

# Conjugate treatment with high concentration normobaric oxygen and hyaluronic acid for vaginal atrophy: a prospective study

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**Abstract. – OBJECTIVE:** Vulvovaginal atrophy is a condition closely related to low circulating estrogen levels, with post-menopause being the main cause. However, patients of childbearing age may also present with these symptoms due to treatments that reduce estrogen production. Local estrogen therapy is the causal treatment of local symptoms, but it is not always accepted and is often abandoned by patients. In recent years, alternative therapies have been proposed: fractional CO<sub>2</sub> laser or the conjugate treatment with normobaric oxygen and hyaluronic acid, the latter being the subject of this study.

The study aimed to evaluate the effectiveness of conjugate topical treatment with normobaric oxygen and hyaluronic acid.

**PATIENTS AND METHODS:** 50 patients were evaluated and treated with 5 applications of 15 minutes each, every 15 days, with Carressflow®. All patients presented at least one of the symptoms related to vulvovaginal atrophy: dryness, burning, and dyspareunia. In all cases, vulvoscopy, colposcopy, and cervicovaginal cytology were performed. The patients were interviewed with an analogic scale (VAS) concerning the severity of symptoms before and after the treatment. Colposcopy and PAP-smear were assessed by mean of Vaginal Health Index Score (VHI) at baseline and at the end of the treatment.

**RESULTS:** All patients completed the treatment scheme and presented with a significant improvement in subjective symptoms. The colposcopy and PAP-smear performed 10 days after the end of the last treatment showed a significant improvement in the appearance and elasticity of the vaginal epithelium and the cytological picture, which showed, in the sample taken after treatment, hyaluronic acid vesicles within the cell cytoplasm.

**CONCLUSIONS:** This study corroborates the data presented in the latest published papers on the effectiveness of treatment with normobaric O<sub>2</sub> and hyaluronic acid on vaginal atrophy. Effi-

cacy has been confirmed both in terms of subjective symptoms reported by the patients and objective improvement at colposcopy and PAP-smear cytology.

*Key Words:*

Vulvovaginal atrophy, Hyaluronic acid, Normobaric oxygen, Non-hormonal treatment, Menopause, Carressflow®.

## Introduction

Vulvovaginal atrophy (VVA) is a condition closely related to the deficiency of circulating estrogen, with post-menopause being the main cause. More than 50% of women 2-4 years after the end of their fertile period present with this condition and report typical symptoms such as vaginal dryness, burning, itching, and dyspareunia, sometimes associated with urinary disorders. Bleeding may also occur in more severe cases, especially after microtrauma<sup>1-5</sup>.

VVA can also affect women suffering from premature interruption of ovarian production of estrogens. Examples include cancer treatments, such as surgery and pelvic radiation therapy, that remove ovaries or make them inactive, either temporarily or permanently. Although in the recent years a great effort has been made to promote fertility-sparing surgery in young patients with gynecological malignancies<sup>6-8</sup>, some of them may still develop atrophy-related symptoms due to subsequent treatments that reduce estrogen production such as chemotherapy or endocrine therapies. Also, the therapies for endometriosis and the use of low estrogen oral contraceptives have been shown to induce, in some patients, vaginal discomfort.

Estrogen deficiency not only causes atrophy of the vaginal epithelium but also significantly alters the vaginal environment by decreasing the count of lactobacilli, increasing the pH, reducing blood supply, and consequently decreasing the vaginal fluid secretion<sup>9</sup>.

All these alterations mainly affect the vaginal and vestibular mucosa, and much less the entire vulvar region, which is more than 60% covered by skin and therefore less influenced by the presence of estrogens.

