SANTEC
Gynecology Energy Company

COMPENDIUM DEGLI STUDI SCIENTIFICI

Elettroporazione in Ginecologia

per

GSM (Sindrome genito-urinaria della menopausa)

Giugno 2024

Titolo . L'elettroporazione vaginale nella terapia della vulvodinia localizzata al vestibolo (vestibolodinia): studio pilota

F.Murina
Servizio di Patologia Vulvare-Osp.V.Buzzi-ICP-Università di Milano

Introduzione. L'eziologia della vestibulodinia (VBD) non è ancora pienamente nota.
Diversi fattori causali sono stati proposti, ed un ruolo rilevante è rappresentato dall'infiammazione neurogenica con conseguente sensibilizzazione periferica e centrale.
Non esiste un trattamento standard della malattia, e pochi studi randomizzati e controllati sono stati effettuati. Le raccomandazioni sono a favore di un approccio multi-dimensionale, concentrandosi sulla gestione del dolore e sul ripristino di una corretta funzione del pavimento pelvico.
La terapia farmacologica (topica od orale) è un pilastro della gestione della VBD.
L' amitriptilina è un antidepressivo triciclico che tratta efficacemente molte condizioni di dolore cronico neuropatico. La sua attività nella modulazione del dolore include l'inibizione centrale della ricaptazione neuronale dei neurotrasmettitori e nell' inibizione dei canali del sodio.
Sebbene la somministrazione orale di amitriptilina è il gold standard per il trattamento del dolore neuropatico e spesso viene utilizzata nella VBD, gli effetti collaterali sistemici quali ipotensione posturale, sedazione, e gli effetti anticolinergici ne limitano il raggiungimento delle dosi terapeutiche.
L'uso a lungo termine della lidocaina topica è stato suggerito come una terapia specifica per la VBD, con il razionale che l'applicazione regolare di lidocaina interrompe gli impulsi dolorosi e riduce l'amplificazione del dolore .
L 'uso di anestetici locali a lungo termine può causare prurito o sensibilizzazione, con grave reazione e dermatite da contatto, in particolare con prodotti come la benzocaina.
La stimolazione elettrica transcutanea (TENS) con correnti bifasiche di frequenze tra 2 e 100 Hz e 50-100 micros di durata dell'impulso, è stata ampiamente utilizzata nel trattamento di VBD con un' elevata efficacia (75%) superiore al placebo.
L'elettroporazione (EP) è la variazione strutturale transitoria delle membrane cellulari a seguito dell'applicazione di impulsi ad alta tensione. La sua applicazione cutanea ha dimostrato di aumentare il rilascio transdermico di farmaci con diverso ordine di grandezza.
Inoltre l' EP amplia la gamma di farmaci (macromolecole, lipofile o idrofile, cariche o molecole neutre) che possono essere rilasciate per via trans dermica.
Obiettivo del nostro studio è stato quello di valutare l'efficacia dell' EP applicata tramite sonda vaginale, utilizzando un mix di due farmaci: amitriptilina + lidocaina, in pazienti con una diagnosi di VBD. Le premesse sono quelle di raggiungere l'area di interesse clinico con una concentrazioni più elevata di principi attivi , superiore alla somministrazione sistemica ma con un minor numero di effetti collaterali.

Materiali e Metodi. Le pazienti arruolate nello studio sono state 17. Tutte avevano una diagnosi di VBD caratterizzata dalla coesistenza delle seguenti condizioni: anamnesi di dolore vulvare cronico alla stimolazione od al tentativo di penetrazione, ipersensibilità al toccamento vestibolare con l'apice di un cotton e l'assenza di manifestazioni obiettive clinicamente evidenziabili.
Sono state escluse le donne in gravidanza, portatrici di pacemaker, con infezioni vulvo-vaginali ed affette da patologie neurologiche. Le pazienti arruolate hanno compilato un questionario riguardante la loro situazione vulvo-vaginale, la salute in generale, caratteristiche demografiche ed alcune domande inerenti fattori psicosessuali.
Successivamente ogni paziente è stata sottoposta ad esame obiettivo ginecologico previa valutazione sintomatologica vulvo-vaginale, riguardante in dettaglio:
-Scala analogica visiva del dolore (VAS) graduata da 0 a 10 (0=assenza dolore, 10=massimo dolore)
-Dispareunia graduata tramite score di Marinof (0=assente; 1=lieve; 2=dolore più intenso con riduzione frequenza dei rapporti; 3=penetrazione pressoché improponibile).
Le pazienti, inoltre, sono state sottoposte ad una valutazione elettrodiagnostica sensoriale della conduzione nervosa delle terminazioni vestibolari attraverso la misurazione della minima soglia di percezione dello stimolo sensitivo (CPT) vestibolare.
Il valore della CPT è stato misurato attraverso la strumentazione Neurometer (Neurotron, Inc., Baltimore,MD), stimolatore elettrodiagnostico che emette stimoli di corrente alternata alla frequenza di 2,000 Hz (specifica per le fibre mieliniche Aβ), 250 Hz (specifica per le fibre Aδ) e 5 Hz (specifica per le fibre C) a livelli d'intensità da 0.001 a 9.99 mA.
Ogni donna inserita nello studio ha ricevuto una sessione settimanale di EP vaginale per un totale di otto sedute attraverso una sonda vaginale in plastica (Bluemoon-Italia) con due anelli metallici trasversali come elettrodi collegata ad un' unità calibrata per l' EP (Bluemoon-Italia).
La nuova sonda è stata realizzata con un alloggiamento per una siringa da 2,5 ml, dove è stato posto il prodotto da veicolare nella mucosa vestibolare.
Nella siringa monouso è stato inserito un mix di farmaci costituito da: amitriptilina alla dose di 60 mg e lidocaina al dosaggio di 40 mg, il tutto dissolto in un gel conduttore.
L'unità di EP è stata settata con i seguenti parametri TENS, in accordo a precedenti studi che hanno utilizzato la tecnica nella terapia della VBD:
- I fase: frequenza di 100 Hz - durata impulso 50 µs-durata 15 min.
- II fase: frequenza di 5 Hz - durata impulso 100 µs -durata 15 min.
Le pazienti sono state rivalutate a fine trattamento sia dal punto di vista sintomatologico (VAS e score di Marinof della dispareunia), che obiettivamente tramite ri-valutazione della CPT.

Risultati. Tutte le pazienti arruolate hanno concluso il protocollo di cura ,e nessuna è stata persa al follow-up. Le caratteristiche delle pazienti studiate sono riassunte nella Tab 1.
La VAS si è ridotta da un valore basale di 7.4 (1.2) ad uno post terapia di 6.3 (1.8), e malgrado la variazione apparentemente non di ampia entità questa si è dimostrata statisticamente significativa (p 0.05). Anche la dispareunia ha subito una riduzione, passando dal valore basale di 2.3 (1.0) ad uno post-cura di 1.8 (1.1) senza però una significatività statistica (Tab.2).
La Tab.3 riassume i valori della CPT basali e post-terapia. La maggior variazione ha riguardato la sottopopolazione delle fibre nocicettive C con il 24.4% di riduzione della sensibilità.
Nel gruppo di fibre Aβ ed Aδ la riduzione percentuale è stata rispettivamente del 15.5% e 20.4%.
Non ci sono stati effetti collaterali rilevanti che hanno indotto la sospensione del trattamento; un lieve bruciore transitorio post-terapia si è avuto solo nel 7% circa dei casi, ed una transitoria sedazione nel 10% circa delle pazienti.

