Performance and Safety of Vaginal Administration of Tocopherol Acetate (Vitamin E) in Pre-, Peri-, and Post-Menopausal Women

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ABSTRACT

The vaginal microbiota has a significant bearing on a woman's mental and physical health at every stage of life. A balanced microflora of the vaginal environment (eubiosis) consists of a predominant presence of lactic acid bacteria. Lactobacilli ensure the maintenance of an acidic pH that effectively makes the environment inhospitable to pathogens by preventing infection and protecting the genital tract. At the end of the reproductive period, the reduction of sex hormones, particularly estrogen, results in a decrease in the lactobacillus component with consequent depletion of the vaginal microbiota and increased susceptibility to pathogens.

Tocopherol (vitamin E) is an essential human nutrient with high antioxidant power. Topically administered, it not only plays a key role in protecting epithelial cell membranes from oxidative damage but is also able to modulate bacterial growth by contributing to pH acidification. The latter feature, in the vaginal canal, creates a favorable environment for the lactobacilli flora, positively affecting the microbial population and thus contributing to the prevention of all those problems related to increased vaginal sensitivity to pathogens during "menopause." In addition, recent data show that vitamin E can counteract the production of biofilm produced by certain pathogens, which has detrimental effects on the homeostasis of the vaginal environment.

The purpose of this non-controlled Investigator Initiated Trail (IIT), with a retrospective design is to evaluate the performance and safety of the vaginal administration of tocopherol acetate in pre-peri and postmenopausal women. The main objectives of the study will be the evaluation of the vaginal pH, of the vaginal eubiosis improving the lactobacilli flora and microbiota, and of sign and symptoms of women. The patients were visited at baseline (with the collection of vaginal swabs) and treated with vaginal administration of tocopherol acetate for 14 days. At the end of treatment (final visit) the patients were visited with the collection of vaginal swabs.

The period of observation is from January 1st, 2020, to December 31st, 2022, while the Data Collection period is planned from March 30th, 2024 to May 31st, 2024.

Observational Study: GEMMOLGYNE 01_2023 Version: 1.0 (final)– November 15, 2023

Title: Performance and Safety of Vaginal Administration of Tocopherol Acetate (Vitamin E) in Pre-, Peri-, and Post-Menopausal Women

CIP code: GEMMOL GYNE 01_2023

Version: 1.0 (final) – November 15, 2023

Register No.: NCT05918848; www.ClinicalTrials.gov

Type: Retrospective Investigator Sponsored Research (IIT Investigator Initiated Trial)

Phase: Post marketing

MD Class: Class IIa, already CE marked in Italy

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Introduction and Rationale:
The vaginal microbiota has a considerable relevance on the psychophysical health of women
at every stage of life. A balanced microflora of the vaginal environment (eubiosis) consists of a predominant presence of lactic acid bacteria.
Lactobacilli guarantee the maintenance of an acidic pH that makes the environment inhospitable for pathogens, preventing infections and protecting the genital tract (1–4).
At the end of the reproductive period, the reduction of sex hormones, in particular oestrogen, determines a decrease in the lactobacillary component with consequent depletion of the vaginal microbiota and increase in susceptibility to pathogens

(5–8).
Vitamin E (tocopherol) is an essential nutrient for humans present especially in oily fruits. It is a fat-soluble molecule with high antioxidant power. Topically, vitamin E not only plays a key role in protecting epithelial cell membranes from oxidative damage, but it is also able to modulate bacterial growth by contributing to pH acidification (9,10). This last feature, in the vaginal canal, creates a favourable environment for the lactobacillary flora (11), positively affecting the microbial population and thus contributing to the prevention of all those problems related to greater vaginal sensitivity to pathogens during menopause. In addition, recent data show how vitamin E is able to counteract the production of biofilm produced by certain pathogens that has detrimental effects on the homeostasis of the vaginal environment (9,10).

A medical device containing tocopherol acetate (vitamin E) (Filme Gyno-V®) has been recently developed; this observational study aims to verify whether a treatment with Filme Gyno V® ovules can have a positive effect on the vaginal microbiota in women in pre-, peri- and post-menopausal, promoting the maintenance or even the increase of the lactobacillary colonisation and therefore a state of eubiosis.

Objectives:

- Primary objective of the study

Assess whether the application of Filme Gyno-V® ovules can contribute to the acidification of vaginal pH, providing an inhospitable environment for any pathogens.