Hormonal replacement therapy (HRT), be it topical or oral, is considered the gold standard treatment for VVA. However, many women decline the use of these hormonal substances due to the risk and fear of increased incidence of breast cancer and the need of a prolonged treatment. Moreover, HRT is contraindicated in oncological patients, even if some data show good results on VVA with little systemic absorption of local estrogens<sup>10</sup>. A recent report of The North American Menopause Society was inconclusive on the modality of treatment for women affected by premature menopause after breast, ovarian or endometrial cancer, suggesting that for these women the management depends on individual preference or need, and it must follow a consultation with oncologist considering the potential risks of hormonal treatment choices<sup>11</sup>.

Over the years, many non-hormonal treatments have been used, including ordinary lubricants/emollients, local hyaluronic acid, soy and red clover derivatives oral compounds, vitamins, etc.

In recent years, alternative therapies have been proposed, such as the use of fractional CO<sub>2</sub> laser<sup>12</sup> or lipofilling with sometimes encouraging results. However, the pain associated with the procedures,

the high costs, and the need for reinterventions are major drawbacks to the widespread use of these methods. Recently, warnings from American scientific societies, due to the risk of stimulating the progression of pre-cancerous lesions, have induced a reduction in the use of CO<sub>2</sub> laser<sup>13-16</sup>.

Hyaluronic acid is a natural polysaccharide present in large quantities in the extracellular matrix of the skin and cartilage. By binding to many water molecules, it allows adequate hydration of the mucous membranes. Oxygen promotes tissue repair processes and collagen synthesis by stimulating neoangiogenesis, promoting a good nourishment of the tissues<sup>17</sup>.

Our investigation aimed to confirm the effectiveness of combined treatment with hyaluronic acid and high concentration normobaric oxygen administered intravaginally through the Caress-flow<sup>®</sup> device (Bologna, Italy).

## Patients and Methods

Fifty-seven patients who had been menopausal (spontaneous/surgical/chemotherapy) for at least 6 months were enrolled during the period March 2021-March 2022.

All patients had vulvovaginal atrophy (VVA) and presented at least one of the following symptoms: vaginal dryness, vaginal burning, and dyspareunia unrelated to infectious/inflammatory pathology. In all cases, the following investigations were performed: vulvoscopy, colposcopy, and sampling for cervical-vaginal cytological examination (PAP-smear).

**Table I.** VAS scale of assessment of subjective symptoms (scores from 1 to 4).

Subjective Symptoms	1 Maximum intensity	2 Medium intensity	3 Low intensity	4 Absent
Malaise				
Burning				
Dryness				
Dyspareunia				

**Table II.** Healthcare provider rating scale of VHI symptoms (scores from 1 to 4).

Objective signs	1	2	3	4
Vaginal epithelium	Reddened with petechiae	Bleeding on contact	Slightly reddened	Normal
Elasticity	Absent	Poor	Medium	Good
Atrophy on cytology	Intense	Medium	Poor	Normal trophism

Patients with vulvar dermatological changes, signs of vulvovaginal infections, or pre-neoplastic/neoplastic changes in the lower genital tract were excluded from the study.

The treatment consisted of five sessions of 15 minutes each, every 15 days of oxygen and hyaluronic acid administration using Caressflow®.

Before starting the treatment, patients filled out a form (Visual Analogic Score: VAS scale) in which the following symptoms were scored from 1 to 4 (1 Highest intensity, 2 Medium intensity, 3 Low intensity, 4 No symptom):

- Malaise
- Burning
- Dryness
- Dyspareunia

The colposcopy and PAP-smear cytology performed to assess the status of the cervicovaginal epithelium were evaluated with a score called Vaginal Health Index (VHI). The appearance of the epithelium and the cells was graded from 4 to 1 according to the severity of the alteration observed:

- Epithelium
  - 1) Reddened with petechiae
  - 2) Bleeding on contact
  - 3) Slightly reddened
  - 4) Normal
- Vaginal elasticity:
  - 1) absent
  - 2) poor
  - 3) medium
  - 4) good
- Atrophy at cytology:
  - 1) intense
  - 2) medium
  - 3) poor
  - 4) normal

All subjective parameters were reported before and after the end of the treatment; colposcopy and cervical-vaginal cytological examination were performed 10 to 30 days after the end of the last treatment.