Discussione. In sintesi possiamo porre in risalto le seguenti considerazioni:

-Nella PVD, un dato fisiopatologico unanime è la proliferazione delle terminazioni nervose nella mucosa vestibolare, considerata una reazione aspecifica di precedenti fattori scatenanti mucosi, come infezioni, traumi e fattori ormonali . Un altro fattore osservato nelle donne con questa malattia è la disfunzione dei muscoli del pavimento pelvico, contratti attorno alla parte distale della vagina, probabilmente come reazione al dolore persistente.

-L'amitriptilina è il farmaco orale maggiormente utilizzato, sebbene non è ben chiara la sua reale utilità nella terapia della VBD. I dosaggi comunemente impiegati sono variabili (30-100 mg) come pure variabile è la percentuale di risposta ,che può avere un range tra 30-60%.
Ciò deriva ,principalmente, dalla difficoltà nel raggiungimento di un ottimale soglia terapeutica derivante dall'elevato numero di drop-out conseguente alla comparsa di effetti collaterali.

-La lidocaina, anestetico locale, è stato impiegato oltre che come topico locale anche in infiltrazioni del nervo pudendo a vari livelli con la finalità di ridurre l'attività ectopica neuronale, alla base dell'allodinia ed iperalgesia , elementi peculiari sia delle sindromi da dolore neuropatico che della VBD.

-L'approccio multimodale alla VBD è quello ritenuto essere oggi ottimale, ed in quest'ottica associare la TENS all'uso di farmaci può rivelarsi una strategia vincente.

-L'EP consente di abbinare l'elettrostimolazione alla veicolazione tran mucosa di macromolecole anche in elevate concentrazioni, che si concentrano nel sito di scatenamento del dolore, il vestibolo vaginale, riducendo l'incidenza di effetti collaterali.

-I nostri risultati suggeriscono l'efficacia della tecnica. I dosaggi di amitriptilina e lidocaina impiegati sono stati elevati e nessuna paziente ha sospeso il trattamento per la comparsa di effetti collaterali, e l'effetto sinergico della TENS ha ulteriormente contribuito al "reset" del sistema nocicettivo alterato.

-La dimostrazione dell'assorbimento ed azione dei farmaci a seguito della veicolazione tramite EP vaginale, è dimostrato dai valori della CPT. La media di riduzione della sensibilità delle fibre

nervose vestibolari del 20% circa, è prova dell'effetto obiettivo del binomio TENS-farmaci nella gestione della disfunzione del sistema di percezione del dolore propria della VBD.

-La variazione non elevata dei sintomi potrebbe essere correlata alla frequenza ridotta (1 appl./settimana) ed al numero relativamente basso di sedute di EP vaginale.
Infatti, precedenti studi sulla TENS hanno portato a risultati brillanti con una media di applicazioni pari a circa 20. Inotre, le pazienti reclutate avevano già utilizzato precedenti terapie con efficacia limitata o transitoria, portando a definirle come "difficili".

-Alla luce di quanto sovra descritto l' EP vaginale può definirsi come tecnica efficace e promettente nella gestione della VBD, sebbene ulteriori studi sono necessari per definire il numero e la frequenza delle sedute necessarie, come pure il mix di farmaci da utilizzare. Inoltre, ciò pone le basi per l'utilizzo della tecnica in altre problematiche vulvo-vaginali che necessitano l'uso di farmaci localmente.

Bibliografia

- Tympanidis P, Terenghi G, Dowd P. Increased innervation of the vulval vestibule in patients with vulvodynia. Br J Dermatol 2003;148(5):1021–7.

- Groysman V. Vulvodynia: new concepts and review of the literature. Dermatol Clin. 2010 Oct;28(4):681-96.

- Graziottin A , Murina F. Clinical Management of Vulvodynia - Tips and Tricks. Milan: Springer-Verlag; 2011:61-62.

- Haefner HK , Collins ME, Davis GD, Edwards L, Foster DC, Hartmann ED, et Al. The vulvodynia guideline. J Low Genit Tract Dis. 2005;9(1):40-51

- Zolnoun, D.A., Hartmann, K.E. and Steege, J.F., Overnight 5% lidocaine ointment for treatment of vulvar vestibulitis, Obstet Gynecol, 102 (2003) 84-7.

- Murina, F., Bianco, V., Radici, G., Felice, R., Di Martino, M. and Nicolini, U., Transcutaneous electrical nerve stimulation to treat vestibulodynia: a randomized controlled trail, BJOG, 115 (2008) 1165-70.

- A.-R. Denet et al. Skin electroporation for transdermal and topical delivery. Advanced Drug Delivery Reviews 56 (2004) 659–674

- Lombry C., Dujardin V. Transdermal Drug Delivery of Macromolecules Using Skin Electroporation. Pharmaceutical Research, Vol. 17, No. 1, 2000

Table 1. Characteristics of the Study Population.

Age, mean (range) y	32.5 (20-48)
Nulliparous, n (%)	15/17 (88)
Duration of symptoms, mean (range), mo	27.5 (6-80)
VAS, mean (SD)	7.4 (1.2)
Marinoff dyspareunia scale, mean (SD)	2.3 (1.0)

VAS, visual analogue scale

Table 2. Post treatment scores of VAS and Marinoff dyspareunia scale

	n. 17 Patients	p
VAS, mean (SD)	6.3 (1.8)	0.05
Marinoff dyspareunia scale, mean (SD)	1.8 (1.1)	NS

VAS, visual analogue scale

Table 3. Results of CPT measurement (100 = 1.0 mA) at 3 selected stimulation frequencies before and after therapy

N. 17 patients	2,000 Hz (Aβ fibers)	250 Hz (Aδ fibers)	5 Hz (C fibers)
Basal	442,2	201,5	90,8
After therapy	523,4	253,3	120,2
Difference, %	*15,5*	*20,4*	*24,4*

Data are expressed as means (Hz = cycles per second).
CPT, current perception threshold

L' Elettroporazione Vaginale : integrazione ed innovazione nel trattamento di patologie ginecologiche complesse

Filippo Murina - Raffaele Felice
Servizio di Patologia Vulvare-Osp.V. Buzzi - ICP - Università di Milano