- Secondary objectives of the study

Verify whether Filme Gyno-V® ovules can promote vaginal eubiosis by acting on the lactobacillary flora. This evaluation will be done in the first instance by determination of vaginal lactobacillary grade according to Ison Hay classification and then by culture examination of the vaginal swab.

- Ancillary objective of the study

Evaluate the effects of the application of Filme Gyno-V® on the microbiota. For this reason, a vaginal swab will be performed and stored at -80°C for subsequent molecular analyses that will be performed in subordination to the clinical and microbiological data obtained.

Endpoints:

Primary endpoint

- Acidity measure of vaginal secretions (pH), with respect to baseline, at day 14; evaluation performed by Investigator.

Secondary and ancillary endpoints

- Change in vaginal mucosal dryness (presence or absence of total symptomatology) and detailing the presence of which symptoms (itching, burning, dryness, dyspareunia) are present compared to baseline on day 14; evaluation performed by Investigator.
- Change in vaginal mucosal integrity, evaluated through a 4-point ordinal scale, with respect to baseline, at day 14; evaluation performed by Investigator.
  Change in mucosal colour, evaluated through a 4-point ordinal scale, with respect to baseline, at day 14; evaluation performed by Investigator.

- Change in mucosal trophism, evaluated through a 4-point ordinal scale, with respect to baseline, at day 14; evaluation performed by Investigator.
- Quantity of vaginal discharge assessment, evaluated through a 4-point ordinal scale, with respect to baseline, at day 14; evaluation performed by Investigator.
- Whiff test, evaluated by means of detection of presence / absence of odour, with respect to baseline, at day 14; evaluation performed by Investigator.
- Change in Lactobacillus grade according to Ison Hay classification by bacterioscopic smear, evaluated according to a 4-grade scoring system, with respect to baseline, at day 14; evaluation performed by Investigator.
- Microbiological evaluation by culture examination of the vaginal swab, evaluated by means of detection of presence / absence of specific microorganisms, with respect to baseline, at day 14; evaluation performed by Investigator.
- Molecular sequencing-based microbiological analysis of vaginal microbiota by next-generation sequencing (NGS) method, evaluated by comparison of microbiota, with respect to baseline, at day 14; evaluation performed by Investigator (for
  24 patients).
- Evaluation of AE, SAE, ADE, SADE (Adverse Events, Serious Adverse Events, Adverse Device Effects, Serious Adverse Device Effects)

Design: Retrospective, Non-Controlled, Monocentric, IIT study

Indication: Maintaining and possibly improving the health and functioning of the female genital tract

Study population: Pre-, peri- and post- menopausal (according to STRAW criteria) women, aged between 45 and 75 years, admitted as outpatients to the study centre for gynaecological visits from January 1^{st,} 2020, to December 31st, 2022, and, after a confirmation of eligibility, consenting to the retrospective collection of their data after a confirmation of eligibility.

Inclusion criteria:
All the following criteria must be fulfilled:
- Women aged ≥ 45 to ≤ 75 years included.
- Premenopausal, perimenopausal, postmenopausal women (according to STRAW criteria) (12) attending the study center for gynecological visits from 1st January, 2020, to 31 December, 2022 and treated with the tested MD (Filme Gyno-V®).
- Patients able to comply with the requirements for the study and freely willing to provide written informed consent to the retrospectively collection of their data.

Exclusion criteria:
Any of the following must be excluded:
- Malignancy (also leukemic infiltrates) within 5 years prior to day 0 (except for treated basal cell/squamous cell carcinoma of the skin).
- Genital bleeding.
- Oestrogen topical (vaginal) treatment during the study period (it must have been terminated at least 6 months before the beginning of the study).

- Systemic oestrogen therapy during the study period (it must have been terminated at least 6 months before the beginning of the study).
- Patients with any illness, or any other medical condition, that, in the opinion of the Investigator, would compromise the participation or be likely to lead to hospitalisation during the study.
- Clinical evidence of acute infection currently requiring treatment (syphilis, herpes simplex, human papilloma virus, gonorrhoea, chlamydia, lymphogranuloma venereum, etc.); clinical evidence or history of chronic infectious disease (e.g., tuberculosis).
- Psychosis, schizophrenia, mania, depressive disorders, history of suicide attempt or suicidal ideation, or any other psychiatric illness (except for intermittent anxiety).
- Known allergy to tested medical device (tocopherol).
  Participation in an interventional clinical study or administration of any investigational agents in the 30 days prior to day 0.
- Presence of any clinically significant medical condition judged by the Investigator to preclude the patient's inclusion in the study.