### Statistical Analysis

The statistical significance of the variance trend in values between treatments was analyzed using the Kruskal-Wallis' test for one-way analysis of variance. The significance of pairwise comparisons between treatment sessions was analyzed using the Wilcoxon test for non-parametric data.

Two-sided *p*-values <0.05 were considered statistically significant.

### Results

Seven patients were excluded from the study because they had:

- Vulvar lichen sclerosus (4)
- Abnormal PAP-smear (3)

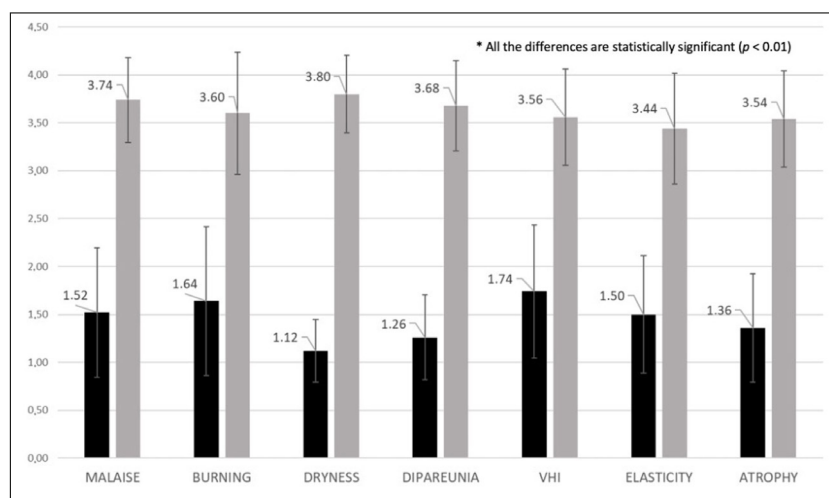
The mean age of the patients at enrollment was 55.5 years +/- 6.4 SD [45-66].

Four patients had chemotherapy-induced menopause for breast cancer.

All patients completed the treatment regimen and showed a marked and statistically significant (*p*<0.001 for all symptoms) improvement in subjective symptoms, achieving their complete disappearance in the 70% of cases (Figure 1).

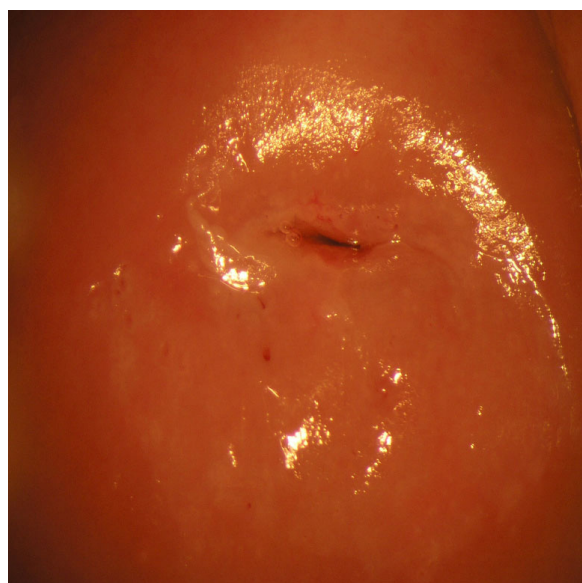
The colposcopy and PAP-smear performed 10-30 days after the end of the last treatment showed a significant change in the appearance and elasticity of the vaginal epithelium (Figures 2 and 3).