L'elettroporazione (EP) è una tecnica di terapia fisica caratterizzata da una variazione transitoria della membrana cellulare conseguente all'applicazione di impulsi ad alto voltaggio, che consentono la veicolazione intracellulare di sostanza ad elevato peso molecolare.
L'applicazione cutanea dell' EP si è dimostrata efficace e facilmente utilizzabile, anche in sedi corporee contraddistinte da un consistente strato corneo, tanto da superare i limiti di penetrabilità di tecniche quali la ionoforesi o gli ultrasuoni. La veicolazione transdermica consente di ottenere un'elevata biodisponibilità del principio attivo nella sede dove si vuole esercitare il massimo effetto, e nella riduzione di effetti collaterali conseguente alla somministrazione sistemica dei farmaci.
Gli elementi determinanti l'elevata veicolazione di principio attivo sono principalmente il peso molecolare delle sostanze che devono oltrepassare la barriera dermica, e le caratteristiche del reservoir all'interno del quale queste vengono disciolte. L' EP si è dimostrata non avere un cut-off di peso molecolare, consentendo il trasporto di molecole anche oltre i 40 kDa (es. buprenorfina e fentanyl). Nell'EP vengono applicati degli impulsi elettrici che generano un potenziale di membrana tra 0.5-1.0 V che ha durata tra 10 μs e 10 ms. Questa reversibile caduta di potenziale trans-membrana della cellula induce la formazione di "pori" che inducono la facilitazione nel passaggio molecolare. Il dispositivo di elettroporazione EPV® (Blue-Moon® Italia), è dotato di due applicatori specifici a uso vaginale, uno standard e uno ridotto da utilizzare in caso di sub-stenosi vaginale. Questi sono composti da una struttura in materiale plastico opportunamente studiata per favorire l'introduzione e comprendente l'alloggiamento di una siringa standard da 2.5 e 1 ml, dove viene inserito il prodotto da veicolare disciolto in un gel conduttore (Fig1). Gli impulsi di EP sono generati su due anelli di acciaio inox chirurgico, posizionati a una distanza ottimale per investire un'ampia zona senza la necessità di muovere l'applicatore. La veicolazione di principi attivi in sede vestibo-vaginale è resa ancora più agevole dal ridotto spessore dello strato epiteliale, che consente di raggiungere organi pelvici adiacenti, sovente interessati da sindromi dolorose di difficile gestione terapeutica. I farmaci che l'EPV® può veicolare sono molteplici, ma soprattutto è possibile combinare più principi attivi a target terapeutico differente, creando una sorta di "infiltrazione virtuale" innocua, riproducibile ed in grado di garantire l'elevata biodisponibilità del farmaco dove è necessaria la sua massima azione. Inoltre, l 'EPV® appare tecnica interessante, versatile ed innovativa per la veicolazione di principi attivi nella sede vestibolo e vaginale, aree dove in prevalenza vengono utilizzati prodotti topici. La penetrazione trans mucosa incrementa la persistenza del principio attivo con un effetto "simil depot", in grado di svolgere un'azione propedeutica nell'impiego di prodotti somministrati per via topica.

<u>Impieghi in ginecologia.</u>

Una delle più importanti ed innovative applicazioni di EPV® è rappresentata dalla ***sindrome da dolore pelvico cronico*** , e dalle condizioni ad esso correlate.
Il dolore pelvico cronico (CPP) è definibile come dolore non derivante da neoplasie maligne riferito ad organi e strutture della pelvi, sia femminile che maschile. Il CPP è caratterizzato da un impatto negativo sulla sfera cognitiva, comportamentale sessuale ed emotiva. Nella donna il CPP trova

classificazione appropriata come persistente o ricorrente dolore pelvico associato a sintomi riferibili al tratto genito-urinario inferiore, con disfunzioni intestinali e/o ginecologiche. All'origine del CPP si evidenzia una condizione di dis-regolazione qualitativa e quantitativa delle afferenze nocicettive provenienti dall'area pelvica. Ripetuti stimoli pelvici di varia natura (infiammatori, infettivi, ormonali,traumatici,etc) ,possono provocare la progressiva attivazione sincrona di quote crescenti di fibre nervose nocicettive, con una conseguente condizione di ipereccitabilità centrale , tendente all'auto mantenimento (neural-axial central-sensitization). I bersagli potenziali della sindrome da CPP sono rappresentati dagli organi pelvici, dalle funzioni da questi espletate e dalla muscolatura della pelvi che svolge funzione di supporto e di coordinamento funzionale. Diverse definizioni vengono usate per identificare la sindrome da CPP, in relazione all'organo che è più sintomatico ed alla funzione che risulta più alterata: sindrome della vescica dolorosa, uretrodinia, vulvodinia e sindrome da dolore della muscolatura del pavimento pelvico. La terapia della sindrome da CPP deve essere multimodale, personalizzata e finalizzata al "reset" del sistema nocicettivo alterato, ed alla modulazione dei processi infiammatori di tipo neurogenico. L'EPV® si è dimostrata efficace nella terapia della sindrome da CPP in uno studio clinico utilizzando un mix di principi attivi rappresentato da lidocaina+tramadolo+diazepam; le 20 pazienti trattate avevano tutte una diagnosi di CPP caratterizzata dalla coesistenza di almeno due delle condizioni organo specifiche proprie della sindrome. Dopo otto sedute settimanali di EPV® la scala analogica visiva del dolore (VAS) si è ridotta da un valore basale di 7.4 ad uno post terapia di 2.3, in assenza di effetti collaterali tali da interrompere il ciclo completo di trattamento. Nella sindrome da CPP è possibile veicolare anche principi attivi ad azione antineuropatica quali l'amitriptilina, che sovente per via sistemica comporta un'elevata incidenza di drop-out per la comparsa di effetti collaterali. I principi attivi possono essere utilizzati anche ad elevato dosaggio compatibilmente con la capienza del reservoir, grazie alla possibilità di concentrarli dove serve con una forte limitazione dell'assorbimento sistemico (Tab.1).

La sindrome genitourinaria della menopausa (genitourinary syndrome of menopause,GSM) é definita come un insieme di segni e sintomi associati con la riduzione degli estrogeni circolanti che determinano cambiamenti a livello vaginale, vulvare, dell'uretra e della vescica.

La sindrome include sintomi genitali quali la secchezza vaginale, il bruciore e l'irritazione, sintomi della sfera sessuale come la mancanza di lubrificazione e la dispareunia e sintomi urinari di urgenza, disuria e infezioni ricorrenti delle vie urinarie.

I cambiamenti anatomici in menopausa includono l'aumentato turnover del collagene, la riduzione dell'elastina, l'aumento del tessuto connettivo e la riduzione del flusso sanguigno a livello vaginale con conseguente assottigliamento dell'epitelio (Fig2). Principale presidio per agire sul trofismo e sulla nocicezione vestibolare è il ricorso ad una sostituzione di tipo estrogenico.

L'azione degli estrogeni si può realizzare direttamente anche sull'inibizione delle terminazioni nervose nocicettive vestibolo-vaginali. Interessanti evidenze hanno dimostrato come una corretta terapia ormonale sostitutiva sistemica può essere insufficiente nel ridurre la dispareunia fino al 25% dei casi. Si è dimostrato in uno studio pilota in 15 pazienti in menopausa da almeno 1 anno, lamentanti bruciore e secchezza vaginale, come la veicolazione tramite EPV® di 50 microgr. di estriolo in gel complessato a 3 g di ac.ialuronico 0.2% in gel, sia efficace nel ridurre il bruciore vulvo-vaginale e la dispareunia, in un ciclo di 8-10 sedute. Ciò apre nuovi scenari nel trattamento della GSM in protocolli integrati e sinergici con terapie topiche, e nuove tecniche quali il laser frazionato vaginale.

Bibliografia essenziale.