Sample size and sample size calculation: The number of enrolled patients should be 50. Vaginal swabs data will be collected for 24 women. A formal sample size calculation was not carried out given the nature of the study.

Number of centres: One (1) centre in Italy will be involved.

Medical device (MD):

Filme Gyno-V® help to form a continual, homogenic and protective film, restoring normal moisture balance in the vaginal surface of women who feel an irritating sensation, such as vulvar itching or burning, soreness, dryness and discomfort during intercourse (not of the pathologic type), consequent of vaginal secretion insufficient or absent; this may be caused by external factors such as stress, menopause, following topical medication or due to the use of harsh vaginal cleansing wash, oral
contraceptives, breastfeeding or after childbirth. Can be used during breastfeeding, suitable with the use of contraceptives. Promotes trophic and reparative physiological processes of the vaginal mucosa. Promotes the growth of *Lactobacillus acidophilus* (of Döderlein).

Filme Gyno-V® is manufactured as vaginal ovules with a shell made by vegetal gelatine and filling with tocopherol acetate Tocopheryl Acetate or Tocopherol Acetate or Vitamin E acetate is the synthetic form of Vitamin E). The shell is made by hydroxypropyl starch, glycerol, carrageenan, and disodium phosphate. The shell it is bound to break, releasing the contained tocopherol acetate (500 mg) and to be expelled mechanically.

MD administration:

All the patients received the medical device (vaginal ovules to be self-administered) containing tocopherol acetate (Vitamin E acetate) according to the Instruction for Use (IFU) of the product (1 vaginal ovule once a day at night before going to bed) before entering the study. The device was product was self-administered by the patients.

Administration duration:

The duration of the administration was 14 days, according to the Instruction for Use (IFU) for the product.

Study duration (period of data collection):
The observation period is from January 1st, 2020, to December 31st, 2022.

Chronogram of visits:
The study will not envisage any visits, being a retrospective collection of data.
Following the usual clinical practice of the Center, each patient attended the following visit:

Procedures performed during visits:
Each patient should give her consent to the retrospective collection of her data signing the Informed consent form. The Investigator will assess for eligibility (according to inclusion and exclusion criteria envisaged by the participation in the study). Patients not meeting the eligibility criteria will not take part in the study and their data will not be retrospectively collected.
The data of patients who attended the visits were collected in the hospital medical charts. All the collected data will be retrospectively recorded in the study CRF.
The patients were visited following the clinical practice of the site as follows:

Visit 1 – Baseline (day 0)

- Demographics and medical history.
- Vital signs and physical examination (to be reported only if abnormal findings have been detected). Gynaecological examination.
- Concomitant treatments evaluation.
- Evaluation of clinical parameters by Investigator:
  - Visual and tactile examination of the genital system mucosa (trophism, colour).
  - Vaginal mucosal dryness evaluation.
  - Vaginal discharge assessment.
  - Whiff test.
  - Evaluation of the integrity of the vaginal epithelium.
- Vaginal pH assessment by Investigator.
- Three (3) vaginal swabs were collected by Investigator to perform the following:
  - Bacterioscopic smear for evaluation of lactobacillary grade (according to Ison Hay classification).
  - Microbiological culture examination.
  - Molecular microbiological examination for evaluation of the microbiota.

These swabs were stored at -80°C and subjected to molecular analysis only subordinate to the result of previous clinical and laboratory data.

The treatment with Filme Gyno V® started at day 0, after this visit, and continued for 14 days, up to the second visit.

Visit 2 - End of Study visit (day 14 ± 1 day)

- Gynaecological examination.

- Concomitant treatments evaluation.
- Evaluation of clinical parameters by Investigator:

- Visual and tactile examination of the genital system mucosa (trophism, colour).
- Vaginal mucosal dryness evaluation.
- Vaginal discharge assessment.
- Whiff test.
- Evaluation of the integrity of the vaginal epithelium.

- Vaginal pH assessment by Investigator.
- Vaginal swabs by Investigator to perform the following:

- Visual and tactile examination of the genital system mucosa (trophism, colour).
- Vaginal mucosal dryness evaluation.
- Vaginal discharge assessment.
- Whiff test.
- Evaluation of the integrity of the vaginal epithelium.

These swabs were stored at -80°C and subjected to molecular analysis only subordinate to the result of previous clinical and laboratory data.

- Evaluation of AE, SAE, ADE, SADE.

