

HISTOLOGICAL CHANGES IN THE EPITHELIUM OF THE VAGINA: ARE THEY IMPORTANT IN THE RESULT OF THE INTRAVAGINAL LASER TREATMENT?

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Abstract

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Objectives: 1. To demonstrate that vaginal tightening results improve at the progestational phase of the cycle. 2. To assess if menstrual cycle can be a conditioning factor in the results of vaginal tightening.

Method: Descriptive, comparative, cross-sectional study conducted on 20 women who attended consultations between October 2017 and December 2017. Two sessions with fractionated CO₂ laser and intravaginal handpiece were performed. The day of the cycle was taken into account to carry out the vaginal tightening, and Likert's scale was applied to the month of each session.

Results: The sample group included 20 women, most of them between 29 and 39 years of age (60% = 12 cases). A statistically significant association between the phase of the menstrual cycle and the level of friction during sexual intercourse after the second session was found. Women who reported a high degree of friction during sexual intercourse at the second tightening session registered a higher average of days of menstruation than those who reported a moderate degree of friction. This difference was statistically significant.

Conclusion: It was possible to determine in the study that the progestational phase improves the results of vaginal tightening with up to 100% and that menstrual cycle can be considered a conditioning factor for the results.

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Introduction

The beginning of laser in gynecology has been known since the 1980s, when the CO₂ laser began to be used as a tool in the treatment of pathologies of the lower genital tract.

Later, in 2010, the use of lasers in gynecology has been revolutionized, when an intravaginal device that would be used in different gynecological conditions appeared.

The purpose of using laser in the vaginal area is due to its capacities to stimulate the cells causing energy increase (ATP), normalization of the membrane potential, DNA activation and protein synthesis, leading to an accelerated cell multiplication of the fibroblasts, endothelial and epithelial cells. When this accelerated multiplication in fibroblast cells is produced, an activation of pre-collagen I and III, dilation of the endoplasmic reticulum, an

increase in the number of mitochondria and in the production of collagen substance also occur - therefore, an increase in collagen and elastic fibers is promoted. There is an increase in vascular neoformation on the side of endothelial cells, and epithelial cells lead to an accelerated re-epithelialization. These changes cause the accelerated and complete repair of the tissues (1).

The vaginal wall is composed of four layers (2): superficial non-keratinized stratified squamous epithelium; lamina propria, a layer of dense connective tissue; muscular layer composed of internal and external fibers of longitudinal smooth muscle; and the adventitia, a layer of tissue rich in connective, collagen and elastic fiber that supports the vaginal wall. The mucosal epithelium shows an estrogen-dependent behavior and function that reacts naturally to the hormonal fluctuations that occur during a woman's life, as well as during the menstrual cycle (3). The estrogenized epithelium is rich in glycogen, which is fermented by lactobacilli, lowering the vaginal pH.

However, it is important to point out that, just as the vaginal epithelium in the estrogenic phase has all its cellular layers (basal, parabasal, intermediate and superficial), in the luteal or progestational phase, they decrease with the epithelium being predominant over the basal and parabasal cells, especially when approaching to the period of menstruation, described as a phase of desquamation. This results in a modification of the thickness of the epithelium, ranging from a thickness of 230 to 300 microns in the estrogenic phase and of 150 to 180 microns in the progestational phase (4, 5). In addition, a modification of 2 to 5 mm is also described according to the hormonal stimulus, because epithelial cells contain intranuclear receptors for sex steroids. That is why the thickness and maturation of the epithelium vary in each menstrual cycle (6).

The lamina propria is composed mainly of collagen and elastin fibers and contains a dense plexus of small blood vessels, lymphatic vessels and nerves. It is denser towards the surface and freer towards the muscular layer. In the anterior vaginal wall, the papillae of the lamina propria are scarce, but in the posterior wall they are prominent and deep. Collagen and elastin participate in the control of the biomechanical properties of vaginal tissue (2). Collagen fibers are rigid and do not distort easily, while elastin fibers provide elasticity to the tissues.