**Figure 1.** Subjective symptoms and objective evaluation before and after treatment: comparison.





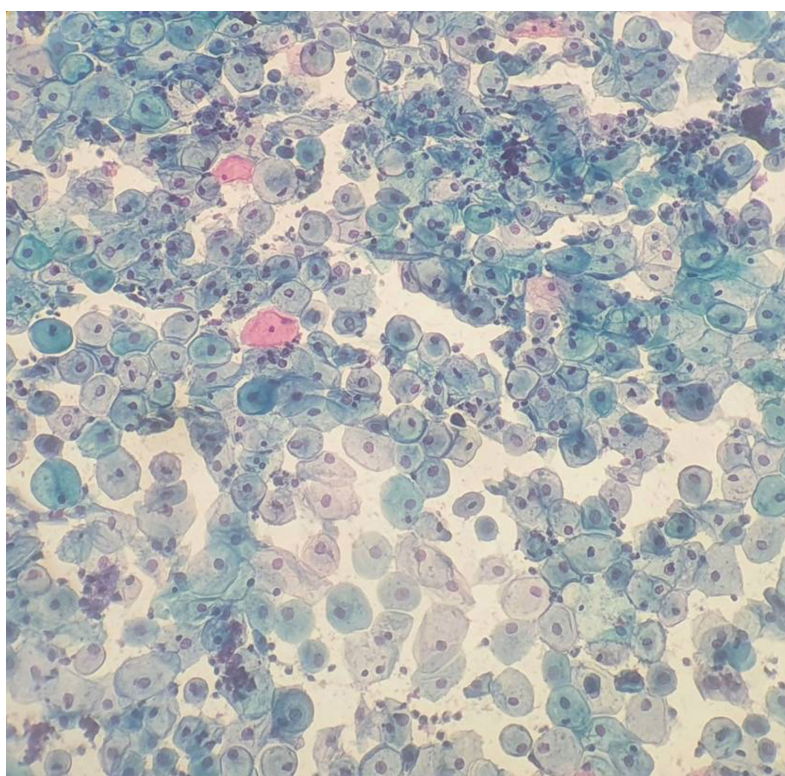
**Figure 2.** Intense atrophy, reddened epithelium with petechiae.



**Figure 3.** Normal epithelium after treatment.

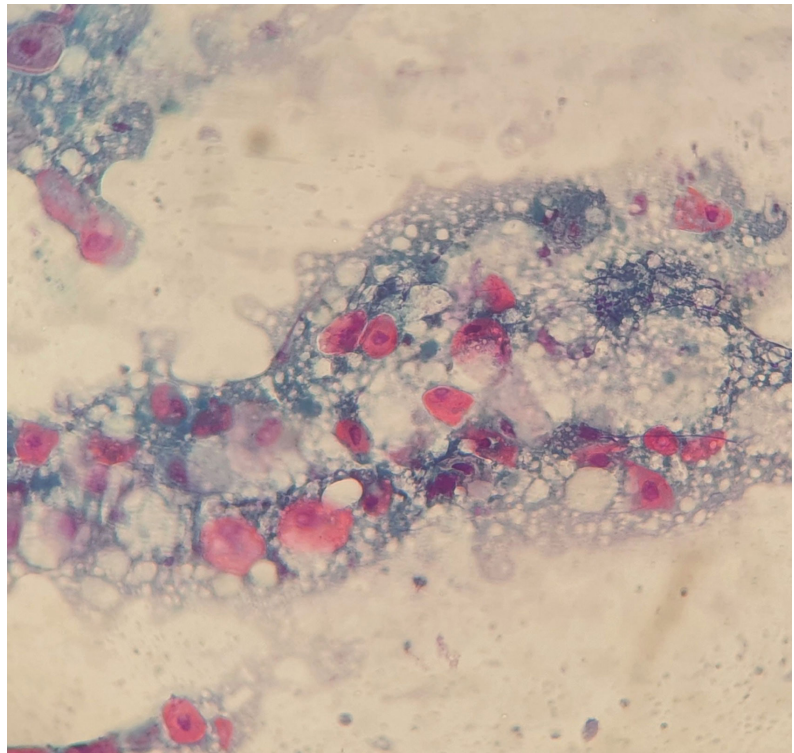
The cervicovaginal cytological examination carried out in patients at the end of the last treatment, showed the disappearance of the atrophy which was present in the pre-treatment cytology (Figure 4), and the presence of cytoplasmic inclusions of hyaluronic acid (Figure 5).

