**CLINICAL INVESTIGATION REPORT CYRON/01/2021**

# A PROSPECTIVE, OPEN-LABEL, PILOT, MULTICENTRIC CLINICAL INVESTIGATION TO EVALUATE THE PERFORMANCE AND SAFETY OF CERVIRON® OVULES IN THE LOCAL TREATMENT OF NON-SPECIFIC OR ENDOGENOUS, SYMPTOMATIC VAGINITIS

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| **Document Type:** | **Clinical Study Report** |
| **Version Number:** | **1.0** |
| **Medical Device Name:** | **Cerviron®** |
| **Medical Device Class:** | **IIb** |
| **Medical Device Manufacturer:** | **Perfect Care Distribution** |
| **Medical Device Manufacturer Contact Details:** | **17-19 Scarlatescu Street, 3rd Floor, 011158 Bucharest, Romania** |
| **Country of Study:** | **Romania** |
| **Release Date:** | **05 Apr 2022** |
| **ClinicalTrials.gov Identifier:** | **NCT04735705** |
| **CIP Identification:** | **CIP version V1.0 dated 06 Jan 2021** |
| **ISO 14155:2020 Compliance Statement:** | **This investigation was conducted in compliance with legal requirements for interventional studies** |

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# 1 GENERAL

## 1.1 TITLE OF THE CLINICAL INVESTIGATION

A Prospective, Open-Label, Pilot, Multicentric Clinical Investigation to Evaluate the Performance and Safety of Cerviron® ovules in the Local Treatment of Non-Specific or Endogenous, Symptomatic Vaginitis.

## 1.2 SCIENTIFIC APPROACH IN CLINICAL STUDY DESIGN, PLANNING, CONDUCT, ANALYSIS AND REPORTING

Cerviron® is a class IIb medical device indicated as an adjuvant treatment in atrophic, irritant, and inflammatory vaginitis caused by the imbalance of the vaginal pH and changes of the vaginal microflora. It favors the healing and re-epithelialization processes of the vaginal mucosa and reduces the proliferation of endogenous pathogens.

The present clinical investigation was design as a national, open-label, pilot, multicentric clinical investigation following the provisions of good clinical practice stated in the international standard ISO 14155:2020 (1) and the applicable national legislation.

All risks associated with the investigational device were estimated in accordance with ISO 14971:2019 (2) prior to initiating the clinical investigation. The justification for the design of the clinical investigation was based on the description and specification of the medical device and the evaluation of pre-clinical data included in the General Description. Emerging non-clinical and pharmaceutical quality data were reviewed and evaluated by qualified experts to assess the potential implications for the safety of study participants.

Following the principles of ICH E8 (R1), Guideline on general considerations for clinical studies, the clinical investigation design was informed by input from a broad range of stakeholders, including participants and healthcare providers. Several discussion sessions were opened from the side of the sponsor organization with the clinical investigators and other site staff, with the scope of not only building quality into the clinical investigation, but also to select the best eligibility criteria and clinically meaningful endpoints for the participants.

The Clinical Investigation Plan version 1.0 dated 06 January 2021 was followed during the execution of the clinical investigation. The Ethics Committee favourable opinion is listed in **ANNEX 2.**

No major protocol deviations were reported.

No amendments to the Clinical Investigation Plan were performed during the study conduct.

All investigators involved in study conduct received training commensurate with their role in the study prior to their becoming involved in the study. Adherence to the CIP and other relevant documents was monitored throughout the study performance and documented.

## 1.3 PROTECTION OF THE CLINICAL INVESTIGATION PARTICIPANTS

The clinical investigation was carried out following the principles stated in the Declaration of Helsinki. (3) The National Ethics Committee (CNBMDM) evaluated all participant-related documentation and

released its favorable opinion prior to the site initiation. All clinical investigators and all parties involved in the conduct of the clinical investigation shared the responsibility for its ethical conduct.

### 1.4 OVERALL SYNOPSIS OF THE CLINICAL INVESTIGATION REPORT

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| **Name of Sponsor** | PERFECT CARE DISTRIBUTION (ROMANIA) |
| **Title** | A Prospective, Open-Label, Pilot, Multicentric Clinical Investigation to Evaluate the Performance and Safety of Cerviron Ovules® in the Local Treatment of Non-Specific or Endogenous, Symptomatic Vaginitis |
| **Protocol Code** | **CYRON/01/2021** |
| **Indication** | **Non-Infectious, Symptomatic Vaginitis** |
| **Hypothesis** | The primary hypothesis is that the medical device can be effectively and safely administered in human participants for the treatment of non-specific or endogenous, symptomatic vaginitis. |
| **Clinical Investigation Objectives** | **Primary Objectives**<br>The primary objective is to assess the therapeutic performance and tolerability of Cerviron® Ovules in participants with symptomatic, non-specific, non-infectious vaginitis, and endogenous symptomatic infections.<br>**Secondary Objectives**<br>The secondary objective of this clinical investigation is the assessment of performance of the medical device by several additionally clinical outcomes (vaginal discharge, vaginal pH, microscopic characteristics of inflammatory cells and characteristics of vaginal microflora).<br>Participants will also evaluate the degree of satisfaction related to the use of the medical device. |
| **Clinical Investigation Method** | This investigation used as a clinical research method a pilot, intervention study with the primary purpose of treating an existing condition and an additional goal of optimizing intervention delivery, with specific attention to adherence and fidelity. |
| **Trial Design Overview** | The clinical investigation was conducted in Romania, in two geographically different research institutions (Bucharest and Timisoara), in a hospital-based setting.<br>Voluntary participants were recruited onsite and included women aged 18 years and older, presenting vaginal symptoms and a diagnosis of endogenous vaginitis.<br>The clinical data was collected using the electronic platform OpenClinica. OpenClinica is compliant with FDA and EMA regulations, including 21 CFR Part 11, HIPAA, GDPR, ICH-GCP, as well as with data practices and security standards (ISO, SOC). |

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| | The safety evaluable individuals included Intention-to-Treat (ITT) cohort consisting of all the participants who received the investigational medical device.<br><br>The efficacy analysis was done in Per-Protocol (PP) population, which included only participants who have attended the 3rd follow-up visit (at 3 months) and did not record any major protocol deviations. |
| **The Medical Device** | The medical device is Cerviron® Vaginal Ovules.<br><br>Perfect Care Distribution has developed a formulation of vaginal ovules containing a combination of active compounds and plant extracts, with an adjuvant role in the treatment of vaginitis of mechanical etiology, caused by imbalances in vaginal pH or disturbances of the vaginal microflora. Cerviron® Vaginal Ovules as per its Instructions for Use is indicated also for re-epithelization and repair of the cervical mucosa after cervical lesions, ulcerations and wounds of mechanical etiology. The composition of the ovules is as follows: Bismuth subgalate 100 mg, Hydrolyzed collagen 15 mg, Thyme extract (Thymus vulgaris) 10 mg, Hydrastis canadensis extract 10 mg, Marigold extract (Calendula officinalis) 10 mg, Turmenic extract (Curcuma longa ) 10 mg and Hexylresorcinol 2 mg.<br><br>Cerviron® Vaginal Ovules has obtained the European Conformity marking during the assessment performed in March 2020 under the requirements of the Medical Device Directive (MDD 93/42/EEC), that was replaced by the new MDR (Medical Device Regulation) 2017/745.<br><br>The recommended use of Cerviron® is one ovule per day, inserted on the first day after the menstruation and during 15 consecutive days. Its adjuvant role is consolidated during the administration for a period of 3 consecutive months. |
| **Participant population** | **Inclusion criteria**<br><br>1 Adult females, aged 18 years to 65 years;<br>2 Participants presenting two or more vaginal symptoms such as leucorrhoea, pruritus, burning sensation, erythema, pain, odour, dysuria, or dyspareunia;<br>3 Participants with a diagnosis of either non-infectious vaginitis, or endogenous, symptomatic infection;<br>4 Negative for Gardnerella vaginalis, Candida albicans, Trichomonas vaginalis;<br>5 Participants willing to provide signed informed consent to clinical investigation participation.<br><br>**Exclusion criteria**<br><br>1 Participants in menstrual period or suffering from menorrhagia;<br>2 Colpectomy;<br>3 Participants with undiagnosed abnormal genital bleeding; |

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| | 4 Participants with vulvar, vaginal or cervical cancer;<br>5 Known, active sexually transmitted infection (STI) in partner, as per anamnesis;<br>6 Participants with HIV or other immunodeficiency;<br>7 Participants with any pathology of the female reproductive organs;<br>8 Known allergy or hypersensitivity to the medical device ingredients;<br>9 Use of spermicides;<br>10 Use of diaphragm;<br>11 Concomitant topical or systemic anti-infective treatment;<br>12 Unable to comply with visit procedures;<br>13 Participants included in other clinical investigations;<br><br>A total number of 50 subjects were screened and 47 participants were considered eligible for the completion of this study. The 47 participants fully met the inclusion and exclusion criteria, signed the Inform Consent, and performed the Baseline visit. Out of 50 subjects enrolled in the clinical study, three subjects did not meet the inclusion and exclusion criteria, as a result they were not included in the clinical trial. The safety population included the 47 subjects that took the treatment; more precisely 39 subjects had a 100% compliance, 6 subjects had a 75% compliance, and 2 participants had a 50% compliance. The ITT population included 47 subjects that received the treatment of the study medical device. The Per Protocol Set population, subjects who completed all the visits without major protocol deviations, was 47 subjects. |

**Clinical investigation endpoints**

**The primary endpoints are:**

- Clinical Performance assessed by the investigator by gynecological examination and participant's interview at 90 days, or after 3 consecutive months of treatment.

  Success was defined by **resolution** (return to participant's usual gynecological conditions, i.e., before the episode which warranted inclusion in the study) **OR substantial improvement** of clinical signs of infectious vaginitis (i.e., abnormal vaginal discharge), and/or vaginal symptoms (vaginal burning and/or vaginal pain, and/or vaginal irritation and/or pruritus and/or odour).

  Failure is defined by persistence or worsening of symptoms and clinical signs or requirement of an alternative or specific treatment.

- The number of treatment-related Adverse Events in volunteers participating in the clinical investigation collected during 90 days.

**The secondary endpoints are:**

- Change in vaginal discharge aspect during clinical exam, assessed by the investigator at 1, 2 and 3 months by using a score between 0 = absent and 4=purulent.
- Change in vaginal pH values at 1, 2 and 3 months, compared to its normal values (3.8 - 4.5).
- Change in vaginal microflora and Lactobacilli count by microscopy performed at 3 months.

- Change in vaginal inflammation by microscopy performed at 3 months.
- Participant Satisfaction performed at 3 months by using a five-point Likert Scale.

**Statistical analysis**

All statistical analyses were be performed using the R statistical software (version 4.1.1) with a few extra statistical packages added, all of which have been revised and updated to the latest version. The final analysis was completed after all subjects have finalized the study, all queries have been resolved, and the database has been locked.

The overall type I error was preserved at 5%.

Statistical analyses were conducted on all subjects who have successfully completed the study without a CIP deviation that is regarded as impacting the assessment of the key variables (as per CIP).

Quantitative variables (i.e., demographic) if normally distributed were described through mean ± Standard Deviation (SD), otherwise median, minimum, maximum, and interquartile range were showed. Qualitative variables were evaluated using frequencies and percentages.

To evaluate changes of proportions over time before and after the treatment, for categorical variables two-proportions z test were performed.

The quality and completeness of the collected data were evaluated preliminary compared to data analysis.

No study participant has been involved in any violation of inclusion/exclusion criteria.

**Primary endpoint**

As defined in the CIP, the primary efficacy outcomes to evaluate the tested Cerviron® were: Clinical Performance assessed by the investigator by gynecological examination and tolerability related to the therapy at 90 days, or after 3 consecutive months of treatment. The primary hypothesis is that, after 3 months after using the medical device, the clinical symptoms will be improved by 100.00% of the treated participants.

**Secondary endpoints**

As secondary hypotheses we assume that several additionally clinical outcomes will be improved with 100.00% of the participants using the medical device. The secondary objective of this clinical investigation included the assessment of performance of the medical device by several additionally clinical outcomes, such as change in vaginal discharge aspect, change in vaginal pH values, change in vaginal microflora and Lactobacilli count, change in vaginal inflammation, and Participant Satisfaction at the end of 3 months of treatment.

**Safety endpoints**

The safety of Cerviron® was measured by the rate of treatment-related adverse events, including serious adverse events (SAEs).

**Safety analysis**

There were a total number of 3 adverse events unrelated to the medical device usage reported for participants 0203, 0213 and 0224.

At the end of study, no treatment-related AE were reported.

**Results**

**Primary outcome**

For 34 (72.34%) study participant using Cerviron® the medical device had a beneficial effect on the score of Vaginal Symptoms, while for only 13 participants (27.66%) the score remained the same ($p < 0.001$).

**Secondary outcomes.**

*Change in Vaginal Discharge Aspect*. The difference in change for Vaginal Discharge Aspect between baseline visit and final visit was statistically significant at 5% significance level ($p < 0.05$).

*Change in Vaginal pH values.* Determination of pH values compared to its normal values (3.8 – 4.5). The difference in change of Vaginal pH values between the baseline and final visit, at 5% significance level was statistically significant ($p < 0.05$).

*Change in vaginal microflora and Lactobacilli count.* The change in Lactobacilli species throughout the study was not statistically significant ($p > 0.05$).

*Change in vaginal inflammation*. The difference in change in vaginal inflammation between baseline and final visit, at 5% significance level was statistically significant ($p < 0.001$).

*Participant satisfaction*. The degree of satisfaction when using the medical device was assessed using a five-point Likert Scale. A total number of 32 out of 47 participants (82.98%) were very satisfied, 6 participants (12.77%) were satisfied, and 2 participants were neutral. No participants were reported as unsatisfied or very unsatisfied.

**Trial Duration:**

The regulatory submission of the clinical investigation dossier was performed on 24 February 2021.
The NEC favorable opinion for the clinical investigation performance was received on 01 April 2021.
The total study duration was between 27 April 2021 and 28 February 2022.
Each participant was followed for 3 months or approximately 90 days.

The Visit Flow Chart is detailed in Table 1.