Currently, laser therapy has a therapeutic role in various medical conditions; more recently, it has gained interest as a non-hormonal treatment for the genitourinary syndrome of menopause (GSM), as a non-invasive option for stress urinary incontinence (SUI) and in the syndrome of vaginal laxity (SLV). Both the microablative fractional CO₂ laser

and the non-ablative vaginal Er: YAG laser (VEL) induces morphological changes in the vaginal tissues (7).

Regarding the Vaginal Laxity Syndrome, few works have been published with different results, however, in the pursue to improve this condition, we must take into account those factors that may influence it, such as: personal history, obstetric history of the woman, energy density that we apply, the wavelength we are using; also, an important factor studied by us is the complex of histological changes of the vaginal epithelium (cycle vaginal) under the influence of estrogen, since there is a significant change in the thickness of the epithelium, which can modify the penetration of the laser beam between one phase and another.

Reasons for which the following objectives are proposed: 1. To demonstrate that the results of vaginal tightening for vaginal laxity improve when performed in the progestational phase of the cycle, and 2. To study if the menstrual cycle can be a conditioning factor in the results of vaginal tightening.

Method

We have performed a descriptive and comparative, cross-sectional prospective study with a two-time (first session and second session), non-experimental design. The study included 20 patients who attended the consultation at Centro Clínico Dr. Rafael Guerra Méndez, Tower E, due to the syndrome of vaginal laxity between October 2017 and December 2017, in the city of Valencia, Venezuela. Patients were included after explaining the study and after signing the informed consent. Two sessions with fractionated CO₂ laser and intravaginal handpiece were performed as treatment, at an interval of 30 to 45 days between each session. The day of the cycle was taken into account to asses out the vaginal tightening and Likert's scale was applied in regard to the improvement of friction during sexual intercourse one month after each session. The following question was asked: *How is the friction during sexual intercourse post-treatment with intravaginal laser?* Patients had the option to answer: None, Mild, Moderate or High. Menopausal patients and those who were under exogenous hormonal stimulation as contraceptives were excluded from this study. The approval of the Ethics Committee of Clinical Center Dr. Rafael Guerra Mendez has also been obtained before the treatment.

Once obtained, results were systematized in a master table made in Microsoft® Excel to be presented in association and average tables. The level of friction during sexual intercourse after each tightening session was associated according to the age groups and according to the phase of the menstrual cycle presented by the patient from

DATA OBTAINING CHART

Age	Pregnancies	Day cycle	Answer first session	Day cycle	Answer second session
38	2L	17 PP	High	10 FE	Moderate
42	1L	19 PP	High	14 PE	Moderate
29	0	8 PE	Moderate	20 PP	High
44	1C	23 PP	High	24 PP	High
50	1C	20 PP	High	17 PP	High
39	1L1C	11 PE	Mild	20 PP	High
39	2L	25 PP	Moderate	23 PP	High
46	1L1C	20 PP	High	20 PP	High
29	2L	11 PE	Moderate	25 PP	High
35	1C	8 PE	Moderate	10 PE	Moderate
43	1L1C	11 PE	High	11 PE	High
32	1L	22 PP	High	22 PP	High
36	1C	9 PE	Moderate	9 PE	Moderate
37	3L	23 PP	High	23 PP	High
35	2L	10 PE	High	11 PE	High
42	1L	23 PP	Moderate	23 PP	High
35	3L	28 PP	High	26 PP	High
38	2C	12 PE	Moderate	12 PE	Moderate
40	3L	7 PE	High	22 PP	High
45	2L	10 PE	Moderate	27 PP	High

Source: own research data (Gómez; 2017).

PE: Phase Estrogenic; PP: Phase Progestational; L: labor; and C: Cesarean

the non-parametric analysis of Chi square (χ^2) for independence between variables. The same way, the exact day after menstruation was compared to the moment of the vaginal tightening session using Kruskal Wallis test (first session) and Mann Whitney (Wilcoxon) (second session) to compare averages. For this process the Statgraphics Plus 5.1 statistical processor was used, adopting values less than 0.05 ($P < 0.05$) as a P level of statistical significance.