-A. Denet, et Al. - Skin electroporation for transdermal and topical delivery. Advanced Drug Delivery Reviews -2004-56;659– 674.
-A. Graziottin, F. Murina - Vulvodynia: tips and tricks. Ed. Springer, 2011.
-F. Murina et Al. Transmucosal delivery of macromolecules using vaginal electroporation to treat vestibulodynia: a pilot study.-Pain and Therapies-2015 –In press
-F. Murina. L'Elettroporazione vaginale : un nuovo approccio terapeutico in ginecologia- Minerva ginecologica-4/2014

Tab.1

Principio attivo da veicolare	**Dosaggio medio**	**Principale indicazione**
Amitriptilina	20-30 mg (10-15 gtt)	Dolore pelvico cronico Vulvodinia
Tramadolo Ketorolac	50 mg (1ml) 20 mg/1 ml	Dolore pelvico cronico Vulvodinia
Lidocaina	20 mg /1 ml	Dolore pelvico cronico Vulvodinia Dispareunia post-parto
Estriolo gel	50 mcg	Sindrome Genito Urinaria Menopausa
Ac.ialuronico gel	3 mg	Sindrome Genito Urinaria Menopausa
Diazepam	5 mg/1 ml	Dolore pelvico cronico Vulvodinia Vaginismo

L' elettroporazione vaginale (EPV©) nel trattamento dei disturbi vaginali della menopausa: studio pilota

Responsabile dello studio : Dott. Filippo Murina
Resp. Servizio di Patologia Vulvare-Osp.V. Buzzi-ICP-Università di Milano

Introduzione.

La sindrome genitourinaria della menopausa (genitourinary syndrome of menopause,GSM) é definita come un insieme di segni e sintomi associati con la riduzione degli estrogeni circolanti che determinano cambiamenti a livello vaginale, vulvare, dell'uretra e della vescica.
La sindrome include sintomi genitali quali la secchezza vaginale, il bruciore e l'irritazione, sintomi della sfera sessuale come la mancanza di lubrificazione e la dispareunia e sintomi urinari di urgenza, disuria e infezioni ricorrenti delle vie urinarie.
I livelli circolanti di estradiolo nella donna in premenopausa variano tra i 40 e i 400 pg/ml e si riducono a meno di 20 pg/ml dopo la menopausa. L'elevata concentrazione dei recettori per gli estrogeni a livello vaginale, del vestibolo e del trigono vescicale regolano la maturazione e la proliferazione cellulare.
I cambiamenti anatomici in menopausa includono l'aumentato turnover del collagene, la riduzione dell'elastina, l'aumento del tessuto connettivo e la riduzione del flusso sanguigno a livello vaginale con conseguente assottigliamento dell'epitelio.
Principale presidio per agire sul trofismo e sulla nocicezione vestibolare è il ricorso ad una sostituzione di tipo estrogenico (NAMS,2013). Interessanti evidenze hanno dimostrato come una corretta terapia ormonale sostitutiva sistemica può essere insufficiente nel ridurre la dispareunia fino al 25% dei casi.
Obiettivo dello studio è stato quello di valutare l'efficacia dell' EPV nella veicolazione combinata di estriolo ed acido ialuronico in gel, al fine di consentire una più elevata penetrazione e persistenza dei principi attivi in sede vaginale.

Materiali e Metodi.

Sono state trattate 15 pazienti in menopausa da almeno 1 anno, lamentanti bruciore e secchezza vaginale. Il protocollo di cura è stato condotto sottoponendo le pazienti ad un trattamento settimanale con EPV per un ciclo di 8-10 sedute. Nel gel conduttore è stata disciolta una quantità di estriolo in gel pari a 50 microgr.(Gelistrol) complessato ad ac.ialuronico 0.2% in gel pari a 3g (Hyalos).

Outcome principale è stato la riduzione del bruciore e secchezza vaginale, valutati tramite score crescente : 0=assente; 1=lieve; 2=medio e 3=intenso.

Risultati.

Tutte le pazienti hanno terminato il ciclo di terapia, e si è registrato un lieve bruciore solo in 2 donne. Dopo 8-10 sedute lo score del bruciore si è ridotto da una media di 2.9 ad una di 1.7, mentre la secchezza ha avuto una variazione più rilevante, passando da una media di 2.7 ad una di 0.6.

L' Elettroporazione Vaginale.

Raffaele Felice
Servizio di Patologia del Tratto Genitale Inferiore Osp.V. Buzzi -Università di Milano

L'elettroporazione (EP) è una tecnica di terapia fisica caratterizzata da una variazione transitoria della membrana cellulare conseguente all'applicazione di impulsi ad alto voltaggio, che consentono la veicolazione intracellulare di sostanza ad elevato peso molecolare.
L'applicazione cutanea dell' EP si è dimostrata efficace e facilmente utilizzabile, anche in sedi corporee contraddistinte da un consistente strato corneo, tanto da superare i limiti di penetrabilità di tecniche quali la ionoforesi o gli ultrasuoni. La veicolazione transdermica consente di ottenere un'elevata biodisponibilità del principio attivo nella sede dove si vuole esercitare il massimo effetto, e nella riduzione di effetti collaterali conseguente alla somministrazione sistemica dei farmaci.
Gli elementi determinanti l'elevata veicolazione di principio attivo sono principalmente il peso molecolare delle sostanze che devono oltrepassare la barriera dermica, e le caratteristiche del reservoir all'interno del quale queste vengono disciolte. L' EP si è dimostrata non avere un cut-off di peso molecolare, consentendo il trasporto di molecole anche oltre i 40 kDa (es. buprenorfina e fentanyl). Nell'EP vengono applicati degli impulsi elettrici che generano un potenziale di membrana tra 0.5-1.0 V che ha durata tra 10 μs e 10 ms. Questa reversibile caduta di potenziale trans-membrana della cellula induce la formazione di "pori" che inducono la facilitazione nel passaggio molecolare. Il dispositivo di elettroporazione EPV® (Blue-Moon® Italia), è dotato di due applicatori specifici a uso vaginale, uno standard e uno ridotto da utilizzare in caso di sub-stenosi vaginale. Questi sono composti da una struttura in materiale plastico opportunamente studiata per favorire l'introduzione e comprendente l'alloggiamento di una siringa standard da 2.5 e 1 ml, dove viene inserito il prodotto da veicolare disciolto in un gel conduttore .Gli impulsi di EP sono generati su due anelli di acciaio inox chirurgico, posizionati a una distanza ottimale per investire un'ampia zona senza la necessità di muovere l'applicatore. La veicolazione di principi attivi in sede vestibo-vaginale è resa ancora più agevole dal ridotto spessore dello strato epiteliale, che consente di raggiungere organi pelvici adiacenti, sovente interessati da sindromi dolorose di difficile gestione terapeutica. I farmaci che l'EPV® può veicolare sono molteplici, ma soprattutto è possibile combinare più principi attivi a target terapeutico differente, creando una sorta di "infiltrazione virtuale" innocua, riproducibile ed in grado di garantire l'elevata biodisponibilità del farmaco dove è necessaria la sua massima azione. Inoltre, l 'EPV® appare tecnica interessante, versatile ed innovativa per la veicolazione di principi attivi nella sede vestibolo e vaginale, aree dove in prevalenza vengono utilizzati prodotti topici. La penetrazione trans mucosa incrementa la persistenza del principio attivo con un effetto "simil depot", in grado di svolgere un'azione propedeutica nell'impiego di prodotti somministrati per via topica.