**Table 1. Visit Flow Chart**

| PROCEDURE \ EVENT | Screening Visit 1 (Day -3 to Day 0) | Clinic Visit 1 (Day 0) | Clinic Visit 2 (Day 30) | Clinic Visit 3 (Day 60) | Clinic Visit 4 (Day 90) |
|---|---|---|---|---|---|
| VISIT CODE | SV1 | CV1 | CV2 | CV3 | CV4 |
| Informed consent | X | | | | |
| Demography data | X | | | | |
| Eligibility criteria | X | | | | |
| Medical history | X | | | | |
| Physical examination | X | | X | X | X |
| Participant's Interview (collection of vaginal symptoms) | X | | X | X | X |
| Collection of vaginal swab | X | | X | X | X |
| Vaginal smear (collection of vaginal discharge) | X | | X | X | X |
| Vaginal pH | | X | X | X | X |
| Inflammatory and parabasal cells (microscopy) | | X | | | X |
| Participant satisfaction (Likert Scale) | | | | | X |
| Adverse events | X | X | X | X | X |
| Concomitant medications | X | X | X | X | X |
| Medical device prescription and instructions for use | | X | | | |
| Medical device accountability | | | | | X |

**End of Clinical investigation Definition:**

The end of clinical investigation was considered as the last visit for the last participant in the study (participant no. 0235) that perform the onsite visit in 28 Feb 2022.

**Conclusions**

The purpose of the present clinical investigation was to evaluate the therapeutic performance and tolerability of Cerviron® Ovules in participants with symptomatic, non-specific, non-infectious vaginitis, and endogenous symptomatic infections. The beneficial effects were assessed as changes between the final visit, visit 3, visit 2, and visit 1 (Baseline) in the following performance outcomes: Clinical Performance after thorough gynecological examination and participant's interview at end of treatment visit over 90 days. The results obtained show very clearly that during the 3 months of treatment with Cerviron®, out of the 47 participants who initially showed vaginal symptoms, a proportion of 72.24% unequivocally showed an improvement in symptoms. Significant improvements were also found in Vaginal discharge aspect score, pH values and vaginal inflammation score. Some improvements were found in vaginal microflora and *Lactobacilli* count, but they were not statistically significant.

In terms of adverse events, 3 study participants reported Grade 1 - vaginal infection, that were resolved under treatment with Cerviron®. At the end of study, no treatment - related AE were reported.

In conclusion, administration of Cerviron® vaginal ovules for a period of 3 months significantly alleviates the symptoms of symptomatic, non-specific, non-infectious vaginitis, and endogenous symptomatic infections and its administration is considered safe.

## 2. INTRODUCTION

### 2.1 STUDY RATIONALE AND BACKGROUND

Vaginitis is the general term characterizing a spectrum of disorders of the vagina caused by infection, inflammation, or changes in the normal vaginal flora. Symptoms include abnormal vaginal discharge, odor, pruritus, pain, dysuria, discomfort and dyspareunia. (4,5) Moreover, vaginitis is responsible for the most common problems in clinical medicine, and it is the reason cited most often for visits to obstetricians and gynecologists. In the past years, the problem of vaginitis has all too often been ignored by the medical community or regarded merely as a minor annoyance to women. According to Gardner, "Vaginitis must cause more unhappiness on earth than any other gynecologic disease". In addition to the many physical and emotional problems associated with vaginitis, the economic loss involved is of astronomic proportions."(6)

The most common causes of vaginitis are bacterial vaginosis, vulvovaginal candidiasis, and trichomoniasis. (7) Bacterial vaginosis is the cause in 40% to 50% of cases in which a cause is identified, with vulvovaginal candidiasis accounting for 20% to 25% and trichomoniasis for 15% to 20% of cases.

There is no cause of vaginitis identified in up to 30% of women. These women may have a range of conditions, including irritant or allergic vaginitis, atrophic vaginitis, or physiologic discharge. (8) Notable infective cause include Group A *streptococci*, *Haemophilus*, *Staphylococci* and rarely *Shigella.* (5) The presence of objective signs of vulvar inflammation in the absence of vaginal pathogens after laboratory testing suggests the possibility of mechanical, chemical, allergic, or other non-infectious causes of vulvovaginal signs or symptoms. It is believed that the oxidative stress has a major role in vaginal epithelial cells apoptosis leading to non-specific vaginitis. Normally, a healthy vaginal environment maintains a balance between the protective organisms (*Lactobacillus*) and other anaerobic and aerobic flora, with *Lactobacillus* as the majority organism. The adversely altered balance between protective organisms and potential pathogens in the microenvironment of genital tract results in gynecological disease, such as non-specific vaginitis. In healthy women, $H_2O_2$ is produced by lactobacilli and maintained a typical concentration in the vaginal fluid. This typical concentration of $H_2O_2$ is toxic to many microorganisms and thus provides an intrinsic protective mechanism in the vaginal compartment.

Non-specific vaginitis (also called vaginal dysbiosis) is the disruption of the vaginal microbiome. Recent studies show that the vaginal dysbiosis affects human papilloma virus acquisition, persistence, and progression to related cervical premalignancy. (9)

Several other types of non-infectious vaginitis are encountered in clinical practice: inflammatory vaginitis, desquamative inflammatory vaginitis, irritant vaginitis, atrophic vaginitis, cytolytic vaginitis and aerobic vaginitis.

Inflammatory vaginitis is an uncommon condition characterized by purulent vaginal discharge, burning, and dyspareunia, and should be considered in participants with these symptoms if no infectious cause is found. Inflammatory vaginitis is associated with low estrogen levels, such as in menopausal or perimenopausal women.

Desquamative inflammatory vaginitis is a chronic inflammatory process involving both vagina and vestibule, occurring almost exclusively in white women, that responds well to topical anti-inflammatory therapy. Long-term maintenance therapy is frequently required. (10,11)

Irritant/allergic vaginitis is characterized by symptoms such as burning and soreness and signs such as vulvar erythema.

Atrophic vaginitis is an uncommon condition that occurs as a consequence of estrogen deficiency and is characterized by symptoms such as thin, clear discharge, vaginal dryness, dyspareunia, itching. An examination of the vulva can indicate inflammation and thin and friable vaginal mucosa. Risk factors contributing to atrophic vaginitis are menopause, lactation, oophorectomy, radiation therapy, chemotherapy, immunologic disorders, premature ovarian failure, endocrine disorders, and antiestrogen medications. (7)

Aerobic vaginitis is a distinct vaginal condition, requiring different clinical management and with distinct clinical risks. (12) Aerobic vaginitis is defined by disruption in *Lactobacillus* dominance but is accompanied by more extreme inflammatory changes than bacterial vaginosis and the presence of mainly aerobic enteric commensals or pathogens, including *Group B Streptococcus (S. agalactiae), Enterococcus faecalis, Escherichia coli*, and *Staphylcoccus aureus*. *Streptococcus agalactiae* vaginal pathogenicity is not uniformly acknowledged throughout the literature. Group B *Streptococcus* virulence for vagina was recognized in the past, as the organism has been observed to potentially cause local inflammation and discharge, as well as *Lactobacilli* rarefaction. (13)

Cytolytic vaginosis is another condition which has been recently described as an entity distinct from either normal flora or bacterial vaginosis. This condition, also called Döderlein's vaginitis, has numerous, active lactobacilli damaging epithelial cells because of extreme acidity and low pH. The bare nuclei and cellular debris that are seen during microscopy, should not be mistaken for leucocytes and cocci. (14)

Cerviron ovules® is a class IIb medical device developed as adjuvant in the treatment of the following conditions:

1. *acute and chronic vulvovaginitis* of mechanical etiology, caused by changes of vaginal pH and changes of the vaginal flora.
2. *cervical lesions* of mechanical origin as it favors the healing and re-epithelialization processes of the cervical mucosa and reduces the proliferation of endogenous pathogens.

Cerviron ovules® is indicated as a maintenance treatment to prevent relapse of non-specific vaginitis, as this occurs very often.

The Cerviron® medical device is intended to be used with antibiotics as a treatment for non-specific vaginitis. Cerviron® vaginal ovules prevents relapses and helps restore vaginal pH while preserving *Lactobacill*i species. They are indicated as long-term supportive therapy, either as a stand-alone treatment or in combination with other oral therapies with antibiotics, antifungals or antivirals. The plant alkaloids in its composition (Marigold, Thyme and Turmenic) and in addition, alkylresorcinols exert an excellent support in its antibacterial action. Cerviron® supportive therapy may be prescribed for 10 or 15 consecutive days.

When used as a stand-alone treatment, the medical device Cerviron® performed well in reducing unpleasant symptoms in atrophic vaginitis. Topical agents such as Cerviron ® ovules are preferred in treating these cases versus hormonal treatments that increase the risk of estrogen exposure, Treatment with Cerviron® vaginal ovules showed a beneficial effect in atrophic vaginitis, offering a good local control of the disease without containing any estrogen derivate. (15)

NCT04735705 was the first planned clinical investigation on human volunteers, in which 50 participants were screened and 47 participants were enrolled, design to investigate the performance and safety of Cerviron® ovules when administered in participants with symptomatic, non-specific, non-infectious vaginitis. The primary objective was to assess the therapeutic performance and tolerability of Cerviron® ovules in participants with symptomatic, non-specific, non-infectious vaginitis, and endogenous symptomatic infections. The secondary objective of the clinical investigation was the assessment of performance of the medical device by several additionally clinical outcomes, including vaginal discharge, vaginal pH, microscopic characteristics of inflammatory cells and characteristics of vaginal microflora.

A second clinical investigation, NCT04735718 is currently conducted to evaluate the performance and safety of Cerviron® ovules in cervix lesions postoperative care.

**2.2 BENEFIT/RISK ASSESSMENT**

Cerviron® ovules contains ingredients that provide therapeutic performance as an adjunct in the treatment of acute and chronic vulvovaginitis of mechanical etiology, caused by changes in vaginal pH and changes in vaginal flora. The main mechanism of action of the medical device is the dispersion of ingredients in the vagina, thus forming a protective barrier that promotes and accelerates the healing process of damaged epithelium, restoring the original vaginal ecosystem.

The solutions drafted to design and create the medical device Cerviron® ovules ensures, according to the Medical Device Regulation 2017/745, the elimination of any kind of internal and external risks associated to the use of the product, respectively the medical device was designed and performed not to compromise the participants' health or safety. The risks related to biocompatibility and toxicity are eliminated using materials that fall within the stipulated norms, the microbiological and physio-chemical verification of the raw materials, of the intermediate product and of the finished product.

The risk of toxicity is eliminated from the design of the medical device by setting the doses of active substances below the limit provided by the literature for the occurrence of toxic effects. The active ingredients used have a local therapeutic effect, their systemic absorption being very low, which leads to the elimination of systemic side effects.

The risk associated to the use of the medical device Cerviron® ovules is in the acceptable limits in relation to the participant's benefit, being compatible with a high level of safety and health protection - according to the certificates of analysis and to the physical, chemical and microbiological test reports. Moreover, the medical device was designed, manufactured, and packed so that its features and performances were not affected as a result of the transport and storage according to its instructions for use.

While the performance and safety profile of the Cerviron® ovules are subject to investigation when used in postoperative care, the only contraindication to Cerviron® ovules has been determined as

hypersensitivity to the active substance or to any of the excipients within the medical device. Therefore, participants volunteering for this trial are not considered to be at additional risk related to the administration of the medical device. Collection of other samples, like vaginal swabs, and vaginal smears are considered not to cause any significant harm.

Considering its short-term duration of action as well as the medical device's instructions for use, a small sample size of 50 participants was defined for this clinical investigation.

## 3. INVESTIGATIONAL DEVICE AND METHODS

### 3.1 DESCRIPTION OF THE INVESTIGATIONAL DEVICE

**The generic name of the device:** Vaginal mucosa protection suppository

**The commercial name of the device:** Cerviron®

**Medical device class: IIb**

The medical device comes in contact with the vaginal mucosa for a prolonged exposure time - the cumulative amount of single, multiple or repeated contact time is likely to exceed 24 hours but will not exceed 30 days.

Cerviron® vaginal ovules is a class IIb medical device intended to be used as adjuvant in the treatment of acute and chronic vulvovaginitis of mechanical etiology, caused by changes of vaginal pH and/or changes of the vaginal flora, and in cervical lesions of mechanical origin. It favors the healing and re-epithelialization processes and reduces the proliferation of endogenous pathogens.

**Composition per ovule**

Cerviron® is composed of: Bismuth subgallate 100 mg, hydrolyzed Collagen 15 mg, Thyme extract (Thymus vulgaris) 10 mg, Goldenseal extract (Hydrastis canadensis) 10 mg, Marigold extract (Calendula officinalis) 10 mg, Turmeric extract (Curcuma longa) 10 mg, Hexylresorcinol 2 mg.

**The structural formula**

Semisolid, ovoid ovules, with yellow to intense yellow colour, weighing 2 g per each ovule, with no color spots or areas with agglomerated powder. In longitudinal section, the ovule has a homogenous aspect, no powder agglomerations or air bubbles.

The medical device is essentially a dispersion of active substances in a molten mass (50°C), in a stainless-steel melting device. After homogenization, the melted mass is distributed in PVC/PE shapes and cooled down. This product's formula was prepared using selected excipients, frequently used in the pharmaceutical industry for manufacturing similar products.

The specifications of the most important compounds are defined in the *European Pharmacopeia*. For the others, the features are specified in the internal monographs.

The base is mainly composed by fat, an excipient frequently used to manufacture vaginal ovules and medicinal suppositories. The base contains a modified content of monoglycerides, which offer hydrophilic properties.

Melting characteristic: the fat bases must liquefy only at body temperature.

In order to formulate this product, a hard fat produced by Gattefosse - France, called Suppocire Nas 50, was chosen. The melting point for this base is between 33,5°C and 35°C. The melting point for the finished product is between 35°C and 37°C. This melting interval is adequate for vaginal ovules.

Cerviron® ovules are packaged in cardboard boxes imprinted according to the legislation in force. Each box contains 2 PVC/PVC blisters with 5 ovules each, with instructions of use.

The quality and quantity structure of the medical device is detailed in the Table 2 below.