In addition, samples of vaginal epithelium of the right lateral wall were taken in two women, in different phases of the cycle (Figure 1): (i) First patient stratified flat epithelium day 9 of the cycle; (ii) stratified flat epithelium on day 27 of the cycle (Figure 2): (i) Second patient: Stratified flat epithelium day 8 of the cycle; (ii) Stratified flat epithelium on day 19 of the cycle. Measurements were made with an Olympus Cx-31 microscope at 40x mag-

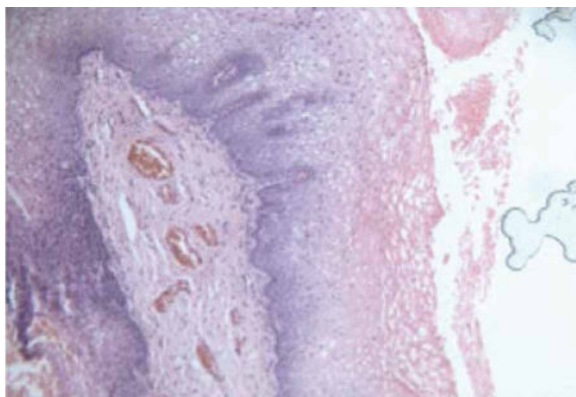
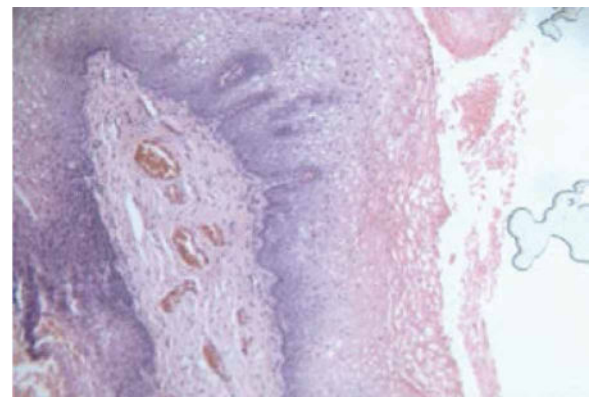


Figure 1. (i) First patient, 40 years old.
Day 9 of cycle (estrogenic phase).
Epithelium measure: 0.04 mm.



(ii) First patient, 40 years old.
Day 27 of cycle (progestational phase).
Epithelium measure: 0.01 mm.

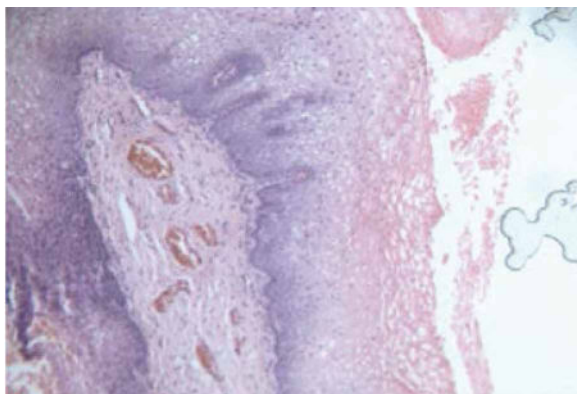
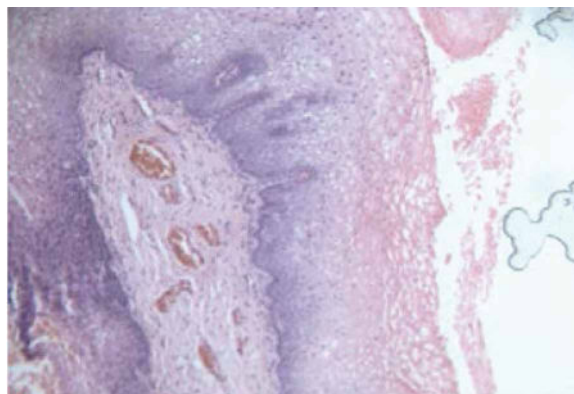


Figure 2. (i) Second patient, 29 years old.
Day 8 of the cycle (Estrogenic phase).
Epithelium measure: 1 mm.