So far, 10 patients have been resubmitted to colposcopy and PAP-smear 6 months after the last treatment: of them, 8 maintained the improvements achieved both in the subjective symptoms and at colposcopy and cytology, while 2 reported a slight increase in vaginal dryness.



**Figure 4.** Pap smear cytology showing mostly immature basal cells, typical for vaginal atrophy.

**Figure 5.** Pap smear cytology 10 days after the end of the last treatment: prevalence of mature superficial cells showing intraplasmic inclusions of hyaluronic acid.



## Discussion

Vaginal atrophy is a very frequent condition in post-menopausal women (about 50%) or women with estrogen deficiency induced by other causes such as surgical castration, chemotherapy, or radiation treatments. This percentage is possibly underestimated as many women do not report some of the disorders associated with vaginal atrophy, especially those related to the sexual sphere<sup>4</sup>.

Post-menopausal hormone replacement therapy can prevent the onset of this condition or at least delay or mitigate it significantly. This option remains, at least in Italy, limited to a few cases, especially in patients with intense autonomic symptoms such as hot flashes, insomnia, decreased libido, anxiety<sup>18</sup>.

Furthermore, there are many situations in which replacement therapy is contraindicated as it represents an important risk factor for metabolic, thromboembolic, and oncological problems, especially in the field of breast cancer<sup>19</sup>.

Local estrogen therapy has played and plays an important role in maintaining good trophism of the vaginal mucosa, but even in this case not all patients accept it, and, in some situations, the effectiveness might not be optimal<sup>20</sup>.

Many topical, non-hormonal products on the market have demonstrated a positive action in promoting lubrication but are often not sufficient to solve all the problems related to alterations of vaginal trophism and allow only transient benefits, with the consequent need for repeated treatments, especially at the time of sexual intercourse<sup>21</sup>.

Even if the most recent treatments with fractionated CO<sub>2</sub> laser and radiofrequency have given significant improvements in the management of the pathology, some difficulties persist regarding the aspect of pain during the procedures<sup>22-24</sup>.

The use of the Caresflow<sup>®</sup> device, which delivers normobaric highly concentrated O<sub>2</sub> and hyaluronic acid, is an alternative to the devices that have so far been available. According to the data emerged from previous studies<sup>25,26</sup>, it appears to be one of the most performing methods to alleviate VVA symptoms. Our study corroborates the previous findings, as we observed a significant improvement both in the subjective symptoms reported by the patients and in the cytology of PAP-smear, the elasticity, and the trophism of the vaginal epithelium. The greatest benefit involved the reduction of vaginal dryness and the disappearance of dyspareunia, thus allowing an increased reported quality of life in over 98% of cases.

Our study is the first, to our knowledge, to have evaluated the actual presence of changes at PAP-smear cytology after the completion of the treatments: the presence of hyaluronic acid vesicles in the cytoplasm of the cells confirms the regenerative action at the tissue level, thus surpassing the surface action of many other methods and suggesting a serious possibility that the improvement in subjective and objective parameters may be lasting over time. This aspect will be analyzed by prolonging the period of follow-up of the treated patients, but the data we have obtained so far by retesting the patients 6 months after the end of treatment is promising.

### Conclusions

The data from this study confirm the information in recently published papers on the effectiveness of treatment with normobaric, highly concentrated oxygen, and hyaluronic acid on vaginal atrophy. Efficacy has been confirmed both in terms of subjective symptoms and at colposcopy plus cytology.

The demonstration of the integration of hyaluronic acid in microvesicles in the cytoplasm of vaginal cells constitutes further scientific confirmation of the regenerative activity endowed by Caressflow®.

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### Ethics Approval

All procedures performed in this study involving human participants followed the ethical standards of the institutional, national research committee and were in accordance with the 2013 Helsinki declaration or comparable ethical standards. All the patients participated to the present study on a volunteer basis. According to the study design, the Interprovincial CN-AT Ethical Committee gave exempt to ethical approval with decision protocol number 43/2021 on the 17th of February 2021.