**Table 2: Active ingredients and excipients**

| No. | Ingredients | Quantity/ 100g | Quantity/ ovule (mg) | Function | Reference standard |
|---|---|---|---|---|---|
| 1. | Bismuth subgallate | 5 | 100,0 | Astringent, healing | Eu.Ph.10.0 |
| 2. | Hexylresorcinol | 0,1 | 2,0 | Antimicrobial | Eu.Ph.10.0 |
| 3. | Hydrolyzed collagen | 0.75 | 15.0 | Healing | ST producer |
| 4. | Thyme extract (*Thymus vulgaris*) | 0.5 | 10.0 | Antifungal | ST producer |
| 5. | Goldenseal extract (*Hydrastis canadensis*) | 0.5 | 10.0 | Hemostatic | ST producer |
| 6. | Marigold extract (*Calendula officinalis*) | 0.5 | 10.0 | Healing | ST producer |
| 7. | Turmeric extract (*Curcuma longa)* | 0.5 | 10.0 | Anti-inflammatory | ST producer |
| 8. | Semi-synthetic glycerides/ SUPPOCIRE NAS 50 PELLETS | 84.5 | 1690.0 | Base | Eu.Ph.10.0 |
| 9. | Anhydrous lanolin | 6.15 | 123.0 | Base | Eu.Ph.10.0 |
| 10. | Colloidal silicon dioxide | 1.5 | 30.0 | Stabilizing agent | Eu.Ph.10.0 |
| **TOTAL** | | | **2000 mg** | | |

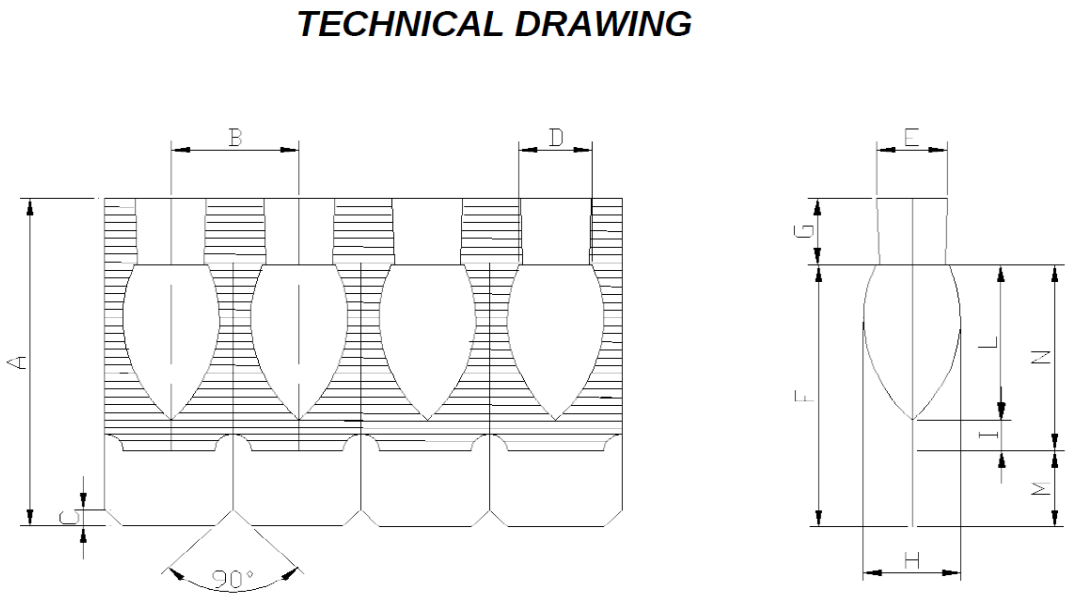


**Figure 1. Technical Drawing**

**Contraindications**

Hypersensitivity to any of the ingredients of the medical device.

**Warnings and Special Cautions for Use**

Considering its composition, in general, no cautions are necessary when using the medical device. Due to insufficient data regarding the use of the medical device during pregnancy, caution and a referral to the specialist physician are recommended in case of pregnancy.

**Pregnancy and Breastfeeding**

Due to insufficient clinical data, it is recommended to consult a specialist before administration to pregnant women. No caution is necessary in case of use during breastfeeding. The active compounds are not excreted in breast milk following the use of Cerviron®.

**Effects on the Ability to Drive and Use Machines**

Cerviron® do not influence the ability to drive vehicles or use machines.

**Side Effects**

Rarely local, light, transient irritations can appear. Hypersensitivity reactions can appear.

**Overdose**

No overdose cases are known.

### 3.1.1 THE INTENDED USE OF THE INVESTIGATIONAL DEVICE(S)

The existing scientific data from the literature regarding the active compounds as well as the structural formula constitute a scientific support for the intended use of the medical device.

Cerviron vaginal ovules is a medical device with local therapeutic effect recommended for adult women suffering from atrophic, aerobic and traumatic vaginitis, cervicitis, cervix erosions and leucorrhea of unspecified cause.

### 3.1.2 DESCRIPTION OF MECHANISM OF ACTION OF THE INVESTIGATIONAL DEVICE, ALONG WITH SUPPORTING SCIENTIFIC LITERATURE

Cerviron® vaginal ovules is a class IIb medical device, intended to be used as an adjuvant in the treatment of acute and chronic vulvovaginitis of traumatic etiology, vulvovaginitis caused by changes of vaginal pH and changes of the vaginal flora, atrophic vulvovaginitis and also of cervical lesions of traumatic origin. It favors the healing and re-epithelialization processes and reduces the proliferation of endogenous pathogens.

During the development of the medical device, the manufacturer aimed to obtain a product with astringent, re-epithelializing and protective properties intended to be used as an adjuvant in the treatment of gynecological diseases without microbial component and to promote the restoration of normal vaginal flora.

Cerviron® vaginal ovules has a content of substances that grants beneficial properties in the treatment of non-infectious vulvovaginitis and cervical erosions. The ovule melts in the vaginal mucosa forming a cream that ensures uniform dispersion of substances contained and acts as a protective barrier with astringent effect, favoring re-epithelialization of damaged tissue and restoration of the initial colpo-ecosystem without affecting the vaginal Döderlein bacilli and thus it creates favorable conditions for their multiplication while preserving the healthy vaginal microenvironment.

**Bismuth subgallate**

Bismuth subgallate is a yellow, odorless powder that fades when exposed to sunlight. Bismuth subgallate is a potent astringent agent, an action which can promote precipitation of some proteins and the formation of a protective film which lines the biopsied area in the time period immediate to the surgery or local trauma. Although the exact mechanism(s) of action by which bismuth compounds are able to cause such antimicrobial effects is not very well defined, a number of experimental observations suggest that bismuth forms a protective barrier that decreases the adhesion of bacteria to epithelial cells. his film can represent a physical protection, reducing the bacteria colonization and preventing the excessive formation of granulation tissue. (16,17) Its astringent, local hemostatic and healing properties recommend Cerviron® as a potential candidate for the treatment of gynecological conditions with an inflammatory component. Its strong astringent ability can facilitate both its deodorizing and hemostatic effects that support its use in a number of over-the -counter products such as hemorrhoidal suppositories or other topical applications.

Studies have shown that bismuth compounds such as bismuth subgallate are able to demonstrate antimicrobial effects against various pathogens such as *E. coli, Salmonella, Shigella, Vibrio cholera, Campylobacter jejuni, H. pylori* and some enteric viruses such as Rotaviruses. (18) Another advantage is its capacity to block the colonization of bacteria, preventing the reinfection and vaginitis recurrence, which occur very often. Bismuth subgallate promotes a high-quality scar formation, very important in women of childbearing age. Studies have shown that bismuth compounds such as bismuth subgallate are able to demonstrate antimicrobial effects against various pathogens such as *E. coli, Salmonella, Shigella, Vibrio cholera, Campylobacter jejuni, H. pylori* and some enteric viruses such as *Rotaviruses*.

Histometric measurements in studies of bismuth subgallate-treated wounds showed a larger area of ulceration of bismuth subgallate-treated wounds on the first day, which could be explained by the presence of bismuth subgallate that fills the wound to the tip and acts as a barrier to the initial contraction. This filling could be a factor that helps protect the wounds from trauma and bacteria. An interesting finding is that, as the test wounds healed, the bismuth subgallate was located deep in the tissues in a small amount. This did not significantly impede the normal healing of this tissue (connective tissue and epithelium). (19)

In the composition of Cerviron® vaginal ovules medical device, the Bismuth Subgallate powder is in the amount of 5% and it is used, due to its astringent properties, to create a physical barrier over the affected area of the vaginal mucosa and therefore to clock oxygen and pathogens to come in contact with it. In this way, the substance creates the premises that allow the damaged tissue to heal naturally and prevents the development of pathogenic bacteria by favoring the restoration of normal vaginal flora.

**Hexylresorcinol**

Hexylresorcinol has local anesthetic, antiseptic and anthelmintic properties.

On July 22, 1991, the US Food and Drug Administration (FDA) has published the Final monograph for first aid antimicrobial- antiseptic products where Hexylresorcinol was identified as 1$^{st}$ category antimicrobial ingredient. The antimicrobial resistance continues to be a universal concern with a huge impact on the therapeutic arsenal. Extended efforts were made to validate new agents for antimicrobial effect. For example, recent studies made by Chaudhuri et al. showed that Hexylresorcinol has DNA topoisomerase inhibition activity (IC50 30 pM), which is well comparable with m-Amsacrine (30 pM) and Purpurin (40 pM). (10) Chaudhuri concluded that Hexylresorcinol has an excellent antimicrobial activity. Specifically, Chaudhuri has performed an assessment to assess the value of the minimum HR inhibiting concentration (MIC in µg / ml) against various relevant organisms, according to the US Pharmacopeia Products Compendia for the 2$^{nd}$ category. (20)

Hexylresorcinol is used in the composition of the medical device Cerviron® vaginal ovules with a consistent role, to prevent the development of endogenous bacteria and possibly those that can reach inside the vagina when administering the medical device. The 0.1% concentration of hexylresorcinol in the medical device inhibits the development of endogenous and exogenous bacteria in the ovule during handling and administration. (21)

Nikolaev and colleagues also demonstrated that alkylresorcinols such as 4- Hexylresorcinol can be used as an adjuvant to increase the efficiency of several known antibiotics. The therapeutic approach where hexylresorcinol is associated with an antibiotic could minimize the risk for development of genetically determined antibiotic resistance and could be assumed that it reduces the episodes of recurrence due to antibiotic treatment failure. (22)

**Vegetable Collagen**

Collagen is the main construction element of living tissues, being a complex of amino acids with nutritive, hydrating, healing, trophic effect. As hydrolysate is easily soluble and absorbable, helping to revitalize the damaged areas and restoring the trophicity of the vaginal mucosa.

Collagen is structurally and functionally a key protein of the extracellular matrix which is also involved in forming the scars during the healing of conjunctive tissues. Many collagen bandages were developed to improve the repair of the wound, especially of non-infected, chronic, idle cutaneous ulcerations.

Collagen fibers form the extracellular framework of all tissues. In the early healing of wounds, type III collagen is established first, the type I proportion growing while the formation of the scar advances and is remodeled. The deposit and remodeling of collagen contributes to increasing the wound's traction resistance, which is approximately 20% at an interval between one to three weeks after the trauma and gradually reaching 70% of the regular skin. (23) Although the epithelial structures can heal through regeneration, the conjunctive tissues cannot depend also on the repair process, especially through the formation of collagenous scar tissue, mainly type I, which serves to restore the continuity, resistance and function of the tissues. (24) Collagen is a fragile substitute of the unwanted tissue, and the scar tissue rarely exceeds 70% of the power of the unwanted tissue.

In vitro, natural collagen can be formed into highly organized, three-dimensional scaffolds that are intrinsically biocompatible, biodegradable, nontoxic upon exogenous application, and endowed with high tensile strength. These attributes make collagen the material of choice for wound healing and tissue engineering applications. (25)

Plant collagen supports a moist environment, conducive to wound healing, encourages the deposition of new collagen fibers, supports the growth of new tissues and the formation of granulation tissue in the wound bed. A key component of chronic wounds is an increased level of matrix metalloproteinases (MMPs). At high levels, MMP not only degrades non-viable collagen, but also viable collagen. In addition, fibroblasts in a chronic wound cannot secrete tissue MMP inhibitors at an appropriate level to control MMP activity. These events prevent the formation of scaffolds necessary for cell migration and ultimately prevent the formation of extracellular matrix (ECM) and tissue granulation. Exogenous collagen action acts as a "sacrificial substrate" in the wound. Also, collagen degradation products have been shown to be chemotactic for a variety of cell types required for granulation tissue formation. (26)

Taking into account the data from the specialized literature, collagen, applied topically in the case of wounds, maintains the level of humidity at the level of the wound, favoring its healing. At the same time, the exogenous intake of collagen favors the degradation of excess MMP and elastase, which, in its absence, would degrade natural collagen, delaying the healing of the wound.

In the medical device Cerviron®, the hydrolysed vegetable collagen is in the amount of 0.75%.

**The thyme extract** (*Thymus vulgaris*)

The thyme extract (*Thymus vulgaris*) has a strong antifungal activity on the Gram-positive and Gram-negative bacteria. The activity is mainly attributed to thymol and carvacrol. (27) The extracts with a higher percentage of phenolic compounds show a higher inhibiting activity. The connections between the thymol concentration and the minimum concentration of bactericides suggest that the formation of the membrane forming perforations is the main manner of action of thymol against oral bacteria. (28,29)

Thymus vulgaris extract is used in the medical device Cerviron® ovules to prevent the development of bacteria and fungi that may come in contact with the vaginal mucosa during handling of the medical device. This substance acts as a preservative for the ovule.

Numerous scientific citations support the antimicrobial properties of thyme oil and rank it among the most powerful essential oils in this regard. (30) Its effectiveness has been attributed mainly to thymol and carvacrol, two phenolic compounds present in the essential oil of *T. vulgaris*, as well as other species of Lamiaceae mentioned under the common names of thyme and oregano. (31) Thymol

comprises the main component of herbal essential oil belonging to the so-called thymol chemotype of *T. vulgaris*, while a high thymol content is generally found in commercial thyme oil.

Thyme essential oil has shown a strong antibacterial action against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *E. coli* in formulary O/W as well as W/O. Data from the literature demonstrate an antifungal activity against the mycelia and spores of *Aspergillus flavus, Aspergillus niger* and *Aspergillus ochraceus*, at a minimum inhibitory concentration of 0.25 - 1 mg / ml. (32) The thyme oil presented high invitro activity, with values below 0.50mg/mL for almost all the microorganisms tested. (33)

The LC50 of thyme oil against mice was recorded as 7142.85 µl kg− 1 body weight indicating its non-mammalian toxicity and strengthening its safe exploitation as preservative. For industrial relevance, the thyme essential oil may be recommended for large scale application as a plant-based preservative because of its strong antifungal as well as antiaflatoxigenic efficacy. Because of its broad antimicrobial spectrum, more efficacy over prevalent synthetic preservatives as well as non-mammalian toxicity, the thyme essential oil may be formulated as a safe and economical plant-based preservative against fungal infestation and aflatoxin contamination. (34)

In the composition of Cerviron® vaginal ovules, *Thymus vulgaris* extract is found in the concentration of 0.5% and has a preservative role, in particular, to prevent attacks by exogenous germs during the administration of the medical device.