(ii) Second patient, 29 years old.
Day 19 of the cycle (Progestational phase).
Epithelium measure: 0.02 mm.

nification with an Olympus graduated micrometer eyepiece.

Results

Of the 20 patients subject to vaginal tightening, an average age of $38.7 \text{ years} \pm 1.23$ was recorded, with a median of 39 years, a minimum age of 29 years, a maximum age of 50 years and a coefficient of variation of 14% (homogeneous series between their data). Most of the patients included in the study were between 29 and 39 years of age (60% = 12 cases).

DISTRIBUTION OF PATIENTS SUBJECT TO VAGINAL TIGHTENING WITH FRACTIONED CO₂ LASER IN STUDY

Age (years)	n	%
29 – 39	12	60,0
40 – 50	8	40,0
Pregnancies		
0	1	5
1	7	35
2	9	45
3	3	15
Total	20	100
Labor		
1	6	42,86
2	5	35,71
3	3	21,43
Total	14	100
C-Section		
1	7	87,5
2	1	12,5
Total	8	100

Source: own research data (Gómez; 2017)

Patients with two pregnancies were more prevalent (45% = nine cases), followed by those with one pregnancy (seven cases). Those with one labor (six cases) were more frequent, followed by those with two labors (five cases). Those with one caesarean section (seven cases) were more frequent as well.

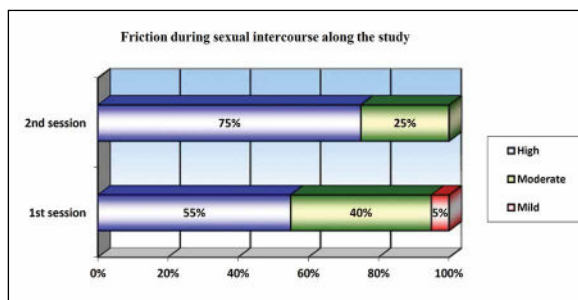
Patients with high friction during sexual intercourse after the first session were more frequent (55% = 11 cases), especially among those between 40 and 50 years of age (6/8). For the second session, high friction during sexual intercourse after vaginal tightening increased to 75% (15 cases), being most frequent in both age groups; however, the highest percentage was registered in those between 40 and 50 years of age (7/8). No statis-

Age	29 – 39		40 – 50		Total	
Friction during sexual intercourse after vaginal tightening (first session)	n	%	n	%	n	%
	1	8,3	0	0,0	1	5,0
	6	50,0	2	25,0	8	40,0
	5	41,7	6	75,0	11	55,0
Friction during sexual intercourse after vaginal tightening (second session)	n	%	n	%	n	%
	4	33,3	1	12,5	5	25,0
	8	66,7	7	87,5	15	75,0
	12	100,0	8	100,0	20	100,0

Source: own research data (Gómez; 2017)

Table 1. Results of the vaginal tightening according to age of the patient in two sessions

tically significant association between the age groups and the level of friction after both sessions was found ($P > 0.05$)



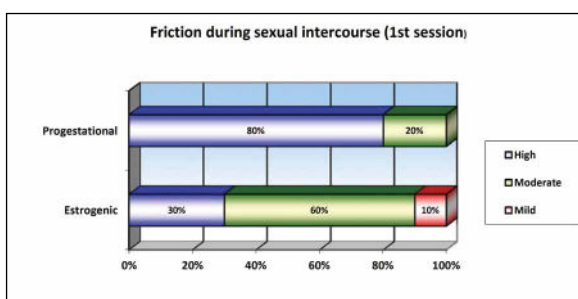
Source: own research data (Gómez; 2017)