<u>Impieghi in ginecologia.</u>

Una delle più importanti ed innovative applicazioni di EPV® è rappresentata dalla ***sindrome da dolore pelvico cronico*** , e dalle condizioni ad esso correlate.
Il dolore pelvico cronico (CPP) è definibile come dolore non derivante da neoplasie maligne riferito ad organi e strutture della pelvi, sia femminile che maschile. Il CPP è caratterizzato da un impatto negativo sulla sfera cognitiva, comportamentale sessuale ed emotiva. Nella donna il CPP trova classificazione appropriata come persistente o ricorrente dolore pelvico associato a sintomi riferibili

al tratto genito-urinario inferiore, con disfunzioni intestinali e/o ginecologiche. All'origine del CPP si evidenzia una condizione di dis-regolazione qualitativa e quantitativa delle afferenze nocicettive provenienti dall'area pelvica. Ripetuti stimoli pelvici di varia natura (infiammatori, infettivi, ormonali,traumatici,etc) ,possono provocare la progressiva attivazione sincrona di quote crescenti di fibre nervose nocicettive, con una conseguente condizione di ipereccitabilità centrale , tendente all'auto mantenimento (neural-axial central-sensitization). I bersagli potenziali della sindrome da CPP sono rappresentati dagli organi pelvici, dalle funzioni da questi espletate e dalla muscolatura della pelvi che svolge funzione di supporto e di coordinamento funzionale. Diverse definizioni vengono usate per identificare la sindrome da CPP, in relazione all'organo che è più sintomatico ed alla funzione che risulta più alterata: sindrome della vescica dolorosa, uretrodinia, vulvodinia e sindrome da dolore della muscolatura del pavimento pelvico. La terapia della sindrome da CPP deve essere multimodale, personalizzata e finalizzata al "reset" del sistema nocicettivo alterato, ed alla modulazione dei processi infiammatori di tipo neurogenico. L'EPV® si è dimostrata efficace nella terapia della sindrome da CPP in uno studio clinico utilizzando un mix di principi attivi rappresentato da lidocaina+tramadolo+diazepam; le 20 pazienti trattate avevano tutte una diagnosi di CPP caratterizzata dalla coesistenza di almeno due delle condizioni organo specifiche proprie della sindrome. Dopo otto sedute settimanali di EPV® la scala analogica visiva del dolore (VAS) si è ridotta da un valore basale di 7.4 ad uno post terapia di 2.3, in assenza di effetti collaterali tali da interrompere il ciclo completo di trattamento. Nella sindrome da CPP è possibile veicolare anche principi attivi ad azione antineuropatica quali l'amitriptilina, che sovente per via sistemica comporta un'elevata incidenza di drop-out per la comparsa di effetti collaterali. I principi attivi possono essere utilizzati anche ad elevato dosaggio compatibilmente con la capienza del reservoir, grazie alla possibilità di concentrarli dove serve con una forte limitazione dell'assorbimento sistemico (Tab.1).

La sindrome genitourinaria della menopausa (genitourinary syndrome of menopause,GSM) é definita come un insieme di segni e sintomi associati con la riduzione degli estrogeni circolanti che determinano cambiamenti a livello vaginale, vulvare, dell'uretra e della vescica.

La sindrome include sintomi genitali quali la secchezza vaginale, il bruciore e l'irritazione, sintomi della sfera sessuale come la mancanza di lubrificazione e la dispareunia e sintomi urinari di urgenza, disuria e infezioni ricorrenti delle vie urinarie.

I cambiamenti anatomici in menopausa includono l'aumentato turnover del collagene, la riduzione dell'elastina, l'aumento del tessuto connettivo e la riduzione del flusso sanguigno a livello vaginale con conseguente assottigliamento dell'epitelio. Principale presidio per agire sul trofismo e sulla nocicezione vestibolare è il ricorso ad una sostituzione di tipo estrogenico.

L'azione degli estrogeni si può realizzare direttamente anche sull'inibizione delle terminazioni nervose nocicettive vestibolo-vaginali. Interessanti evidenze hanno dimostrato come una corretta terapia ormonale sostitutiva sistemica può essere insufficiente nel ridurre la dispareunia fino al 25% dei casi. Si è dimostrato in uno studio pilota in 15 pazienti in menopausa da almeno 1 anno, lamentanti bruciore e secchezza vaginale, come la veicolazione tramite EPV® di 50 microgr. di estriolo in gel complessato a 3 g di ac.ialuronico 0.2% in gel, sia efficace nel ridurre il bruciore vulvo-vaginale e la dispareunia, in un ciclo di 8-10 sedute. Ciò apre nuovi scenari nel trattamento della GSM in protocolli integrati e sinergici con terapie topiche, e nuove tecniche quali il laser frazionato vaginale.

Bibliografia essenziale.

-A. Denet, et Al. - Skin electroporation for transdermal and topical delivery. Advanced Drug Delivery Reviews -2004-56;659– 674.
-A. Graziottin, F. Murina - Vulvodynia: tips and tricks. Ed. Springer, 2011.
-F. Murina. L'Elettroporazione vaginale : un nuovo approccio terapeutico in ginecologia- Minerva ginecologica-4/2014

Tab.1

Principio attivo da veicolare	**Dosaggio medio**	**Principale indicazione**
Amitriptilina	20-30 mg (10-15 gtt)	Dolore pelvico cronico Vulvodinia
Tramadolo Ketorolac	50 mg (1ml) 20 mg/1 ml	Dolore pelvico cronico Vulvodinia
Lidocaina	20 mg /1 ml	Dolore pelvico cronico Vulvodinia Dispareunia post-parto
Estriolo gel	50 mcg	Sindrome Genito Urinaria Menopausa
Ac.ialuronico gel	3 mg	Sindrome Genito Urinaria Menopausa
Diazepam	5 mg/1 ml	Dolore pelvico cronico Vulvodinia Vaginismo

Clinical Obstetrics, Gynecology and Reproductive Medicine

Research Article

Transmucosal delivery of macromolecules using vaginal electroporation to treat vestibulodynia: A pilot study

Filippo Murina*, Raffaele Felice, Stefania di Francesco and Silvia Oneda

Lower Genital Tract Disease Unit, V. Buzzi Hospital–University of Milan, Milan, Italy

Abstract

Background: Provoked vestibulodynia (VBD), is characterized by burning and cutting pain localized to the vulvar vestibule in response to light touch.

Objective: To assess the effectiveness of the electroporation (EP), applied through the vaginal probe using a mix of two drugs: amitriptyline + lidocaine in patients with a diagnosis of VBD.

Method: 17 patients with the diagnosis of VBD received a weekly vaginal EP for a total of eight sessions using the following mixture of drugs: amitriptyline at a dose of 60mg and lidocaine at a dose of 40 mg, all dissolved in a conductive gel. Visual analogue scale (VAS), Marinoff score for dyspareunia, and current perception threshold obtained from the vulvar vestibule were assessed at baseline and at the end of treatment.