**The Goldenseal extract (*Hydrastis Canadensis*)**

The main constituents of the goldenseal root are isoquinoline alkaloids, such as hydrastine (1.5-4%), berberine (2.5%), canine (0.5%) and other alkaloids. Berberine is usually found in goldenseal roots as a sulphate at a concentration of 5000–60,000 ppm (35) Hydrastine is also found in goldenseal in concentrations of 15,000-40,000 ppm. Secondary metabolites include also sideroxylin, 8-desmethyl-sideroxillin and 6-desmethyl-sideroxylin. (36)

Hydrastis Canadensis powder has an astringent effect on the mucous membranes of the upper respiratory tract, the gastrointestinal tract, the bladder, the rectum, and the skin. Astringent herbs are used to reduce blood loss from the reproductive tract as well as the gastrointestinal tract, respiratory tract, and skin. The astringents most effective in uterine blood loss are often those that are high in tannins, although the tannins are most likely not the only constituent responsible.

The mechanism of such activities is complex and involves various cellular kinases and signaling pathways. Berberine has activity against various parasites such as tapeworm, viruses and candida (yeast) infection. Berberine is also known to stimulate the immune system. Rehman et al. have reported that, like Echinacea, Goldenseal has also immunomodulator effects, as shown by the growth of the primary immunoglobulin answer (IgM) against antigen in rats. The authors have reached the conclusion that Goldenseal extract improves the immune answer by increasing the production of immunoglobulin specific for the foreign object (antigen). (37)

In the composition of Cerviron® ovules medical device, the Goldenseal powder is found in the amount of 0.5%, used to promote the natural healing of damaged tissues and to reduce the irritative symptoms that manifest in non-infectious vulvovaginitis. It promotes the natural re-epithelialization processes.

**The Marigold extract** *(Calendula officinalis)*

The Marigold extract contains as active principles carotenoid substances, triterpenic compounds and volatile oil. These give a healing, antitricomonazic, reducing the excessive abnormal vaginal secretion (leucorrhea).

In the composition of Cerviron® ovules medical device, the Calendula officinalis extract is found in the amount of 0.5%, used to promote the natural healing of damaged tissues and to reduce the irritative symptoms that manifest in non-infectious vulvovaginitis. It promotes the natural re-epithelialization processes.

Calendula officinalis extract is traditionally used for the symptomatic treatment of minor inflammations of the skin and as an adjuvant in the healing of minor wounds. (38)

Calendula officinalis, belonging to the family of Asteraceae, commonly known as English Marigold or Pot Marigold is an aromatic herb which is traditionally used for treating wounds, ulcers, herpes, scars, skin damage, frostbite and blood purification. It is also used for gastrointestinal diseases, gynecological problems, eye diseases, skin injuries and some cases of burn. Calendula is used topically as suspension or tincture for treating acne, reducing inflammation, controlling bleeding, and soothing irritated tissue. This plant is rich in many pharmaceutical active ingredients like carotenoids, flavonoids, glycosides, steroids and sterols quinines, volatile oil, and amino acids. The medicinal use of Calendula flower preparations for the symptomatic treatment of minor inflammations of the skin or the oral mucosa, and as an aid in healing minor wounds is documented in a number of scientific references. (39,40)

**Turmenic extract** (*Curcuma longa*)

Turmeric is acquired from Curcuma longa L., a tuberous herbaceous perennial plant with yellow flowers and wide leaves, which is a member of ginger family and grows in tropical climate.

In the Cerviron ovules medical device, Turmeric extract is found in a concentration of 0.5%, used to promote the natural healing and reepithelization of damaged tissues and to reduce the irritating symptoms that occur in non-infectious vulvovaginitis.

Overall, there is early evidence that turmeric/curcumin products and supplements, both oral and topical, may provide therapeutic benefits for skin health. However, currently published studies are limited, and further studies will be essential to better evaluate efficacy and the mechanisms involved. (41–43)

The curcumin found in turmeric can help wounds heal by decreasing inflammation and oxidation. It also lowers the response of the body to cutaneous wounds. This results in in acceleration of the wound healing process. Studies have found that turmeric and collagen can positively impact different biological processes relevant to wound healing.

Curcumin acts on different stages of the natural wound healing process to speed healing. Studies provide evidence of the ability of curcumin to reduce the body's natural response to skin wounds, such as inflammation and oxidation. (44) Recent literature on the healing properties of curcumin also provides evidence of its ability to enhance granulation tissue formation, collagen deposition, tissue remodeling, and wound contraction. Curcumin has been shown to have strong modular effects on wound healing. Studies have shown that curcumin does this by acting on the inflammatory,

proliferative and remodeling phases of the wound healing process and therefore reduces the time required to heal wounds. (45)

In traditional medicine, Turmeric is applied topically for the treatment of acne, wounds, burns, bruises, ulcers, eczema, insect bites, parasitic infections, bleeding and skin diseases such as herpes zoster and pemphigus. It is used as a paste or ointment (mixed with oil or other substances), in the form of a tincture or extract. The healing effects of Curcuma longa extract have been studied in rabbits. The group treated with Curcuma longa showed a significantly higher mean value for wound contraction compared to the controls. Furthermore, the wounds showed lower inflammation and an increasing tendency in collagen formation.

**Synthetic amorphous silica**

Synthetic amorphous silica (SAS), in the form of pyrogenic (fumed), precipitated, gel or colloidal SAS, has been used in a wide variety of industrial and consumer applications including food, cosmetics and pharmaceutical products for many decades. (46) There are no relevant clinical trials and thus the pharmacological, immunological or metabolic effects of colloidal silicon dioxide cannot be confirmed. In the compositon of Cerviron® ovules ovules, this substance is used as a stabilizing agent.

**Lanolin**

Lanolin is listed in the CosIng database of the European Commission with the function of antistatic, hair and skin conditioning (emollient), surfactant cleansing and surfactant emulsifying. Lanolin is antistatic, hair and skin conditioning (emollient), surfactant cleansing and surfactant emulsifying. No relevant in vitro and in vivo studies and clinical trials for the assessment of pharmacological, immunological or metabolic activities are available. For this reason, pharmacological, immunological or metabolic effects cannot be confirmed for anhydrous lanolin. In the composition of Cerviron® ovules, this substance is used for the dispersion of the substances in the fat base.

**Semi-synthetic glycerides**

As a base ingredient the semi-synthetic glycerides are used, a hard fat composed of mono-, di- and triglyceride esters of fatty acids (C10 to C18). Suppocire has been approved as a non-toxic and non-irritating ingredient in medicinal products. It provides good dispersion and compatibility properties for ingredients in the suppository mass as well as softening and diffusion properties for efficient and uniform release of the ingredients (https://www.gattefosse.com/pharmaceuticals-products/suppocire-nas-50). In a study investigating the suppository parameters breaking hardness, disintegration time and release properties of ingredients in vaginal suppositories, Suppocire NA was found to be the best lipophilic base in comparison to similar products. (47)

For detailed investigational device information, please refer to **Investigator's Brochure** version **1.0** dated **04 February 2021**.

### 3.1.3 PREVIOUS INTENDED USES OR INDICATIONS FOR USE, IF RELEVANT

Not applicable.

### 3.1.4 ANY CHANGES TO THE INVESTIGATIONAL DEVICE DURING THE CLINICAL INVESTIGATION OR ANY CHANGES FROM THE IB

Not applicable.

## 3.2 CLINICAL INVESTIGATION PLAN (CIP)

During the clinical investigation CIP version 1.0 dated 06 January 2021 was used. The CIP was drafted according to ISO 14155:2020. No amendments were performed to this version. A copy of the clinical investigation plan is available by request.

### 3.2.1 THE CLINICAL INVESTIGATION OBJECTIVES

**Primary Objectives**

The primary objective was to assess the therapeutic performance and tolerability of Cerviron® Ovules in participants with symptomatic, non-specific, non-infectious vaginitis, and endogenous symptomatic infections.

**Secondary Objectives**

The secondary objective of this clinical investigation was the assessment of performance of the medical device by several additionally clinical outcomes (vaginal discharge, vaginal pH, microscopic characteristics of inflammatory cells and characteristics of vaginal microflora).

Participants also evaluated the degree of satisfaction related to the use of the medical device.

### 3.2.2 THE CLINICAL INVESTIGATION DESIGN

This present clinical investigation was a non-randomized intervention, single-group assignment, open label with the primary purpose of treatment.

Since Cerviron® vaginal ovules has an innovative composition, was preferred an exploratory approach for the design of the present clinical investigation. The main objectives and clinical endpoints were the performance and the safety profile of the investigational medical device.

Fifty (50) participants were recruited during this clinical investigation.

**The primary endpoints were:**

1 Clinical Performance Assessed by the Investigator after thorough gynaecological examination [Time Frame: 3 months]

Success was defined by resolution (return to participant's usual gynecological conditions, i.e. before the episode which warranted inclusion in the study) OR substantial improvement of clinical signs of infectious vaginitis (i.e. abnormal vaginal discharge), and/or vaginal symptoms (vaginal burning and/or vaginal pain, and/or vaginal irritation and/or pruritus and/or odour).

Failure was defined by persistence or worsening of symptoms and clinical signs or requirement of an alternative or specific treatment.

2 Rate of treatment-related Adverse Events in subjects participating in the clinical investigation [Time Frame: 3 months]

The safety of CERVIRON® as measured by the rate of treatment-related adverse events, including serious adverse events (SAEs), in subjects participating in the clinical investigation.

**The secondary endpoints were:**

1 Change in Vaginal Discharge Aspect during Clinical Exam, Assessed by the Investigator [Time Frame: at 1, 2 and 3 months]

The vaginal discharge was assessed by the treating physician using a score between 0 and 4, as follows:

0=absent

1=mild: insufficient for speculum collection

2=moderate: sufficient for speculum collection

3=abundant: visible at the introitus even before speculum introduction

4=purulent, abnormal discharge: visible at the introitus even before speculum introduction

2 Change in vaginal pH values [Time Frame: at 1, 2 and 3 months]

The determination of pH values was performed using the Veneris pH Tests for vaginal infections.

3 Change in vaginal microflora and Lactobacilli count [Time Frame: 3 months]

The change in Lactobacillus species was shown through laboratory microscopy.

4 Change in vaginal inflammation [Time Frame: 3 months]

The change in inflammatory and parabasal cells was performed through laboratory microscopy.

5 Participant Satisfaction (Likert Scale) [Time Frame: 3 months]

The degree of satisfaction when using the medical device was assessed using a five-point Likert Scale.

### 3.2.3 THE ETHICAL CONSIDERATIONS

The present clinical investigation was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki.

These principles protect the rights, safety and well-being of human subjects, which are the most important considerations and shall prevail over interests of science and society. These principles were understood, observed, and applied at every step in the clinical investigation by the Investigator, the Investigator's nominated staff, the Sponsor personnel and the Contract Research Organization personnel.

All parties involved in the conduct of the clinical investigation shared the responsibility for its ethical conduct in accordance with their respective roles in the clinical investigation.

The present clinical investigation was conducted in accordance with the provisions of the International Standard ISO 14155:2020 Clinical Investigation of medical devices for human subjects - Good Clinical Practice. These principles address Good Clinical Practice for the design, conduct, recording and reporting of clinical investigations carried out in human subjects to assess the safety and performance of medical devices. These principles were understood, observed, and applied at every step in the clinical investigation.

Prior to commencing the clinical investigation, the Sponsor obtained documentation of the EC's approval/favorable opinion. The present clinical investigation did not begin until the required approval/favorable opinion from the EC or regulatory authority have been obtained.

### 3.2.4 THE DATA QUALITY ASSURANCE

The collected data was subjected to multiple checks for correctness (entry errors). Every information has been verified by an SDV (Source Data Verification) process, performed by the study monitors. Data clarification queries have been implemented to obtain clean data.

All data collected through the data management system, by eCRFs, were checked for completeness and extreme values (outlier) presence. The data was collected using the electronic platform OpenClinica. OpenClinica is compliant with FDA and EMA regulations, including 21 CFR Part 11, HIPAA, GDPR, ICH-GCP), as well as with data practices and security standards (ISO, SOC). Any anomalies found were forwarded by the study monitor to the Investigator. Such data queries were solved with priority, in no more than 3 days from the query's opening. It was the statistician's decision to use/accept the revised data for the subsequent statistical analyses.

Data for the present clinical investigation is stored according to Annex E of ISO 14155:2020.

All statistical analyses were performed using the R statistical software (version 4.1.1) with a few extra statistical packages added, all of which have been revised and updated to the latest version. The final analysis was completed after all subjects have finalized the study, all queries have been resolved, and the database has been locked.

The overall type I error will be preserved at 5%. Data from unscheduled visits was not included in the analysis.

Statistical analyses were conducted on all subjects who have successfully completed the study without a CIP deviation that is regarded as impacting the assessment of the key variables (as per CIP).
Quantitative variables (i.e., demographic) if normally distributed were described through mean ± Standard Deviation (SD), otherwise median, minimum, maximum, and interquartile range were showed. Qualitative variables were evaluated using frequencies and percentages.

To evaluate changes of proportions over time before and after the treatment, for categorical variables two-proportions z test were performed.

The quality and completeness of the collected data were evaluated preliminary compared to data analysis. No study participant has been involved in violation of inclusion/exclusion criteria.

### 3.2.5 THE PARTICIPANT POPULATION

The target population for this clinical investigation was comprised of females suffering from symptomatic vulvovaginitis, either non-specific or caused by an endogenous imbalance in the vaginal microflora, with abnormal vaginal secretion and with characteristics signs and symptoms, such as pruritus, erythema, burning, pain, odor, dysuria and dyspareunia.

Vulnerable subjects were not enrolled in this clinical investigation.