Phase (first session)	Estrogenic		Progestational		Total	
Friction during sexual intercourse after vaginal tightening (first session)	n	%	n	%	n	%
Mild	1	10,0	0	0,0	1	5,0
Moderate	6	60,0	2	20,0	8	40,0
High	3	30,0	8	80,0	11	55,0
Total	10	100,0	10	100,0	20	100,0

Source: own research data (Gómez; 2017)

Table 2. Results of the vaginal tightening according to the phase of the menstrual cycle after the first session

At the first session of laser tightening, half of the patients were in estrogen phase (10 cases) from which the prevailing friction during sexual intercourse was moderate (six cases). High friction prevailed in 80% (eight cases) of patients who were in the progestational phase. No statistically significant association was found between the phase of the menstrual cycle and the level of friction during sexual intercourse after the first session ($\chi^2 = 5.27$, 2 gl, P value = $0.0716 > 0.05$)

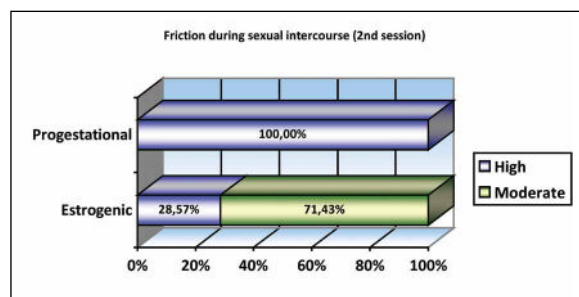


Source: own research data (Gómez; 2017)

Phase (second session)	Estrogenic		Progestational		Total	
Friction during sexual intercourse after vaginal tightening (second session)	n	%	n	%	n	%
Moderate	5	71,4	0	0,0	5	25,0
High	2	28,6	13	100,0	15	75,0
Total	7	100,0	13	100,0	20	100,0

Source: own research data (Gómez; 2017)

Table 3. Results of the vaginal tightening according to the phase of the menstrual cycle after the second session



Source: own research data (Gómez; 2017)

Friction during sexual intercourse after vaginal tightening (first session)	N	Days FUR (first session) Md - RI	min	Max	KW	p
Mild	1	11 - 0	11	---	1,89	0,3879
Moderate	8	10,5 - 9	8	25		
High	11	20 - 12	7	28		
Friction during sexual intercourse after vaginal tightening (second session)	N	Days FUR (second session) Md - RI	min	Max	KW	p
Moderate	5	10 - 2	9	14	71	0,0038*
High	15	22 - 4	11	27		

Source: own research data (Gómez; 2017)

*Denotes a statistically significant difference ($p < 0.05$)

Table 4. Comparison of days subsequent to menstruation at the time of vaginal tightening according to the friction level after both sessions

At the second session of tightening, patients in the progestational phase prevailed, and all of them reported a high friction during sexual intercourse (13 cases); in patients in the estrogenic phase (seven cases), the friction after tightening was moderate (five cases). There was a statistically significant association between the phase of the menstrual cycle and the level of friction during sexual intercourse after the second session ($\chi^2 = 8.86$, 1 gl, P value = 0.0014 < 0.05).

Although the highest average of days after the menstruation was recorded by those women who reported a high degree of friction during sexual intercourse in the first tightening session, this difference was not statistically significant ($P > 0.05$).

In the second session of tightening, those women who reported a high degree of friction during sexual intercourse, recorded an average of days after the menstruation much higher than those that reported a moderate degree of friction, this difference being statistically significant ($P < 0.05$).

Discussion

Of the 20 patients subject to vaginal tightening, an average age of 38.7 years $\pm$ 1.23 was recorded, with a median of 39 years, a minimum age of 29 years, a maximum age of 50 years and a coefficient of variation of 14% (homogeneous series between their data), being more frequent those women with 29 and 39 years (60% = 12 cases). Patients with two pregnancies were more frequent (45% = nine cases), followed by those with one pregnancy (seven cases).