Results: The VAS was reduced from a baseline of 7.4 (1.2) to a 6.3 post therapy (1.8), and despite the variation not apparently of large extent was actually statistically significant (p 0.05). Dyspareunia also reduced, from baseline of 2.3 (1.0) to a post- therapy of 1.8 (1.1) without statistical significance. There were no relevant side effects that prompted discontinuation of treatment.

Conclusions: Based on what aforementioned vaginal EP can be defined as an promising technique in the management of VBD. Although further studies are needed to define the number and frequency of sessions required, as well as the mix of drugs to be used.

Introduction

Vulvodynia is a common multifactorial, heterogeneous, and chronic gynecological disorder that affects a large number of women worldwide. It is estimated that the prevalence rate of vulvodynia is 16% in women aged 18 to 64 years, resulting in constant demand for medical care [1]. The predominant form of vulvodynia, provoked vestibulodynia (VBD), is characterized by burning and

cutting pain localized to the vulvar vestibule in response to light touch [2]. Etiology for vulvodynia has yet to be established, yet several theories exist. Numerous factors have been proposed and a relevant role appears to be that of a dysfunctional state of the somatosensory system, with a peripheral and central neural sensitization [2].

Vulvodynia may result from peripheral sensitization in the skin, central sensitization in the spinal cord, or both of them. An inflammatory process in the vulvar skin could release a cascade of cytokines that sensitize the nociceptors resulting in a physical increase in the number of nerves. Furthermore, the local sensory pain nerves could become progressively more sensitized, and develop abnormal neuro-secretion with ulterior pain deterioration or persistence [2-3]

There is no standard treatment of the disease, and few randomized controlled trials have been performed [4]. The recommendations are in favor of a multi-dimensional approach, focusing on the management of pain and restoration of proper pelvic floor function. Medical therapy (topical or oral) is a mainstay of the management of VBD [5]. Amitriptyline is a tricyclic antidepressant that effectively treats many conditions of chronic neuropathic pain [6]. It modulates the pain by acting on the central inhibition of neuronal reuptake of neurotransmitters and inhibition of sodium channels. Although oral administration of amitriptyline is the gold standard for the treatment of neuropathic pain and it is often used in the VBD, the systemic side effects such as postural hypotension, sedation, anticholinergic effects limit the attainment of therapeutic doses.

The long-term use of topical lidocaine has been suggested as a specific therapy for VBD, with the rationale that the regular application of lidocaine interrupts the pain signals and reduces the pain amplification [7]. The use of local anesthetics in the long term can cause itching or sensitization, with severe reaction and contact dermatitis, especially with products such as benzocaine [8]. Transcutaneous electrical nerve stimulation (TENS) with biphasic currents of frequencies between 2 and 100 Hz and a pulse duration of 50-100 microsec, has been widely used with a high efficacy (75%) as compared to the placebo in the treatment of VBD [9].

Electoporation (EP) is the transitory structural perturbation of lipid bilayer membranes due to the application of high voltage pulses. Its topical application has been shown to increase the transdermal delivery of drugs with different order of magnitude [10]. In addition, the EP expands the range of drugs (macromolecules, lipophilic or hydrophilic, charged or neutral molecules) that can be delivered transdermally [11].

The aim of our study was to evaluate the effectiveness of the EP applied through the vaginal probe, using a mix of two drugs:

Correspondence to: Filippo Murina M.D.; address: V. Buzzi Hospital, Via Castelvetro 32-Milano, Italy, Tel: +390257995464; E-mail: filippomurina@tin.it

Key words: *vulvodynia, transcutaneous electrical nerve stimulation, electroporation, vestibulodynia, transdermal delivery*

Received: October 02, 2017; **Accepted:** October 16, 2017; **Published:** October 19, 2017

amitriptyline + lidocaine in patients with a diagnosis of VBD. The presumption is to reach the area of clinical interest with a higher concentration of active ingredients, reaching higher concentration than systemic administration but with fewer side effects.

Material and methods

A total of 17 patients were enrolled. All had diagnosis of VBD due to the coexistence of the following conditions: a history of chronic vulval pain elicited by stimulation or attempted intercourse and a positive cotton-swab test, that is tenderness at palpation of the vestibular area with a cotton tip applicator and the absence of any clinically detectable manifestation. Exclusion criteria were pregnancy, cardiac pacemakers, vaginal infections and neurological disorders.

The patients completed a questionnaire regarding their vulvovaginal situation, general health, demographic characteristics, and some questions about psychosexual factors.

Subsequently, each patient underwent a gynecological examination and evaluation of vulvovaginal symptoms with details concerning:

-Visual-analogue scale of pain (VAS) graduated from 0 to 10 (0 = no pain, 10 = maximum pain)

-Dyspareunia-graded by Marinof score (0 = absent, 1 = mild, 2 = intense pain with reduced frequency of intercourse, 3 = penetration almost impossible).

Patients were also subjected to a sensory electrodiagnostic evaluation of the vestibular nerve conduction by measuring the minimum threshold of perception of a sensory input on the vestibular mucosa (CPT). The CPT values were measured using the Neurometer CPT/C electro diagnostic neurostimulator (Neurotron, Inc., Baltimore,MD), which emits alternating sinusoid waveform current stimuli at frequencies of 2,000 Hz (specific for large, myelinated AA fibers), 250 Hz (specific for AC fibers) and 5 Hz (specific for C fibers), at intensity levels from 0.001 to 9.99 mA.

Each woman included in the study received a weekly vaginal EP for a total of eight sessions through a plastic vaginal probe (Bluemoon-Italy) with two transversal metal rings as electrodes connected to a unit calibrated for the EP (Bluemoon-Italy).

The new probe has been designed with a slot for a 2.5 ml syringe, in which the product to be conveyed in the vestibular mucosa was placed. In the disposable syringe we inserted following mixture of drugs: amitriptyline at a dose of 60mg and lidocaine at a dose of 40 mg, all dissolved in a conductive gel. The EP unit was set with the following parameters of TENS, in agreement with previous studies that have used the technique in the treatment of VBD [9]:

- Phase I: 100 Hz - 50 ms- pulse duration for a duration of 15 min.

- Phase II: 5 Hz - 100 ms-pulse duration for a duration of 15 min.

The patients were re-evaluated at the end of treatment both from symptomatic point of view (VAS and Marinof score of dyspareunia), and objectively through re-evaluation of the CPT.

Results

All the recruited patients completed the treatment protocol, and none were lost during follow-up. The characteristics of the patients studied are summarized in Table 1. The main age of the patients was 32,5 years (20-48 years). Eighty-eight percent of the patients were nulliparous (15/17). If we consider the mean duration of the symptoms was 27,5 months (6-80 months).

The VAS was reduced from a baseline of 7.4 (1.2) to a 6.3 post therapy (1.8), and despite the variation not apparently of large extent was actually statistically significant (p 0.05). Dyspareunia also reduced, from baseline of 2.3 (1.0) to a post- therapy of 1.8 (1.1) without statistical significance (Table 2).

Table 3 summarizes the values of the CPT baseline and post-therapy.