### 3.2.5.1 INCLUSION/EXCLUSION CRITERIA

#### 3.2.5.1.1 Inclusion criteria for subject selection

The inclusion criteria have been verified at the screening visit (day -3). Each potential subject had to satisfy all the following criteria before being enrolled in the clinical investigation:

- Adult females, aged 18 years to 65 years;
- Subjects presenting two or more vaginal symptoms such as leucorrhoea, pruritus, burning sensation, erythema, pain, odour, dysuria, or dyspareunia;
- Subjects with a diagnosis of either non-infectious vaginitis, or endogenous, symptomatic infection;
- Negative for Gardnerella vaginalis, Candida albicans, Trichomonas vaginalis;
- Subjects willing to provide signed informed consent to clinical investigation participation.

#### 3.2.5.1.2 Exclusion criteria for subject selection

The exclusion criteria have been verified at the screening visit (day -3). Subjects fulfilling one or more of the following exclusion criteria have not been included in the clinical investigation:

- Subjects in menstrual period or suffering from menorrhagia;
- Colpectomy;
- Subjects with undiagnosed abnormal genital bleeding;
- Subject with vulvar, vaginal or cervical cancer;
- Known, active sexually transmitted infection (STI) in partner, as per anamnesis;
- Subjects with HIV or other immunodeficiency;
- Subjects with any pathology of the female reproductive organs;
- Known allergy or hypersensitivity to the medical device ingredients;
- Subjects using spermicides;
- Subjects using diaphragm;
- Concomitant topical or systemic anti-infective treatment;
- Unable to comply with visit procedures;
- Subjects included in other clinical investigations.

### 3.2.6 THE TREATMENT AND TREATMENT ALLOCATION SCHEDULE

This present clinical investigation was a non-randomized intervention, open label, single group assignment with the primary purpose of treatment.

Cerviron vaginal ovules ® is an adjuvant treatment for acute and chronic vulvovaginitis of mechanical etiology, caused by changes of vaginal pH and/or changes of the vaginal microflora. It favours the healing and re-epithelialization processes and reduces the proliferation of endogenous pathogens.

**Composition per ovule**

Cerviron® is composed of: Bismuth subgallate 100 mg, hydrolyzed Collagen 15 mg, Thyme extract (Thymus vulgaris) 10 mg, Goldenseal extract (Hydrastis canadensis) 10 mg, Marigold extract (Calendula officinalis) 10 mg, Turmeric extract (Curcuma longa) 10 mg, and Hexylresorcinol 2 mg.

After the final eligibility check performed by the treating physician (after the collection of the vaginal swabs) the participant was instructed regarding the medical device usage.

As per Instructions for use, the recommended dose prescribed was 1 Cerviron® ovule inserted vaginally with a total number of 15 days. The investigational device, the instructions for use and the primary and secondary packaging were designed following the requirements of national and EU regulations and indicated that the investigational device is intended exclusively for use in the present clinical investigation. Accountability forms were kept separately and were reported by Investigator for each participant at each study visit.

Each participant followed a treatment schema of 3 months (a total of 45 days of treatment).

In case of pregnancy, in general, no specific cautions are necessary. Due to insufficient data regarding the using of the medical device during pregnancy, a specialist should be consulted in case of administration to pregnant women.

There were no cases of pregnancy during our investigation.

### 3.2.7 ANY CONCOMITANT MEDICATIONS AND TREATMENTS

Vaginal tampons usage was prohibited during the clinical investigation because tampons can absorb some of the active substances and as such, they can prevent the ovule from exhibiting its full performance.

Also, a list of restricted concomitant medications was defined in CIP Attachment (Prohibited Concomitant Medication and Treatments) for the purpose of distinguishing between the efficacy of the medical device in questions and other therapies that can exert the same therapeutic effect. The prohibited concomitant medication list is shown below:

1. *Vaginal creams, ointments, lubricants;*
2. *Use of other anti-infectious treatments, either systemic or local;*
3. *Use of hydrocortisone cream or other local, anti-inflamatory therapy;*
4. *Vaginal tampons;*
5. *Use of an etonogestrel/ethinyl estradiol vaginal ring (Nuvaring® 152 ) or other intrauterine device;*
6. *Oral or vaginal antibiotic therapy or other vaginal therapies (like douching, spermicide);*
7. *Oral or vaginal probiotics (e.g. vaginal lactobacilli).*

Without these exceptions in terms of concomitant medications, the treating physician was free to add, withdraw, or alter doses of any kind of medication at his/her own discretion based on standard medical practice. All concomitant medication and treatments were recorded in the appropriate study documents (the eCRF and source data).

### 3.2.8 DURATION OF FOLLOW-UP

The clinical investigation included subjects under treatment with Cerviron® vaginal ovules for approximately 3 months.

Each enrolled subject performed 4 visits. The screening visit (SV) took place with 3-0 days before the baseline visit (day 0). On day 0 the subjects received the medical device.

The study participants had 4 visits at the investigational site, at 30 days interval each. The study visit flow-chart is listed in Figure 2.

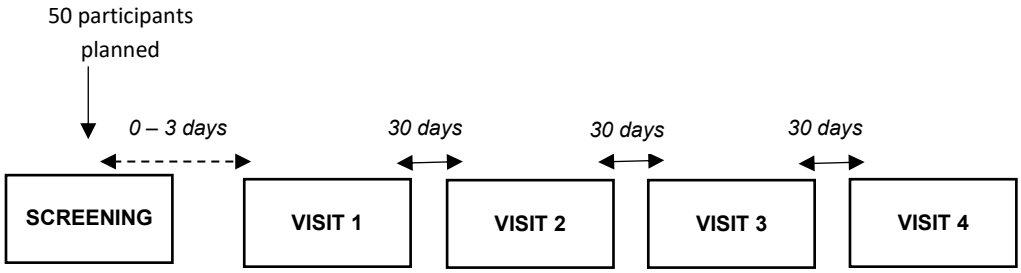


**Figure 2. Study Flow Chart**

### 3.2.8.1 THE STATISTICAL ANALYSIS

Quantitative variables (i.e., demographic) if normally distributed were described through mean ± Standard Deviation (SD), otherwise median, minimum, maximum, and interquartile range were showed. Qualitative variables were evaluated using frequencies and percentages. To evaluate changes of proportions over time before and after the treatment, for categorical variables a two-proportions z test were performed.

The quality and completeness of the collected data were evaluated preliminary compared to data analysis.

No participant subject has been involved in violation of inclusion/exclusion criteria.

Statistical analysis and generation of tables, figures, and patient data listings were performed using R statistical software version 4.1.1 according to the standard operating procedures used during the clinical investigation performance (SOPs).

All variables were analyzed in an explorative manner using descriptive statistics only. Summary statistics consisted of frequency tables for categorical variables. For continuous variables, descriptive statistics (number of available observations, mean and median, standard deviation [SD], minimum, and maximum) were tabulated unless specified otherwise.

### 3.2.8.2 THE CLINICAL INVESTIGATION HYPOTHESIS OR PASS/FAIL CRITERIA

The primary hypothesis is that, after 3 months after using the medical device, the clinical symptoms will be improved by 100.00% of the treated participants (50 out of 50 evaluable subjects). As secondary hypotheses we assume that several additionally clinical outcomes will be improved with 100.00% of the participants using the medical device.

The intended performance that will be verified during this clinical investigation is the medical device's effect in symptomatic, non-specific, non-infectious vaginitis, and endogenous symptomatic infections treatment.

### 3.2.8.3 SAMPLE SIZE CALCULATION

During September 2020 - January 2021, we performed a review of the scientific literature to find similar investigations to determine the minimal clinically difference between Baseline and a similar period of treatment of 90 days. In a similar study, conducted by Murina, 44 samples were collected to achieve a power of 80% and one-sided significance of 5% for detecting a difference of 0.25 between marginal proportions. (48)

To confirm the sample size in the Murina study, we decided to repeat the calculation, and according to Cohen's (1988) calculation formula, considering a drop-out rate of about 10%, we estimated a representative sample around 50 participants.

#### 3.2.8.4 STATISTICAL ANALYSIS METHODS

All statistical analyses were performed using the R statistical software (version 4.1.1) with a few extra statistical packages added, all of which have been revised and updated to the latest version. The final analysis was completed after all subjects have finalized the study, all queries have been resolved, and the database has been locked.

The overall type I error was preserved at 5%. Statistical analyses were conducted on all subjects who have successfully completed the study without a CIP deviation that is regarded as impacting the assessment of the key variables (as per CIP).

## 4. RESULTS

### 4.1 CLINICAL INVESTIGATION INITIATION/CONCLUSION AND SUBJECTS BASELINE CHARACTERISTICS

The Clinical Investigation Initiation was performed during a Site Initiation Visit conducted at each of the selected sites approved by the National Ethics Committee. The sites and the Principal Investigators are listed below:

| | |
|---|---|
| **Name:** | **Associated Professor Daniela-Oana Toader, MD** |
| **Centre:** | Clinical Investigational Site 01 |
| | Institutul National pentru Sanatatea Mamei si Copilului “Alessandrescu-Rusescu” Obstetrics-Gynecology “Polizu” |
| | Department of Obstetrics-Gynecology III |
| | 38-52 Gheorghe Polizu Street, District 1 |
| | 011061 Bucharest, Romania |
| **Name:** | **Associated Professor Izabella Petre, MD** |
| Centre: | Clinical Investigational Site 02 |
| | Spitalul Clinic Judetean de Urgenta “Pius Brinzeu” Timisoara |
| | Department of Obstetrics-Gynecology II |
| | 12 Victor Babes Boulevard |
| | 300226 Timisoara, Romania |

### 4.2 CLINICAL INVESTIGATION CLOSE-OUT

The Clinical Investigation completion was performed on March 3rd, 2022, once all the data of all participants enrolled were collected, checked for correction and the database lock was done.

### 4.3 DISPOSITION OF SUBJECTS

A total number of 50 subjects were screened and 47 participants were considered eligible for the completion of this study. The 47 fully met the inclusion and exclusion criteria, signed the Inform Consent, and performed the Baseline visit.

Out of 50 subjects enrolled in the clinical study, three subjects did not meet the inclusion and exclusion criteria, as a result they were not included in the clinical trial. All 47 participants completed all visits.

The safety population included the 47 subjects that took the treatment; more precisely 39 subjects had a 100% compliance, 6 subjects had a 75% compliance, and 2 participants had a 50% compliance.

The ITT population included 47 subjects that received the treatment of the study medical device.

The Per Protocol Set population, subjects who completed all the visits without major protocol deviations, was 47 subjects. The distribution of participants on the two sites is listed in Table 3.

| Site Number | No of screened participants | No of enrolled participants | First participant IN | First participant OUT | Last participant IN | Last participant OUT |
|---|---|---|---|---|---|---|
| Site 01 | 15 | 15 | 15.07.2021 | 25.09.2021 | 01.09.2021 | 28.11.2021 |
| Site 02 | 35 | 32 | 27.04.2021 | 10.07.2021 | 27.11.2021 | 28.02.2022 |

**Table 3. The distribution of study participants.**

### 4.4 DEMOGRAPHY AND OTHER BASELINE CHARACTERISTICS

The following table 4 shows the demography of the study participants.

| Baseline characteristics | Experimental | |
|---|---|---|
| | *n* | % |
| Age (years): mean [range] | 47 | 41.23 [22 – 64] |
| Gender | | |
| Female | 47 | 100.00 |
| Male | 0 | 0.00 |
| Ethnicity | | |
| Caucasian | 47 | 100.00 |
| Childbirth potential | | |
| Yes | 36 | 76.60 |
| No | 11 | 23.40 |

| Baseline characteristics | Experimental | |
|---|---|---|
| | *n* | % |
| Subject in menopause | | |
| Yes | 9 | 19.15 |
| No | 38 | 80.85 |
| Subject pregnant | | |
| Yes | 0 | 0.00 |
| No | 47 | 100.00 |
| History of cancer | | |
| Yes | 2 | 4.26 |
| No | 45 | 95.74 |
| Sex life | | |
| Yes | 44 | 93.62 |
| No | 3 | 6.38 |
| Reproductive life | | |
| Yes | 25 | 53.19 |
| No | 22 | 46.81 |
| Tobacco consumption | | |
| Yes | 20 | 42.55 |
| No | 27 | 57.45 |
| Alcohol consumption | | |
| Yes | 0 | 0.00 |
| No | 47 | 100.00 |
| Physical activity | | |
| Yes | 32 | 68.09 |
| No | 15 | 31.91 |
| Rest/Day | | |
| Yes | 34 | 72.34 |
| No | 13 | 27.66 |
| Hydration | | |
| Yes | 36 | 76.60 |
| No | 11 | 23.40 |

**Table 4. Demographic data of all study participants**

### 4.5 CLINICAL INVESTIGATION PLAN COMPLIANCE

During the study period no major protocol deviation and/or issue were reported regarding the clinical investigation plan.

### 4.6. DATA QUALITY ASSURANCE

The collected data was subjected to multiple checks for correctness (entry errors). Every information was verified by an SDV process, performed by the study monitors. Data Clarification Forms were implemented to obtain clean data.

The database lock was performed after the study's statistician approved all the data for correctness and completeness.

### 4.7 PRIMARY OUTCOMES

The following primary outcomes were collected in this study: Clinical Performance Assesses by the Investigator After Thorough Gynecological Examination and Participant's Interview at End of Treatment Visit, after 3 months.

Success was defined by resolution (return to participant's usual gynecological conditions, i.e., before the episode which warranted inclusion in the study) or substantial improvement of clinical signs of infections vaginitis (i.e., abnormal vaginal discharge), and/or vaginal symptoms (vaginal burning and/or vaginal pain, and/or vaginal irritation and/or pruritus and/or odor). As per CIP, failure was defined by persistence or worsening of symptoms and clinical signs or requirement of an alternative or specific treatment.

The Presence of Vaginal Symptoms Score Change between Baseline and Final Visit is detailed in Table 5.

| Baseline score | Visits | |
|---|---|---|
| | Baseline | Final |
| Yes Answer | 47 | 13 |
| No Answer | 0 | 34 |
| **Total number of subjects** | **47** | **47** |

**Table 5. The Presence of Vaginal Symptoms score change between baseline and final visit.**

The primary hypothesis is that, after 3 months after using the medical device, the clinical symptoms will be improved by 100.00% of the treated participants.

For 34 out of 47 participants (72.34%) treated with Cerviron©, the medical device had a beneficial effect on the score of Vaginal Symptoms, while for only 13 participants (27.66%) the score remained the same. Performing a test for equality of proportions with continuity correction, at 5% significance level, and considering a null hypothesis that is no statistically significant difference between baseline visit and final visit, we evidenced that the differences between these values were statistically significant (*$p < 0.001$, 95% CI [57.42% - 87.25%]*).