The molecular sequencing-based microbiological analysis of vaginal microbiota by next-generation sequencing (NGS) method was performed for the samples of 24 women, taken both at day 0 and day 14.

Statistical analysis:

A two-sided p-value of 0.05 or less will be used to declare statistical significance for all analyses. Similarly, all confidence intervals will be calculated at the 95% level. If a patient is missing information for one or more variables, the missing data will not be replaced.

All statistical analyses will be performed using the R statistical software v 4.2.1, or the latest stable version at the time of statistical analysis. The overall type I error rate will be preserved at 5%. All tests will be two-sided. Statistical analyses will be conducted on all patients who have successfully completed the study as requested by the protocol without a protocol deviation that is regarded as impacting the assessment of the key variables (as per protocol).

The quality and

completeness of the collected data will be evaluated preliminarily compared to data analysis. If a patient is missing information for one or more variables, even after the resolution of its query, the missing data will not be replaced.

If a patient has been involved in violation of inclusion/exclusion criteria, the respective data will be excluded from the analysis. Quantitative variables (i.e., demographic) if normally distributed will be described through media, standard deviation (SD); non-normally distributed variables will be described using median and range of interquartile. The Student's t-test and the Mann-Whitney U will be employed to perform comparative analysis in accordance to the distribution of these variables. Categorical variables will be finally described using frequencies and percentages and comparative analysis will use the $\chi 2$ test.

In particular, data belonging from swabs will be analyzed to determine intra and inter-individual variations in the vaginal lactobacillary population. The safety analysis will be done on the safety population.

GCP Statement, Guidelines and legislation:

The protocol the present study has been developed in accordance with the following:

- World Medical Association. Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects (64th WMA General Assembly, Fortaleza, Brazil, October 2013).
- ICH Harmonized Tripartite guidelines for Good Clinical Practice (ICH GCP) requirements.
- Regulation EU 2017/ 745 of the European Parliament and of the Council of 5 April 2017 on medical devices.
- MDCG 2020-10/1 Safety reporting in clinical investigations of medical devices under the Regulation (EU) 2017/745 (May 2020).
- ISO 14155:2020 (E). Clinical Investigation of medical devices for human subjects – Good Clinical Practice.
- Italian legislation.

Ethics Committee Approval - Informed Consent and Patient Information

Prior to the initiation of the study, the Investigator (or a delegate) must submit the clinical protocol, the Patient Information Leaflet, the Patient Consent Form and any other documentation as required to the local centre's EC for review and approval. The Investigator is responsible for obtaining the written Patient Consent Form from each patient participating in the study, in accordance with the ICH-GCP Guidelines and the current version of the Declaration of Helsinki.

Timing:

	A	B
	Start of the Project	Time 0
	Protocol Submission to EC	15 days after time 0
	Approval by Competent Authority	60 days after time 0
	Data Collection Start date	80 days after time 0
	Observation period	from January 1st, 2020, to December 31st, 2022
	Data Collection End date	100 days after time 0
	Final Analysis	120 days after time 0

OBSERVATION AND ASSESSMENTS SCHEDULE PERFORMED DURING VISITS

These visits are performed before entering the retrospective, observational study.

Before entering the study, each patient signs an Informed consent form and is assessed for eligibility (according to inclusion and exclusion criteria envisaged by the observational study). Patients not meeting the eligibility criteria does not take part in the study and their data are not retrospectively collected.

	A	B	C
	Procedures	***Visit 1***	***Visit 2***
	Day 0	***Day 14 (± 1)***	
	Demographics and Medical History	X	
	Physical examination (including gynaecological examination) and vital signs	X	X
	Concomitant treatments	X	X
	Visual and tactile examination of the genital system mucosa (trophism, dryness, colour)	X	X
	Presence / absence of leukorrhea	X	X
	Whiff test	X	X
	Evaluation of the integrity and thickness of the vaginal epithelium	X	X
	Vaginal pH assessment	X	X
	Vaginal swab (bacterioscopic smear for evaluation of lactobacillary grade (according to Ison Hay classification)	X	X
	Vaginal swab (microbiological culture examination)	X	X
	Vaginal swab (molecular microbiological examination for evaluation of the microbiota)*	X	X
	AE/SAE and ADE/SADE collection		X

*The molecular sequencing-based microbiological analysis of vaginal microbiota by next-generation sequencing (NGS) method is performed for the samples of 24 women, taken both at day 0 and day 14. The data are collected as well.