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### Informed Consent

All the patients included in the study signed an informed consent prior to the procedure and agreed to the anonymous use of clinical data for research purposes.

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This research received no external funding.

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### Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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### Conflicts of Interest

The authors declare no conflict of interest.

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### References

- 1) Mac Bride MB, Rhodes DJ, Shuster LT. Vulvovaginal atrophy. *May Clin Proc* 2010; 85: 87-94.
- 2) Bachmann GA, Nevadunsky NS. Diagnosis and treatment of atrophic vaginitis. *Am Fam Physician* 2000; 61: 3090-3096.
- 3) Gass ML, Cochrane BB, Larson JC, Manson JE, Barnabei VM, Brzyski RG, Lane DS, LaValleur J, Ockene JK, Mouton CO, Barad DH. Patterns and predictors of sexual activity among women in the hormone therapy trials of the Women's health initiative. *Menopause* 2011; 18: 1160-1171.
- 4) Santoro N, Komi J. Prevalence and impact of vaginal symptoms among postmenopausal women. *J Sex Med* 2009; 6: 2133-2142.
- 5) Simon JA, Kokot-Kierpa M, Goldstein J, Nappi RE. Vaginal health in the United States: results from the vaginal health: insights views and attitudes survey. *Menopause* 2013; 20: 1043-1048.
- 6) Sopracordevole F, Chiossi G, Barbero M, Cristoforoni P, Ghiringhello B, Frega A, Tortolani F, Boselli F, Clemente N, Ciavattini A, Italian Society of Colposcopy and Cervico-Vaginal Pathology. Surgical approach and long-term clinical outcome in women with microinvasive cervical cancer. *Anticancer Res* 2014; 34: 4345-4349.
- 7) Gullo G, Etrusco A, Cucinella G, Perino A, Chiantera V, Laganà AS, Tomaiuolo R, Vitagliano A, Giampaolino P, Noventa M, Andrisani A, Buzzaccarini G. Fertility-Sparing Approach in Women Affected by Stage I and Low-Grade Endometrial Carcinoma: An Updated Overview. *Int J Mol Sci* 2021; 22: 11825.
- 8) Tanos P, Dimitriou S, Gullo G, Tanos V. Biomolecular and Genetic Prognostic Factors That Can Facilitate Fertility-Sparing Treatment (FST) Decision Making in Early Stage Endometrial Cancer (ES-EC): A Systematic Review. *Int J Mol Sci* 2022; 23: 2653.
- 9) Constantine GD, Simon JA, Pickar JH, Archer DF, Kushner H, Bernick B, Gasper G, Graham S, Mirkin S, REJOICE Study Group. The REJOICE trial: a phase-3 randomised controlled trial evaluating the safety and efficacy of a novel vaginal oestradiol soft-gel capsule for symptomatic vulvar and vaginal atrophy. *Menopause* 2017; 20: 888-902.
- 10) Biglia N, Peano E, Sgandurra P, Moggio G, Panuccio E, Migliardi M, Ravarino N, Ponzzone R, Sismondi P. Low-dose vaginal estrogens or vaginal moisturizer in breast cancer survivors with urogenital atrophy: a preliminary study. *Gynecol Endocrinol* 2010; 6: 404-412.