A very important aspect of Cerviron© treatment is that it reduces the symptoms of vaginitis after 30 days. Thus, for 9 out of 47 patients (19.15%) who were treated, the medical device had a beneficial effect on the score of Vaginal Symptoms (*p < 0.05, 95% CI [5.77% - 32.52%]*) measured at Visit 2 (30 days). This improvement in vaginal score is much more evident at 60 days when 25 out of 47 patients (53.19%) showed an improvement in symptoms (*p < 0.001, 95% CI [36.80% - 69.58%]*).

Even if between Visit 3 (60 days) and Visit 4 (90 days) there is a reduction of the score of 19.5%, more precisely the score improves for 9 patients, this improvement is not statistically significant (*p = 0.081*).

The effect of Vaginal Symptoms changes between visits for Cerviron© is graphically presented in Figure 3.

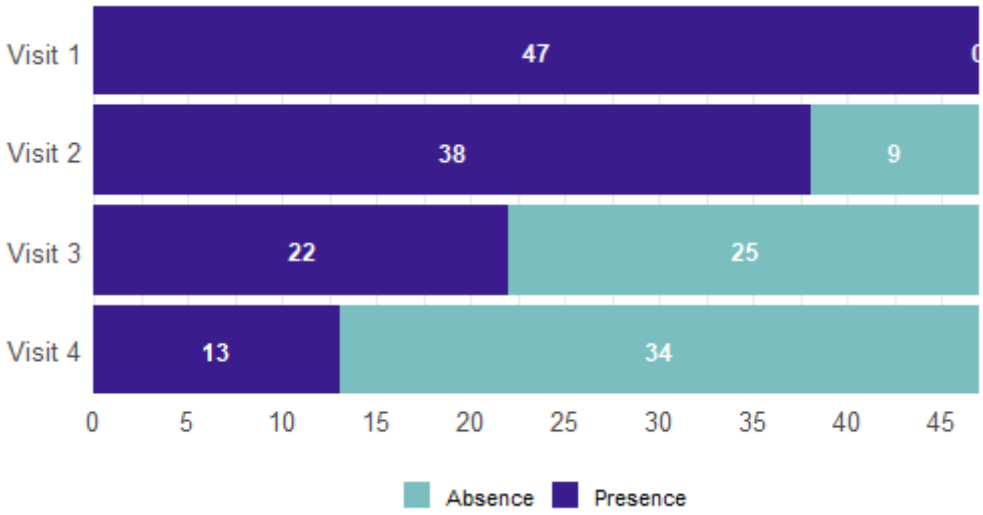


**Figure 3. Change from baseline for Vaginal Symptoms – comparison between Visits**

The variables collected by Investigators were the Vaginal Symptoms and the vaginal swab results. The Investigators collected through Patient's interview the presence or absence of the following symptoms: leucorrhea, pruritus, burn, rash, pain, odour, dysuria, dyspareunia. The vaginal swab results were delivered by the Bioclinica central laboratory. *(*Table 6*)*.

| Vaginal Symptoms | Visit 1 | | Visit 2 | | Visit 3 | | Visit 4 | |
|---|---|---|---|---|---|---|---|---|
| | *YES* | *NO* | *YES* | *NO* | *YES* | *NO* | *YES* | *NO* |
| Leucorrhea | 45 (93.62%) | 2 (4.26%) | 26 (55.32% | 21 (44.68) | 16 (34.04%) | 31 (65.96% | 12 (25.53%) | 35 (74.47%) |
| Pruritus | 8 (17.02%) | 39 (82.98%) | 44 (93.62% | 3 (6.38%) | 1 (2.13%) | 46 (97.87%) | 1 (2.13%) | 46 (97.87%) |
| Burn | 6 (12.77%) | 41 (87.23%) | 1 (2.13%) | 46 (97.87%) | 0 (0.00%) | 47 (100.00%) | 0 (0.00%) | 47 (100.00%) |
| Rash | 9 (19.15%) | 38 (80.85%) | 2 (4.26%) | 45 (95.74%) | 0 (0.00%) | 47 (100.00%) | 0 (0.00%) | 47 (100.00%) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Pain | 24 (51.06%) | 23 (48.94% | 3 (6.38%) | 44 (93.62%) | 1 (2.13%) | 46 (97.87%) | 0 (0.00%) | 47 (100.00%) |
| Bad smell | 13 (27.66%) | 34 (72.34%) | 7 (14.89%) | 40 (85.11%) | 3 (6.38%) | 44 (93.62%) | 3 (6.38%) | 44 (93.62%) |
| Dysuria | 8 (17.02%) | 39 (82.98%) | 4 (8.51%) | 43 (91.49%) | 5 (10.64%) | 42 (89.36%) | 0 (0.00%) | 47 (100.00%) |
| Dyspareunia | 15 (31.91%) | 32 (68.09%) | 9 (19.15%) | 38 (80.85%) | 3 (6.38%) | 44 (93.62%) | 1 (2.13%) | 46 (97.87%) |
| Vaginal swab results* | 45 (95.74%) | 2 (4.26%) | 45 (95.74%) | 2 (4.26%) | 40 (85.11%) | 7 (14.89%) | 42 (89.36%) | 5 (10.64%) |

* *1 – Normal Vaginal Swab Results, 0 – Pathological Vaginal Swab Results*

**Table 6. The Presence or absence of symptoms related to Vaginal Symptoms – comparison between Visits**

At the same time, the effectiveness of Cerviron© on each symptom related to the vaginitis was studied separately. Thus, it was observed that treatment for 90 days with Cerviron© significantly reduced the following symptoms: Leucorrhea *(p <0.001)*, Pruritus *(p < 0.05)*, Burn *(p < 0.05)*, Rash *(p < 0.05)*, Pain *(p < 0.001)*, Bad smell *(p < 0.05)*, Dysuria *(p < 0.05)*, and Dyspareunia *(p < 0.001)*.

In Figure 4 the evolution of each symptom from Baseline, and throughout all the study visits is represented.


**Figure 4. Vaginal Symptoms and Vaginal swab results – comparison between visits**

### 4.8 AE, SAES

During the study conduct, safety was assessed at every visit. Three study participants (with study codes 0203, 0213 and 0224) were reported with mild, grade-1 vaginal infection. Study participant no. 0203 experienced a Streptococcus infection while study participants 0213 and 0224 experienced E. Coli

infection that resolved over time with oral antibiotics and the treatment with Cerviron© was continued, as per the treating physician recommendation.

At the end of study, no treatment-related AEs or SAEs were reported.

### 4.8.1 DEATHS, OTHER SERIOUS ADVERSE EVENTS, AND OTHER SIGNIFICANT ADVERSE EVENTS

#### 4.8.1.1 DEATHS

No deaths occurred during the clinical investigation.

#### 4.8.1.2 OTHER SERIOUS ADVERSE EVENTS

No serious adverse events (SAEs) occurred during the clinical investigation.

#### 4.8.1.3 OTHER SIGNIFICANT ADVERSE EVENTS

No significant adverse events (AEs) were reported during this clinical investigation.

#### 4.8.1.4 NARRATIVES OF DEATHS, OTHER SERIOUS ADVERSE EVENTS, AND OTHER SIGNIFICANT ADVERSE EVENTS

Not applicable.

#### 4.8.1.5 ANALYSIS AND DISCUSSION OF DEATHS, OTHER SERIOUS ADVERSE EVENTS, AND OTHER SIGNIFICANT ADVERSE EVENTS

No deaths or significant AEs were reported during the present clinical investigation.

#### 4.8.1.6 CLINICAL LABORATORY EVALUATION

The Clinical Laboratory evaluation was performed in the Hospital Laboratory for site 01 while a private practice was preferred for site 02.

#### 4.8.1.7 VITAL SIGNS, PHYSICAL FINDINGS AND OTHER OBSERVATION RELATED TO SAFETY

No other observation related to safety was reported during the present clinical investigation.

#### 4.8.1.8 ANALYSIS OF LOCAL AND SYSTEMIC REACTIONS

No systemic reactions were reported during the present clinical investigation.

#### 4.8.1.9 SAFETY CONCLUSION

The safety analysis set included all study participants who were exposed to Cerviron© (N=47).

The intravaginal administration of Cerviron© in participants with symptomatic, non-specific, non-infectious vaginitis, and endogenous symptomatic infections proved safe for the study participants and not accompanied by any significant adverse events.

At the end of study, no treatment-related AEs were reported.

Cerviron© vaginal ovules exert a very good tolerability with the vaginal mucosa and vaginal epithelium. Cerviron© may be a better choice also for pregnant women with aerobic vaginitis, under the supervision of the gynecologist. Future research is needed to confirm its tolerability profile during pregnancy.

### 4.9 SECONDARY OUTCOMES

As secondary hypotheses we assume that several additionally clinical outcomes will be improved with 100.00% of the participants using the medical device.

#### 4.9.1 CHANGE IN VAGINAL DISCHARGE ASPECT

The vaginal discharge was assessed by the treating physician using a score as follows: absent, mild: (insufficient for speculum collection), moderate (sufficient for speculum collection), abundant (visible at the introitus even before speculum introduction), purulent discharge (visible at the introitus even before speculum introduction but with a purulent aspect). The change in vaginal discharge aspect during Visit 1-Visit 4 is reflected in Table 7.

| Change in Vaginal Discharge Aspect | Visit 1 | Visit 2 | Visit 3 | Visit4 |
|---|---|---|---|---|
| Absent | 0 (0.00%) | 2 (4.26%) | 8 (17.02%) | 9 (19.15%) |
| Mild | 6 (12.77%) | 26 (55.32%) | 21 (44.68%) | 13 (27.66%) |
| Moderate | 37 (78.22%) | 19 (40.43%) | 18 (38.30%) | 25 (53.19%) |
| Abundant | 4 (8.51% | 0 (0.00%) | 0 (0.00%) | 0 (0.00%) |
| Purulent | 0 (0.00%) | 0 (0.00%) | 0 (0.00%) | 0 (0.00%) |

**Table 7. Change in Vaginal Discharge Aspect score between each visit**

Due to the small proportions of some visits, such as Visit 1 for Absent (0.00%), Abundant (8.51%) and Purulent (0.00%) symptoms, and finally (visit 4), for Abundant (0.00%) and Purulent (0.00%) symptoms, to highlight the difference between baseline and final visit, data reduction was used, thus, neglecting the small and statistically insignificant proportions. Thus, for the participants, the categories of symptoms were reconsidered in Table 8, as follows:

1. Mild symptoms. Participants with Absent and Mild symptoms were included in a new category called Mild;
2. Moderate symptoms. Participants with Moderate and Abundant symptoms have been included in a new category called Moderate;
3. Purulent symptoms, even if not found, were left in the same way.

| Vaginal Discharge Aspect score | Visits | |
|---|---|---|
| | Baseline | Final |
| Mild symptoms | 6 | 21 |
| Moderate symptoms | 41 | 20 |
| Purulent symptoms | 0 | 0 |
| ***Total number of subjects*** | 47 | 47 |

**Table 8. Change in Vaginal Discharge Aspect score between visit 1 and visit 4**

McNemar's Chi-squared test with continuity correction was performed to assess the status-quo of the Vaginal Discharge Aspect score between the baseline and final visit (Visit 4). At a 5% significance level and considering a null hypothesis that is no statistically significant difference between baseline visit and final visit, we evidenced that the differences between these values were statistically significant ($p < 0.05$).

### 4.9.2 CHANGE IN VAGINAL PH VALUES

The determination of pH values between Visits 1-Visit 4 is showed in Table 9.

| Change in vaginal pH values | Visit 1 | Visit 2 | Visit 3 | Visit4 |
|---|---|---|---|---|
| Normal | 38 (80.85%) | 41 (87.23%) | 44 (93.62% | 47 (100.00%) |
| Abnormal | 9 (19.15%) | 6 (12.77%) | 3 (6.38%) | 0 (0.00%) |

**Table 9. Change in Vaginal pH Values between visit 1 and visit 4**

Performing test for equality of proportions without continuity correction to assess the change of the Vaginal pH values between the baseline and final visit (Visit 4). At a 5% significance level and considering a null hypothesis that is no statistically significant difference between baseline visit and final visit, we evidenced that the differences between these values were statistically significant ($p < 0.05$). There are limitations in performing the proportionality test because the proportion of participants with an abnormal pH is small (9 participants). It is observed that after the 3 months of treatment with Cerviron© all participants presented a normal pH.

### 4.9.3 CHANGE IN VAGINAL MICROFLORA AND LACTOBACILLI COUNT

The change in Lactobacillus species as shown by microscopy is presented in Table 10.

| **Vaginal microflora** | **Visit 1** | | **Visit 2** | | **Visit 3** | | **Visit 4** | | ***p*-value** |
|---|---|---|---|---|---|---|---|---|---|
| | *YES* | *NO* | *YES* | *NO* | *YES* | *NO* | *YES* | *NO* | |
| Lactobacilli | 25 (53.19%) | 22 (46.81%) | 27 (57.45%) | 20 (42.55%) | 27 (57.45%) | 20 (42.55%) | 30 (63.83%) | 17 (36.17%) | *= .131** |
| Leukocytes | 37 (78.72%) | 10 (21.28%) | 39 (82.98%) | 8 (17.02%) | 36 (76.60%) | 11 (23.40%) | 38 (80.85%) | 9 (19.15%) | *= 1** |
| Enterobact. | 0 (0.00%) | 47 (100.00%) | 1 (2.13%) | 46 (97.87%) | 0 (0.00%) | 47 (100.00%) | 0 (0.00%) | 47 (100.00%) | *NA* |
| Squamous epithelial cells | 3 (6.38%) | 44 (93.62%) | 5 (10.64%) | 42 (89.36%) | 0 (0.00%) | 47 (100.00%) | 0 (0.00%) | 47 (100.00%) | *= .241*** |
| Gardnerella vaginalis | 0 (0.00%) | 47 (100.00%) | 0 (0.00%) | 47 (100.00%) | 0 (0.00%) | 47 (100.00%) | 2 (4.26%) | 45 (95.74%) | *= .475*** |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Trich. vaginalis | 0 (0.00%) | 47 (100.00%) | 0 (0.00%) | 47 (100.00%) | 0 (0.00%) | 47 (100.00%) | 0 (0.00%) | 47 (100.00%) | *NA* |
| Candida | 1 (2.13%) | 46 (97.87%) | 0 (0.00%) | 47 (100.00%) | 0 (0.00%) | 47 (100.00%) | 2 (4.26%) | 45 (95.74%) | *= 1** |
| Yeast cells and filaments | 3 (6.38%) | 44 (93.62%) | 6 (12.77%) | 41 (87.23%) | 4 (8.51%) | 43 (91.49%) | 2 (4.26%) | 45 (95.74%) | *= 1** |

* *p* – value was obtained with Mc'Nemar test with continuity correction

** *p* – value was obtained with 2-sample test for equality of proportions with continuity correction

**Table 10. Change in vaginal microflora from Visit 1 to Visit 4**

From the data presented in Table 9 it is observed that some improvements are seen in the vaginal microflora but without statistical significance.

