophy (VVA) is positively improved by topical hyaluronic acid application. A prospective, observational study. Eur Rev Med Pharmacol Sci. 2016;20(20):4190–5.
35. Nappi RE, et al. Treatment of vulvo-vaginal atrophy with hyaluronate-based gel: a randomized controlled study. Minerva Obstet Gynecol. 2022;74(6):480–8.

## Figures

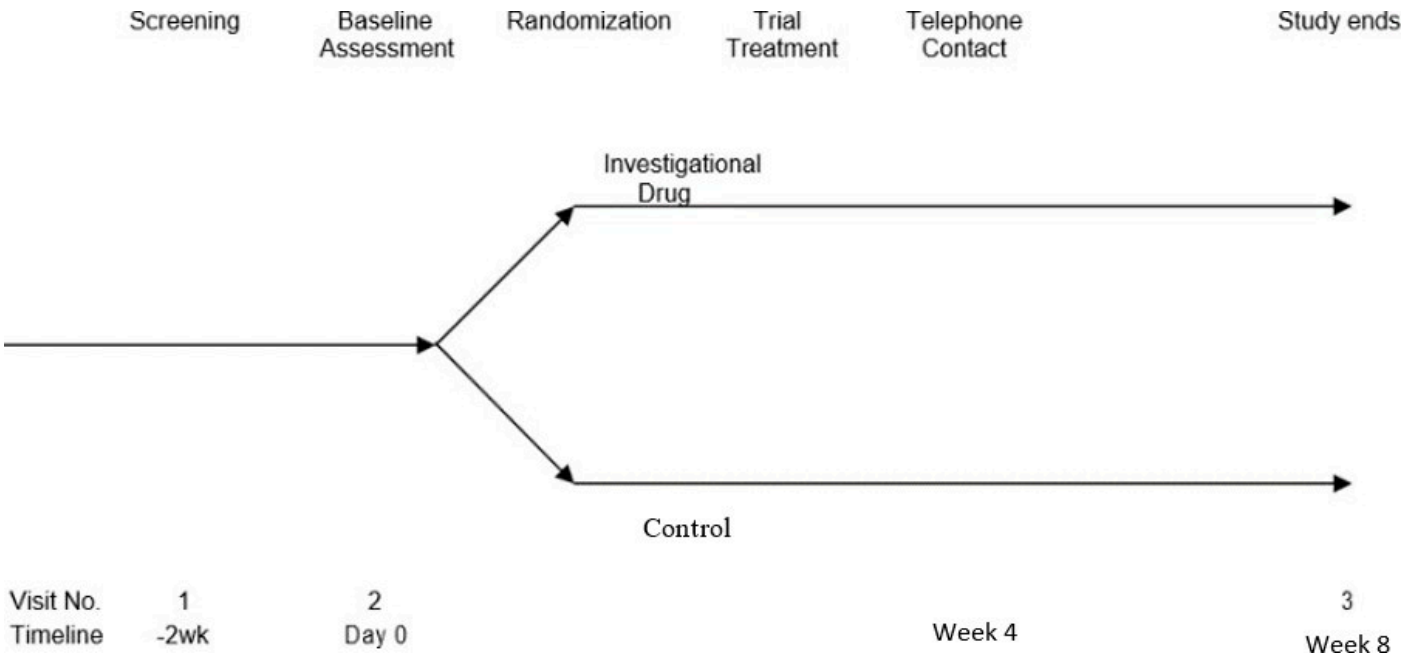



**Figure 1**

Design of a randomised, open clinical trial