Most changes involved the subpopulation of nociceptive C fibers with 24.4 % reduction in sensitivity. In the group of Aß and Aδ fibers, the reduction rate was respectively 15.5% and 20.4 %. There were no relevant side effects that prompted discontinuation of treatment, a post- treatment mild transient stinging was seen in about 7% of cases, and transient sedation in 10 % of patients.

Discussion

In VBD, aunanimous pathophysiological data is the proliferation of nerve endings in the vestibular mucosa, which is considered a non-specific reaction of previous mucosal triggers, such as infection, trauma, and hormonal factors. Another factor observed in women with this disease is the dysfunction of the pelvic floor muscles, contracted around the distal part of the vagina, probably as a reaction to persistent pain. Amitriptilina is the most widely used oral medication, although its real usefulness in the treatment of VBD is not clear. The doses commonly used vary (30-100 mg) as can vary the percentage of the response, ranging between 30-60 % .This mainly derives from the difficulty in achieving an optimal therapeutic threshold resulting in high number of drop-outs due to the occurrence of side effects.

The lidocaine, a local anesthetic, has been used topically as well as in local infiltration of the pudendal nerve with the aim of reducing the ectopic neuronal activity, responsible for allodynia and hyperalgesia, peculiar elements of neuropathic pain syndromes as well as that of the VBD. A multimodal approach towards the VBD is considered to be optimal, and from this point of view association of TENS with the use of drugs may prove to be a winning strategy.

The EP allows to combine the electrical stimulation to the transmucosal transport of macromolecules even in high concentrations,

Table 1. Characteristics of the study population.

Age, mean (range) y	32.5 (20-48)
Nulliparous, n (%)	15/17 (88)
Duration of symptoms, mean (range), mo	27.5 (6-80)
VAS, mean (SD)	7.4 (1.2)
Marinoff dyspareunia scale, mean (SD)	2.3 (1.0)

VAS: Visual Analogue Scale

Table 2. Post treatment scores of VAS and Marinoff dyspareunia scale.

	n. 17 Patients	p
VAS, mean (SD)	6.3 (1.8)	0.05
Marinoff dyspareunia scale, mean (SD)	1.8 (1.1)	NS

VAS: Visual Analogue Scale

Table 3. Results of CPT measurement (100 = 1.0 mA) at 3 selected stimulation frequencies before and after therapy.

N. 17 patients	**2,000 Hz (Aβ fibers)**	**250 Hz (Aδ fibers)**	**5 Hz (C fibers)**
Basal	442,2	201,5	90,8
After therapy	523,4	253,3	120,2
Difference, %	*15,5*	*20,4*	*24,4*

Data are expressed as means (Hz = cycles per second). CPT: Current Perception Threshold

which are then focused at the site of pain elicitation, vaginal vestibule, thus reducing the incidence of side effects.

Our results suggest the effectiveness of the technique. The doses of amitriptyline and lidocaine that we used used were high and no patient had to discontinue the treatment for the occurrence of side effects. The synergistic effect of TENS further contributed to the " reset" of the previously altered nociceptive system.

The demonstration of the absorption and action of drugs as a result of channeling through vaginal EP, is confirmed by CPT values. A 20 % average reduction of the sensitivity of vestibular nerve fibres proves the effect of the combination of TENS with that of drugs in the management of the dysfunction in the pain perception system, proper to the VBD.

A mild improvement of the symptoms may be due to the reduced frequency (1 appl./Week) and a relatively low number of EP vaginal sessions.

In fact, previous studies on TENS led to brilliant results with an average of approximately 20 sessions. Moreover, the patients recruited had already been treated with limited or transient efficacy, leading to define them as "difficult".

Based on what aforementioned vaginal EP can be defined as an effective and promising technique in the management of VBD. Although further studies are needed to define the number and frequency of sessions required, as well as the mix of drugs to be used. It may further lead the foundation for the use of the technique in other vulvovaginal problems that require the use of drugs locally.

References

1. Polpeta NC, Giraldo PC, Teatin Juliato CR, Gomes Do Amaral RL, Moreno Linhares I, et al. (2012) Clinical and therapeutic aspects of vulvodynia: the importance of physicaltherapy. *Minerva Ginecol* 64: 437-445. [Crossref]
2. Ventolini G (2013) Vulvar pain: Anatomic and recent pathophysiologic considerations. Clin Anat 26: 130-133. [Crossref]
3. Tympanidis P, Terenghi G, Dowd P (2003) Increased innervation of the vulval vestibule in patients with vulvodynia. *Br J Dermatol* 148: 1021-1027. [Crossref]
4. Haefner HK , Collins ME, Davis GD, Edwards L, Foster DC, et al. (2005) The vulvodynia guideline. *J Low Genit Tract Dis* 9: 40-51. [Crossref]
5. Groysman V (2010) Vulvodynia: new concepts and review of the literature. *Dermatol Clin* 28: 681-696. [Crossref]
6. Reed BD, Caron AM, Gorenflo DW, Haefner HK (2006) Treatment of vulvodynia with tricyclic antidepressants: efficacy and associated factors. *J Low Genit Tract Dis* 10: 245-251. [Crossref]
7. Dede M, Yenen MC, Yilmaz A, Baser I (2006) Successful treatment of persistent vulvodynia with submucous infiltration of betamethasone and lidocaine. *Eur J Obstet Gynecol Reprod Biol* 124: 258-259. [Crossref]
8. Zolnoun DA, Hartmann KE, Steege JF (2003) Overnight 5% lidocaine ointment for treatment of vulvar vestibulitis. *Obstet Gynecol* 02: 84-87. [Crossref]
9. Murina F, Bianco V, Radici G, Felice R, Di Martino, et al. (2008) Transcutaneous electrical nerve stimulation to treat vestibulodynia: a randomized controlled trail. *BJOG* 115: 1165-1170. [Crossref]
10. Denet AR, Vanbever R, Préat V (2004) Skin electroporation for transdermal and topical delivery. *Adv Drug Deliv Rev* 56: 659-674. [Crossref]
11. Lombry C, Dujardin N, Préat V (2000) Transdermal Drug Delivery of Macromolecules Using Skin Electroporation. *Pharm Res* 17: 32-37. [Crossref]

Journal of
Clinical Medicine

IMPACT FACTOR 3.9
Indexed in: PubMed
CITESCORE 5.7

Article

Treatment of Vulvovaginal Laxity by Electroporation: The Jett Plasma Medical for Her II Study

Tomas Fait, Tivadar Baltazár, Leona Bubenickova, Jan Kestranek, Martin Stepan, Miroslav Muller and Pavel Turcan

Topic
The Use of New Technologies for Health and Clinical Practice

Edited by
Dr. Luca Marin, Dr. Matteo Vandoni and Dr. Vittoria Carnevale

MDPI

https://doi.org/10.3390/jcm12196234

Journal of
Clinical Medicine

MDPI

Article

Treatment of Vulvovaginal Laxity by Electroporation: The Jett Plasma Medical for Her II Study

Tomas Fait [1,2], Tivadar Baltazár [3], Leona Bubenickova [4], Jan Kestranek [5,6], Martin Stepan [6], Miroslav Muller [7] and Pavel Turcan [8,*]