### 4.9.4 CHANGE IN VAGINAL INFLAMMATION

The change in inflammatory and parabasal cells by laboratory microscopy was statistically significant, with a number of 32 study participants recording an improvement.

| Response | Visits | |
|---|---|---|
| | Baseline | Final |
| Yes | 32 | 15 |
| No | 15 | 32 |
| ***Total number of subjects*** | 47 | 47 |

**Table 11. Change in vaginal inflammation score between baseline and final visit**

McNemar's Chi-squared test with continuity correction was performed to assess the difference of the Vaginal Inflammation score between the baseline and final visit (Visit 4). At a 5% significance level and considering a null hypothesis that is no statistically significant difference between baseline visit and final visit, we evidenced that the differences between these values were statistically significant ($p < 0.001$).

### 4.9.5 PARTICIPANT SATISFACTION

The degree of satisfaction when using the medical device was assessed using a five-point Likert Scale, as very satisfied, satisfied, neutral, unsatisfied, and very unsatisfied.

The evaluation of participant satisfaction was summarized in the following Table 12 and Figure 5.

| Participant Satisfaction | Very Satisfied | Satisfied | Neutral | Unsatisfied | Very Unsatisfied |
|---|---|---|---|---|---|
| *SCORE* | 39 (82.98%) | 6 (12.77%) | 2 (4.26%) | 0 (0.00%) | 0 (0.00%) |

**Table 12. Participants response regarding Medical Device Usage Satisfaction**

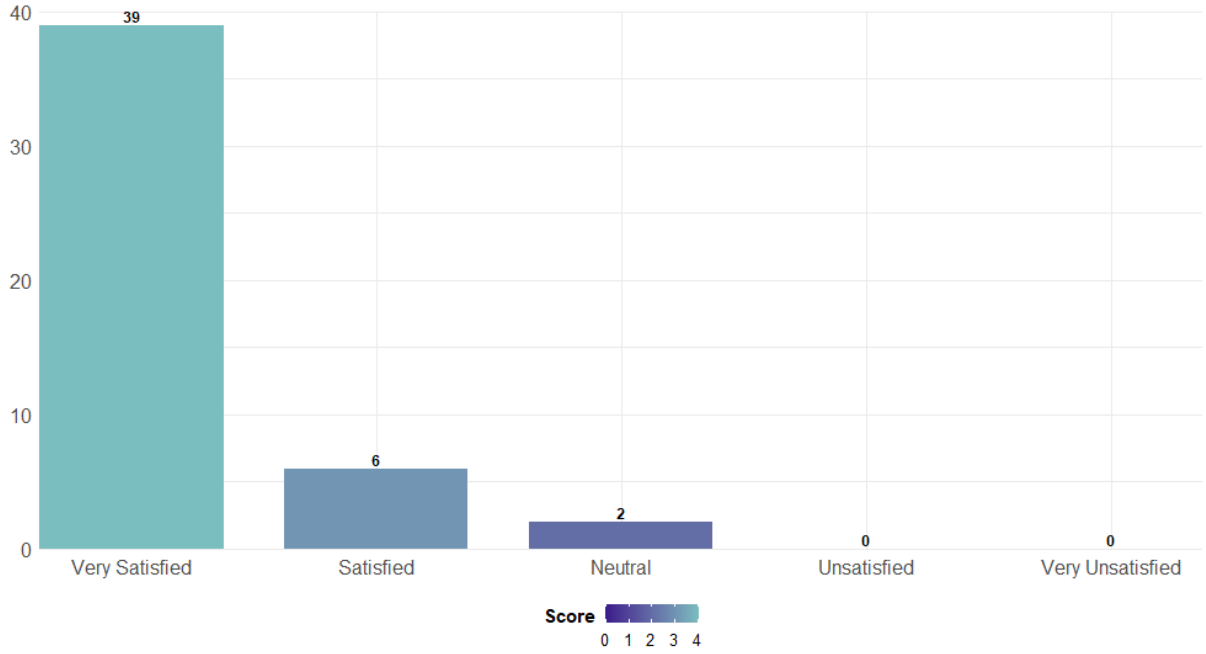


**Figure 5. Participant Satisfaction Score – Likert Scale**

Thirty-nine study participants (82.98%) were very satisfied, 6 participants (12.77%) were satisfied, and 2 participants (4.26%) were neutral. No participants declared themselves unsatisfied or very unsatisfied.

### 4.9.6 EXPLORATORY ANALYSIS

#### 4.9.6.1. PERFORMANCE IN POSTMENOPAUSAL WOMEN

##### 4.9.6.1.1 DYSURIA

A normal feature of natural aging is the postmenopausal syndrome. Menopause is one of the most significant events in a woman's life bringing a broad spectrum of physiological changes caused mainly by the lack of estrogens. The hypoestrogenic state results in hormonal and anatomical changes in the genitourinary tract, with vaginal dryness, dysuria and dyspareunia being some of the most frequent symptoms that can have a great impact on the quality of life of the affected women.

The effectiveness of Cerviron© at 30 days and 90 days on specific symptoms, such as dysuria and dyspareunia in menopausal versus non-menopausal patients, has also been investigated. At the same time, a correlation was suggested between the evolution of symptoms and the response to treatment between these groups. During the clinical investigation, there were 9 participants with postmenopausal syndrome enrolled. Participants that accused dysuria and their symptom evolution between visits are represented in Table 13.

| Patient groups | Vaginal symptom: Dysuria | | | | | |
|---|---|---|---|---|---|---|
| | Baseline | | After 30 days | | Final Visit (90 days) | |
| | Presence | Absence | Presence | Absence | Presence | Absence |
| Menopause | 3 | 6 | 3 | 6 | 0 | 9 |
| No Menopause | 5 | 33 | 1 | 37 | 0 | 38 |

**Table 13. Comparison between menopausal and non-menopausal patients with Dysuria at Visit 1, Visit 2, and Visit 4**

In the first group, there were 9 menopausal patients, of which at baseline 3 patients had dysuria (33.33%), and 6 patients did not show this symptom. The second group consisted of non-menopausal patients (N = 38). At baseline, 5 patients (13.16%) had dysuria, while 33 patients did not have this condition (Table 13).

In the first stage, the proportion of menopausal patients who responded to Cerviron© treatment after 30 days was determined. It was observed that the proportion of patients in this group remained unchanged after 30 days of treatment *(p = 1*).

In the second stage, the proportion of non-menopausal patients who responded to treatment after 30 days was determined. From the analyzed data it was observed that the proportion in this group decreased from 13.16% (N = 5) at baseline to 2.63% (N =1) after 30 days. This decrease of 10.53 percentage points between the two groups is not statistically significant (*p = 0.202*).

At the final visit, after 90 days of treatment, it is observed from table 13 that both menopausal and non-menopausal patients have an identical response to treatment. Basically, the effectiveness of Cerviron© after 90 days of treatment is identical in the two groups.

**4.9.6.1.2 DYSPAREUNIA**

From out of 9 menopausal patients, 4 patients (44.45%) had baseline dyspareunia as a symptom, and 11 out of 38 non-menopausal patients (28.96%) also had this condition.

After 30 days of treatment with Cerviron©, it was observed that although there was a 22.22% (N = 2) reduction in symptoms in the group of menopausal patients, this was not statistically significant (*p = 0.617*).

In the group of non-menopausal patients, after 30 days of treatment, it was found that there was a reduction in dyspareunia in 4 patients (10.53%). However, this reduction is not statistically significant (*p = 0.418*).

In contrast, at the final visit versus baseline in the group of menopausal patients the reduction was from 4 cases to 0 cases of dyspareunia, but from a statistical point of view this reduction is not significant (*p = 0.378*), and in the group of women who do not at menopause the cases decreased from 28.95% (N = 11) at baseline to 2.63% (N = 1) (*p < 0.05*).

The evolution of dyspareunia is represented in Table 14.

| Patient groups | Vaginal symptom: Dyspareunia | | | | | |
|---|---|---|---|---|---|---|
| | Baseline | | After 30 days | | Final Visit (90 days) | |
| | Presence | Absence | Presence | Absence | Presence | Absence |
| Menopause | 4 | 5 | 2 | 7 | 0 | 9 |
| No Menopause | 11 | 27 | 7 | 31 | 1 | 37 |

**Table 14. Comparison between menopausal and non-menopausal patients with Dyspareunia at Visit 1, Visit 2, and Visit 4**

In postmenopausal women, irritation and trauma occurring during sexual intercourse are the result of the hypoestrogenic environment of urogenital tract tissue, with thicker vaginal epithelium and lamina propria, atrophy of smooth muscles, reduction of vaginal-area blood flow, and loss of tissue elasticity causing bothersome symptoms. For postmenopausal women, during the clinical investigation a significant improvement was seen in the most bothersome symptoms (dysuria and dyspareunia) as shown. However, there is no correlation between this physiological condition and the response to treatment with Cerviron©. Both menopausal and non-menopausal women have a similar response to treatment at 30 days of treatment (*p = 0.795*) and at the end of the treatment period (*p = 0.998*).

#### 4.9.6.1.3 CHANGE IN VAGINAL INFLAMMATION SCORE

The response to treatment with Cerviron© in terms of the score of vaginal inflammation in menopausal subject versus non-menopausal subjects was considered. The change in vaginal inflammation score is represented in Table 15.

| Patient groups | Change in Vaginal Inflammation Score | | | |
|---|---|---|---|---|
| | Baseline | | Final Visit (90 days) | |
| | Presence | Absence | Presence | Absence |
| Menopause | 7 | 2 | 1 | 8 |
| No Menopause | 25 | 13 | 14 | 24 |

**Table 15. Comparison between menopausal and non-menopausal patients in terms of changes in vaginal inflammatory score**

At baseline, 7 from 9 patients (77.78%) in the menopausal group had a symptom of vaginal inflammation, and in the group of non-menopausal patients, 25 patients (65.79%) out of a total of 38 had symptoms.

After 90 days of treatment with Cerviron©, the score improved significantly in both groups studied. Thus, in the group of menopausal patients the inflammatory symptoms were reduced to 1 case (11.11%) ($p < 0.05$), while in the other group there were 14 cases (36.84%) ($p < 0.05$).

However, there is no correlation between menopausal and non-menopausal women ($p = 0.166$) in terms of improving vaginal inflammation score.

The medical device can therefore be considered a safe and effective alternative for the treatment of specific signs and symptoms in postmenopausal women (dysuria, dyspareunia and vaginal inflammation), especially when hormonotherapy is not recommended.

#### 4.9.6.2 PERFORMANCE IN BALANCING VAGINAL PH IN SEXUALLY ACTIVE PATIENTS

It has also been determined whether sexual activity can have any effect on changes in vaginal pH and on the effectiveness of treatment with Cerviron©. Figure 6 shows the evolution of pH in sexually active patients during the 90 days of treatment. After 30 days of treatment, a slight decrease was observed in cases with abnormal pH from 20.45% (*N = 9*) to 13.64% (*N = 6*).

In contrast, at 60 days of treatment, the rate of patients with abnormal pH decreased to only 6.82% (*N = 3*), which shows that treatment with this medical device is effective (*p < 0.05*).

At the end of the clinical investigation, all patients in this group had normal vaginal pH.

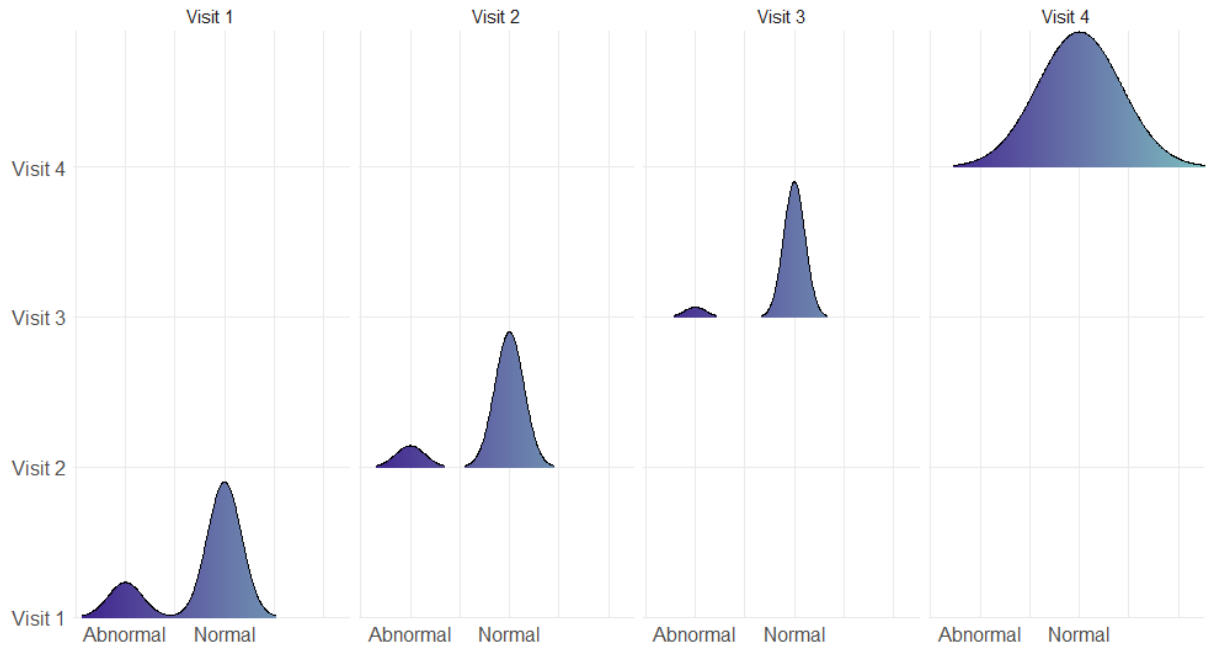


**Figure 6. PH changes between visits in the group of participants with sexual activity**

## 5. DISCUSSIONS AND OVERALL CONCLUSIONS

The purpose of this study was to investigate the therapeutic performance and tolerability of Cerviron© in participants with symptomatic, non-specific, non-infectious vaginitis, and endogenous symptomatic infections. The effects were assessed as changes between Baseline, Visit 2, Visit 3, and Final Visit in the following primary performance outcomes: clinical performance after thorough gynecological examination and participant's interview at end of treatment visit *(regarding the presence of vaginal symptoms)* and rate of treatment-related AE in subjects participating in the clinical investigation.