A pilot study conducted in Venezuela in 2012 by Dr. Gaviria P. Jorge E, Lanz Z. José, on "Laser Tightening (LVT) - evaluation of a novel noninvasive laser treatment for vaginal relaxation syndrome", was carried out in 21 patients, of whom five suffered prolapse, showing improvement after two laser sessions in the five of them, by reducing the prolapse. Regarding the relaxation of the vagina with respect to sex, 76.2% reported a moderate improvement and 19% reported an important improvement. A questionnaire about sexual satisfaction was also applied and if answered affirmatively, they were four options to check: more friction/sensitivity, better orgasms, more orgasms and no improvement. 95.2% chose the first option and 57.1% the second option (8).

Although this study does not describe whether there is a correlation between the age and the increased friction, it agrees that intravaginal laser treatment improves the symptoms of vaginal laxity. In the sample studied, no statistically significant association was found between the age groups and the level of friction after both sessions ($P > 0.05$). In the first session those patients with high friction during sexual intercourse were more frequent (55% = 11 cases), symptom which was most

prevalent among those between 40 and 50 years of age (6, 8). For the second session, high friction during sexual intercourse after vaginal tightening increased to 75% (15 cases), being the most frequent result in both age groups; however, the highest percentage was registered in those between 40 and 50 years of age (7/8).

In the first session of tightening, half of the patients were in the estrogenic phase (10 cases) and in this group the friction during sexual intercourse was moderate (six cases). High friction prevailed in 80% (eight cases) among the patients who were in the progestational phase. No statistically significant association was found between the phase of the menstrual cycle and the level of friction during sexual intercourse after the first session ($\chi^2 = 5.27$, 2 gl; P value = 0.0716 > 0.05).

In the second session of the tightening, patients who were in progestational phase prevailed, and all of them reported high friction during sexual intercourse (13 cases). For patients in the estrogenic phase (seven cases), the friction after tightening was moderate (five cases). A statistically significant association was found between the phase of the menstrual cycle and the level of friction during sexual intercourse after the second session ($\chi^2 = 8.86$, 1 gl, P value = 0.0014 < 0.05).

In the first session of tightening, although the highest average number of days after the menstruation was recorded by those women who reported a high degree of friction during sexual intercourse, this difference was not statistically significant ($P > 0.05$).

In the second tightening session, those women who reported a high degree of friction during sexual intercourse recorded an average number of days after menstruation (or menstruation) much higher than those who reported a moderate degree of friction, this difference being statistically significant ($P < 0.05$).

A study carried out in the Department of Gynecology of the Hospital de Raffaele performed by S. Salvatore in patients with urogenital atrophy, reported an improvement in laxity in 40% of patients after the first session, followed by 60% in the second session and 90% in the third session (9); these results demonstrate the utility of laser treatment in these gynecological conditions. However, when searching for bibliographic references, no studies related to the results of vaginal tightening and the improvement of friction taking into account the vaginal cycle have been found so far, neither in Spanish nor in English.

Lucia H. Cardenal describes a variation of the epithelium that may range from 2 to 5 mm thickness according to the hormonal stimulus. The estrogen stimulus increases the thickness of the epithelium and the progestational makes it mature. In the present work, when samples of the epi-

thelium of two women who were not exposed to exogenous hormones, both in the estrogenic and progestational phases were taken, the change in thickness was observed (6).

Conclusion

To obtain optimal results in the treatment of vaginal laxity with laser we must take into account different factors that may or may not be related to the patient, in this case no bibliography was found related to the histological changes of the vaginal epithelium (vaginal cycle) and the result of the treatment. In the present study, it was possible to determine that when performing this procedure in the progestational phase, the results can improve up to 100% and that the menstrual cycle can be

taken into consideration as a conditioning factor for the results.

We conclude it is not imperative that the treatment of vaginal tightening has to be performed in the estrogenic phase, but we suggest that the energy density must be increased in order to achieve a deeper penetration of the ray in the epithelium during this phase. This is a factor that can be taken into account when performing laser treatments or other technologies for intravaginal use, as in the case of Stress Urinary Incontinence.

Conflicts of interest: none declared.

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Informed consent obtained.

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