- 11) North American Menopause Society. Management of symptomatic vulvovaginal atrophy: 2013 position statement of the North American Menopause society. *Menopause* 2013; 24: 409-416
- 12) Athanasiou S, Pitsouni E, Grigoriadis T, Michailidis G, Tsiveleka A, Rodolakis A, Loutradis D. A study protocol of vaginal laser therapy in gynecological cancer survivors. *Climacteric* 2020; 23: 53-58.
- 13) Nappi RE, Biglia N, Cagnacci A, Di Carlo C, Luisi S, Paoletti AM. Diagnosis and management of symptoms associated with vulvovaginal atrophy: expert opinion on behalf of the Italian VVA study Group. *Gynecol Endocrinol* 2016; 17: 1-5.
- 14) Santoro N, Worsley R, Miller KK, Parish SJ, Davis MR. Role of estrogens and estrogen-like compounds in female sexual function and dysfunction. *J Sex Med* 2016; 13: 305-316.
- 15) Portman D, Palacios S, Nappi RE, Mueck AO. Ospemifene, a non-oestrogen selective oestrogen receptor modulator for the treatment of vaginal dryness associated with postmenopausal vulvar and vaginal atrophy: a randomised, placebo-controlled, phase III trial. *Maturitas* 2014; 78: 91-98.
- 16) Salvatore S, Leone Roberti Maggiore U, Athanasiou S, Origoni M, Candiani M, Calligaro A, Zerbinati N. Histological study of the effects of microablative fractional CO2 laser on atrophic vaginal tissue: an ex vivo study. *Menopause* 2015; 22: 845-849.
- 17) Jokar A, Davari T, Asadi N, Ahmadi F, Foruhari S. Comparison of the hyaluronic acid vaginal cream and conjugated oestrogen used in treatment of vaginal atrophy of menopause women: a randomised controlled trial. *Int J Community based Nurs Midwifery* 2016; 4: 69-78.
- 18) Manzoli L, Di Giovanni P, Del Duca L, De Aloysio D, Festi D, Capodicasa S, Monastra G, Romano F, Staniscia T. Use of hormone replacement therapy in Italian women aged 50-70 years. *Maturitas* 2004; 49: 241-251.
- 19) Deli T, Orosz M, Jakab A. Hormone Replacement Therapy in Cancer Survivors - Review of the Literature. *Pathol Oncol Res* 2020; 26: 63-78.
- 20) Lethaby A, Ayeleke RO, Roberts H. Local oestrogen for vaginal atrophy in postmenopausal women. *Cochrane Database Syst Rev* 2016; 2016: CD001500.
- 21) Sinha A, Ewies AA. Non-hormonal topical treatment of vulvovaginal atrophy: an up-to-date overview. *Climacteric* 2013; 16: 305-312.
- 22) Salvatore S, Nappi RE, Zerbinati N, Calligaro A, Ferrero S, Origoni M, Candiani M, Leone Roberti Maggiore U. A 12-week treatment with fractional CO2 laser for vulvovaginal atrophy: a pilot study. *Climacteric* 2014; 17: 363-369.
- 23) Zerbinati N, Serati M, Origoni M, Candiani M, Iannitti T, Salvatore S, Marotta F, Calligaro A. Microscopic and ultrastructural modifications of postmenopausal atrophic vaginal mucosa after fractional carbon dioxide laser treatment. *Lasers Med Sci* 2015; 30: 429-436.
- 24) Salvatore S, Mappi RE, Parma M, Chionna R, Lagona Z, Zerbinati N, Ferrero S, Origoni M, Candiani M, Leone Roberti Maggiore U. Sexual function after fractional microablative CO2 laser in women with vulvovaginal atrophy. *Climacteric* 2015; 18: 219-225.
- 25) Origoni M, Cimmino C, Carminati G, Iachini E, Stefani C, Girardelli S, Salvatore S, Candiani M. Postmenopausal vulvovaginal atrophy (VVA) is positively improved by topical hyaluronic acid application. A prospective, observation study. *Eur Rev Med Pharmacol Sci* 2016; 20: 4190-4195.
- 26) Condemi L, Di Giuseppe J, Delli Carpini G, Garroia F, Frega A, Ciavattini A. Vaginal natural oxygenation device (VNOD) for concomitant administration of hyaluronic acid and topical hyperbaric oxygen to treat vulvo-vaginal atrophy: a pilot study. *Eur Rev Med Pharmacol Sci* 2018; 22: 8480-8486.