The primary objective of the clinical investigation was met. For 34 (72.34%) study participant using Cerviron® the medical device had a beneficial effect on the score of vaginal symptoms, while for only 13 participants (27.66%) the score remained the same ($p < 0.001$) over the 3 treatment sessions (each of 15 days).

As secondary performance outcomes, the clinical investigation proposed several paraclinical investigations (vaginal discharge, vaginal pH, microscopic characteristics of inflammatory cells and vaginal microflora characteristics).

The symptoms for which patients were enrolled in the study have certainly improved. No persistence or worsening of symptoms /clinical signs were noted in any participant. The presence of vaginal symptoms has improved statistically significant from Baseline to the 90-day Visit. The medical device also significantly improved the vaginal discharge aspect score, pH values, and vaginal inflammation symptoms. Treatment success (evaluated through participant questionnaires) was relevant in the assessment of the degree of satisfaction with the medical device usage. In terms of the degree of satisfaction offered, participants in the largest proportion (around 96%) said they were satisfied with this medical device. Only 2 participants declared themselves neutral in terms of satisfaction.

In terms of safety, the medical device was used according to the data supplied by the Manufacturer on the labelling, in the instructions and/or in Investigator's Brochure. Three patients experience mild vaginal Cocci and E. Coli infections and the study team decided to continue the treatment with Cerviron©. At the final study visit, no study participants presented treatment-related AEs or SAEs, which makes the medical device safe to administer.

Data from the secondary variables supported the results from the primary variable, as follows:

- *Change in vaginal discharge aspect*. The difference in change for Vaginal Discharge Aspect between baseline visit and final visit was statistically significant at 5% significance level ($p < 0.05$).

The vaginal discharge aspect is useful in the management of vaginal infections. The change in vaginal discharge aspect was assessed by the investigator as an objective finding and was verified by laboratory examination during every study visit. The vaginal discharge aspect improved during the medical device usage. While at the Baseline visit, participants were evaluated with abundant discharge (N=4), moderate discharge (N=37) and mild discharge (N=6), after 90 days, some participants had no vaginal discharge (N=9), and other participants were reported with mild vaginal discharge (N=13) or moderate discharge (N=25). As such, Cerviron© ovules proves its performance in improving the vaginal discharge aspect after 3 months of usage.

- *Change in vaginal pH values.* Determination of pH values compared to its normal values (3.8 – 4.5). The difference in change of Vaginal pH values between the baseline and final visit, at 5% significance level was statistically significant ($p < 0.05$).

The vagina has a dynamic microbial ecosystem with varying vaginal pH levels. An imbalance in that ecosystem can alter the vaginal pH and upscale to the point of causing medical attention. It is observed that after the 3 months of treatment with Cerviron© all participants presented a normal pH. The normal vaginal pH level (between 3.8 – 4.5) is very important for its protective role in blocking yeast and bacteria multiplication. Thus, the supportive role of the medical device in preventing reinfection is proven on the basis of its effective role in correction of the unbalanced vaginal pH.

The vaginal pH can be affected by overall health conditions, such as age, taking antibiotics, vaginal hydration status, vaginal douching, unprotected sex, variations in the menstrual cycle, daily diet. (49) The semen is relatively alkaline, with a pH value of approximately 8.0. and can alter the vaginal pH during unprotected intercourse. As such, the vaginal defense system is altered, causing a change in vaginal pH that remains elevated even after 10–14 h. (50) This alteration leaves the vagina les protected against infection. Several applications of Cerviron© will maintain a healthy vaginal environment and conserve the microbial ecosystem. Moreover, Cerviron© can be used as an adjuvant

treatment in patients requiring antibiotic treatment in order to maintain a healthy, more acidic vaginal pH level.

- *Change in vaginal microflora and Lactobacilli count.* The change in Lactobacilli species throughout the study was not statistically significant ($p > 0.05$).

The common vaginal microbiome, the *Lactobacilli* species, can produce acidic pH and bacteriocins to kill other bacteria in the vagina. *Lactobacilli* can produce an acidic environment in the vagina, which is designed to protect women from sexually transmitted pathogens and opportunistic infections. During the present clinical investigation, some improvements were found in vaginal microflora and *Lactobacilli* count, but they were not statistically significant.

- *Change in vaginal inflammation*. The difference in change in vaginal inflammation between baseline and final visit, at 5% significance level was statistically significant ($p < 0.001$).

Vaginal inflammation is often present in the diagnosis of vaginitis, especially in desquamative inflammatory vaginitis. Signs and symptoms include abundant discharge, vestibulo-vaginal irritation, and dyspareunia. The physical examination of vaginal walls shows signs of inflammation with increased erythema and petechiae. The vaginal sample is characterized by the absence of clue cells, hyphae, trichomonads and *lactobacilli* with increased number of parabasal cells. Parabasal cells are typically round or oval in shape with a high nuclear/cytoplasmic ratio.

Cerviron© medical device reduces vaginal inflammation as shown by laboratory microscopy. The change in inflammatory and parabasal cells was relevant, with a number of 32 study participants recording an improvement.

In conclusion, Cerviron® ’s clinical performance as adjuvant treatment in non-specific or symptomatic vaginitis was demonstrated throughout the present 90-days clinical investigation. Administration of Cerviron© significantly alleviates the symptoms of symptomatic, non-specific, non-infectious vaginitis, and its administration can be considered safe. Cerviron® can be used as long-term supportive therapy, either as a stand-alone treatment or in combination with other oral therapies with antibiotics, antifungals or antivirals. Cerviron® supportive therapy may be prescribed for 10 or 15 consecutive days. While balancing the vaginal pH, Cerviron reduces vaginal inflammation and improves the vaginal discharge aspect after 3 months of therapy.

Cerviron® beneficial effects were correlated with participants’ different baseline characteristics, such as premenopausal or menopausal status, normal hydration and nutrition status and age. There was no statistical difference reported between the groups. Cerviron® was safely administrated across all age groups, with or without a healthy diet and hydration.

Regarding sexual activity, Cerviron® can be safely administered to both sexually active women as well as to abstinent women.

Despite the fact that during the clinical investigation no pregnancy cases were managed, the usage of Cerviron® is recommended under the close supervision of the gynecologist. As shown, Cerviron® can be safely applied in different forms of vaginitis. When added to the standard-of-care in aerobic vaginitis, Cerviron® will restore the vaginal pH, will reduce inflammation and will reduce the proliferation of aerobic bacteria. A good management of aerobic vaginitis during pregnancy significantly improves perinatal outcomes and can prevent treatment failures and severe

complications, such as pelvic inflammatory disease, infertility, miscarriage, chorioamnionitis, premature rupture of membranes and preterm delivery. (52)

Further studies need to be planned to confirm the tolerability profile of Cerviron® in pregnant participants.

## 6. ABBREVIATED TERMS AND DEFINITIONS

| | |
|---|---|
| **AE** | Adverse Event |
| **CIP** | Clinical Investigation Plan |
| **CNBMDM** | National Ethics Commitee (Comisia Nationala De Bioetica a Medicamentului si a Dispozitivelor Medicale) |
| **CRF** | Case Report Form |
| **CRO** | Contract Research Organization |
| **eCRF** | Electronic Case Report Form |
| **GCP** | Good Clinical Practice |
| **IB** | Investigator Brochure |
| **ICF** | Informed Consent Form |
| **ICH** | International Council for Harmonization |
| **ISO** | International Organization for Standardization |
| **LC50** | Lethal Concentration 50 |
| **MMP** | Matrix Metalloproteinases |
| **NEC** | National Ethics Committee |
| **PHI** | Personal Health Identifiers |
| **PI** | Principal Investigator |
| **Ppm** | Parts Per Million |
| **SAE** | Serious Adverse Event |
| **SDV** | Source Data Verification |
| **SAR** | Serious Adverse Reaction |
| **SOC** | Service Organization Control |
| **SUSAR** | Suspected Unexpected Serious Adverse Reaction |

## 7. ETHICS

The clinical investigation and its updates were reviewed by the National Ethics Committee from Romania (CNBMDM). A list of the NEC members is available by request. The study was conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki. The Participant Information and Consent was obtained prior to participant enrollment (at Screening Visit)

in most cases by the Principal Investigator or the delegated that were also the consulting physician and a member of the clinical site team.

A sample of the participant consent form is available by request.

**Data Protection**

Participants were assigned a unique identifier by the sponsor. Any participant records or datasets that were transferred to the sponsor contain the identifier only. Participant names or any information which would make the participant identifiable were not transferred. Sponsor staff that required access to personal data agreed to keep confidentiality. Only the relevant data to fulfil the objectives of the study were collected. Study participants were informed that they have the right to request access to their personal data and the right to request rectification of incorrect or incomplete data. No personal details were recorded during the clinical investigation, except for the data included in the medical charts.

## 8. INVESTIGATORS AND ADMINISTRATIVE STRUCTURE OF CLINICAL INVESTIGATION

This clinical investigation was conducted in Romania, in a hospital-based setting, in two investigational centres, located in Bucharest and Timisoara, Romania.

The details of the clinical sites, affiliation, principal investigators and subinvestigators' names can be found below:

| Clinical site | Affiliation | Name | Surname |
|---|---|---|---|
| Site 01 | INSTITUTUL NATIONAL PENTRU SANATATEA MAMEI SI COPILULUI " ALESSANDRESCU-RUSESCU"<br>Obstetrics-Ginecology „Polizu"<br>Department of Obstetrics-Gynecology III<br>38-52 Gheorghe Polizu Street, District 1<br>011061 Bucharest, Romania | Daniela-Oana<br><br>Raluca | Toader<br><br>Olaru |
| Site 02 | SPITALUL CLINIC JUDEȚEAN DE URGENȚĂ "PIUS BRINZEU" TIMISOARA<br>Department of Obstetrics-Gynecology II<br>12 Victor Babeș Boulevard<br>300226 Timisoara, Romania | Izabella<br><br>Lavinia | Petre<br><br>Stelea |

**Table 16. Clinical sites and affiliation**

### 8.1 SITE PERFORMANCE

**Site 01 INSTITUTUL NATIONAL PENTRU SANATATEA MAMEI SI COPILULUI " ALESSANDRESCU-RUSESCU" BUCHAREST (Principal Investigator Dr. Daniela-Oana Toader)**

The site screened 15 patients. All 15 patients received the medical device CERVIRON. The site conducted the trial according to ICH-GCP and study protocol requirements. There were no critical or major findings identified. The eCRF was completed in a timely manner for patients 101-115 for all the visits. Primary and secondary objectives, safety and concomitant medication were reported as per CIP.

Patient 108 is reported with 3 unreturned, unadministered ovules. Three patients (101, 105 and 109) out of 15 did not return the used boxes of the clinical investigation medical device.

During the study, the PI has had excellent oversight and implication.

**SITE 02 SPITALUL CLINIC JUDEȚEAN DE URGENȚĂ "PIUS BRINZEU" TIMISOARA (Principal Investigator Dr. Izabella Petre)**

The site screened 35 patients. Only 32 patients met the inclusion and exclusion criteria. Three patients (0206, 0215 and 0218) were screen-failure. There were 8 onsite monitoring visits performed between 24 May 2021 - 28 Feb 2022.

For participant 0213 Visit 1 was performed on 19.05.2021 and visit 2 was performed in a serious delay, on 24.09.2021. The PI explained that the patient was detached with work. Also, participants no. 0216 and 0217 have a delay of 3 months between Visit 1 and Visit 2. There were no critical or major findings identified. The eCRF was generally completed with some delays from the study team. The site team members were compliant with GCP principles, CIP and the current clinical trials legislation. During the study, the PI has had a good oversight and implication.

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**ANNEX 1. STUDY-RELATED ESSENTIAL DOCUMENTATION**

**ANNEX 2. THE EC FAVOURABLE OPINION AND ESSENTIAL CORRESPONDENCE**

## AUTHORS AND SPONSOR SIGNATURE PAGE

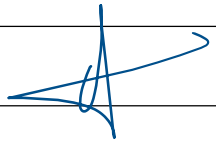
Statement of Agreement

I have written this report and confirm that to the best of my knowledge it accurately describes the conduct and results of the clinical investigation entitled "A Prospective, Open-Label, Pilot, Multicentric Clinical Investigation to Evaluate the Performance and Safety of Cerviron® ovules in the Local Treatment of Non-Specific or Endogenous, Symptomatic Vaginitis" (Version 1.0 dated 05 Apr 2022).

**Pharm. Ramona Petrita**

05- Apr - 2022

Medical Writer's Name — Date



Medical Writer's Signature

Statement of Agreement

I have written this report and confirm that to the best of my knowledge it accurately describes the conduct and results of the clinical investigation entitled "A Prospective, Open-Label, Pilot, Multicentric Clinical Investigation to Evaluate the Performance and Safety of Cerviron® ovules in the Local Treatment of Non-Specific or Endogenous, Symptomatic Vaginitis" (Version 1.0 dated 05 Apr 2022).

**Pharm. Alexandru - Remus Pinta**

06 - Apr - 2022

Biostatistician's Name — Date

Biostatistician's Signature

Statement of Agreement

I have read this report and confirm that to the best of my knowledge it accurately describes the conduct and results of the clinical investigation entitled "A Prospective, Open-Label, Pilot, Multicentric Clinical Investigation to Evaluate the Performance and Safety of Cerviron® ovules in the Local Treatment of Non-Specific or Endogenous, Symptomatic Vaginitis" (Version 1.0 dated 05 Apr 2022).

**Pharm. Ema Peta**

07 - Apr - 2022

Sponsor's Name — Date

Sponsor's Signature