1 Department of Obsterics and Gynaecology, 2nd Faculty of Medicine, Charles University, 150 00 Prague, Czech Republic; tfait@seznam.cz
2 Department of Health Care Studies, College of Polytechnics Jihlava, 586 01 Jihlava, Czech Republic
3 Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Faculty of AgriSciences, Mendel University in Brno, 613 00 Brno, Czech Republic
4 Faculty of Medicine, Masaryk University, 625 00 Brno, Czech Republic; bubenickova@jett.eu
5 Department of Obsterics and Gynaecology, Hospital Trutnov, 541 01 Trutnov, Czech Republic; kestranek.jan@nemtru.cz
6 Department of Obsterics and Gynaecology, Medical Faculty in Hradec Kralove, Charles University, 500 05 Hradec Kralove, Czech Republic; martin.stepan@fnhk.cz
7 Gynedology Pardubice Muller, 530 02 Pardubice, Czech Republic; info@gynmuller.cz
8 Mediol Centre—Olomouc, 779 00 Olomouc, Czech Republic
* Correspondence: turcanp@seznam.cz

Abstract: Introduction: Vaginal laxity is a widespread and undertreated medical condition associated especially with vaginal parity. Aim: To evaluate the efficacy and safety of electroporation therapy treatment of vulvovaginal laxity by the Jett Plasma for Her II device. Methods: The Jett Plasma for Her II Study is a multicentric, prospective, randomized, single-blinded, and controlled study. Women presenting with vaginal laxity were randomized to receive electroporation therapy delivered to the vaginal tissue (active—82 patients) vs. therapy with zero intensity (placebo—9 patients). Results: A total of 91 subjects whose average age was 48.69 ± 10.89 were included. Due to the results of a one-way analysis of variance, it may be concluded that in the case of the vaginal laxity questionnaire (VLQ), there is a statistically significant difference between actively treated patients and the placebo group ($F_{1,574} = 46.91$; $p < 0.001$). In the case of the female sexual function index (FSFI), a one-way ANOVA test also showed a statistically significant difference between the actively treated patients and the placebo group ($F_{1,278} = 7.97$; $p = 0.005$). In the case of the incontinence impact questionnaire-7 (IIQ-7), a one-way ANOVA test showed a statistically significant difference between the actively treated patients and the placebo group ($F_{1,384} = 15.51$; $p < 0.001$). It confirms that improvement of vaginal laxity is conjoined with benefits in symptoms of urinary incontinence. Biopsy performed after the end of the treatment shows an increase in the vaginal mucosa thickness by an average of 100.04% in the active group. The treatment was well tolerated with no adverse events. No topical anesthetics were required. Conclusions: Treatments of vulvovaginal laxity by electroporation therapy achieved significant and sustainable 12-month effectiveness. Responses to the questionnaires also suggest subjective improvement in self-reported sexual function, incontinence, sexual satisfaction, and urogenital distress.

Keywords: vaginal laxity; vulvovaginal laxity; nonsurgical vaginal tightening; incontinence; sexual function; urogenital distress; electroporation therapy

check for updates

Citation: Fait, T.; Baltazár, T.; Bubenickova, L.; Kestranek, J.; Stepan, M.; Muller, M.; Turcan, P. Treatment of Vulvovaginal Laxity by Electroporation: The Jett Plasma Medical for Her II Study. *J. Clin. Med.* **2023**, *12*, 6234. https://doi.org/10.3390/jcm12196234

Academic Editors: Luca Marin, Matteo Vandoni and Vittoria Carnevale

Received: 10 September 2023
Revised: 19 September 2023
Accepted: 25 September 2023
Published: 27 September 2023

1. Introduction

Vaginal laxity (vaginal looseness, vaginal relaxation syndrome, wide vagina syndrome) is a common condition characterized by a loss of tone or elasticity of the vagina [1]. This can be caused by a variety of factors, but the most important of them is vaginal childbirth [2].

It may also be partly caused by aging and oestrogen deficiency [3], but this idea is not generally approved. The pelvic floor muscles play a critical role in maintaining the vaginal tone and supporting the pelvic organs, but they can become weakened or damaged due to childbirth. As a result, the vaginal walls may become stretched or widened, leading to a decrease in excitement during sexual activity, difficulty achieving orgasm, and a loss of confidence [4,5].

Studies show prevalence of vaginal laxity is about 35%. Personal history of a single delivery relates to 5.6 (CI 1.67–15.3, $p = 0.004$) times more likely to report laxity than in nulliparous women. Women who had caesarean delivery were protected against vaginal laxity (adjusted odds ratio 0.39, 95% CI 0.17–0.9) [6]. There is an extraordinarily strong association with musculus levator ani extensive distensibility and all measures of anterior and posterior compartment descent [7]. However, it is not an early symptom of pelvic organ prolapse [8].

Aging is another common cause of vaginal laxity. As women age, their bodies undergo several changes, including a decrease in oestrogen levels. Oestrogen plays a crucial role in vaginal health and elasticity, and a decrease in oestrogen can lead to thinning of the vaginal walls and decreased lubrication. These changes can contribute to vaginal laxity and discomfort during sexual activity. On the other side, the main sign of vulvovaginal atrophy (genitourinary syndrome of menopause) caused by oestrogen deficiency is vaginal tightness [9].

In addition to childbirth and aging, obesity is another risk factor for vaginal laxity. Women who are overweight or obese may experience increased pressure on their pelvic floor muscles, leading to stretching and weakening. This can contribute to symptoms of vaginal laxity and may increase the risk of urinary incontinence and other pelvic floor disorders [10].

There are several treatment options available for vaginal laxity, ranging from non-invasive methods such as pelvic floor exercises and vaginal rejuvenation devices to more invasive approaches like vaginal surgery [11]. Pelvic floor physical therapy is a common nonsurgical treatment option that involves exercises designed to strengthen the pelvic floor muscles and improve vaginal tone.

Vaginal rejuvenation devices, such as thermal therapy and radiofrequency energy, can also be used to stimulate collagen production and tighten the vaginal walls [12].

Jet Plasma for Her II (Compex Ltd., Brno, Czech Republic) works on the basis of electroporation. The base of this mechanism is the creation of small temporary nanopores in the cell walls. It is induced by the application of high voltage electrical pulse to the cell membrane. Macromolecules and other ions could pass through these nanopores in both directions. The cells increase their volume [13]. It decreases the atrophy of vaginal mucosa and submucosal tissue and improves the tissue cohesion. Electroporation is also used for the transport of drugs into tumor cells or in irreversible ones for the destruction of cells. In this situation, we used another voltage and length of application [14].

The purpose of the Jett Plasma for Her II study was to determine the efficacy and safety of this type of therapy in women with vaginal laxity. We have three years of experience with this type of therapy. We started with this therapy in 2020 when a vaginal application device was created. The same therapy has been used in beauty care at Jett Plasma Lift Medical since 2015 with other devices.

2. Materials and Methods

The Jett Plasma for Her II Study is a multicentric, prospective, randomized, single-blinded, and controlled study which started in November 2019 and currently includes 91 patients. The study has been authorized by the Ethics Committee of University Hospital Ostrava, code number 16.10-TF-JPH II. The study was completed without any additional payment from patients or reimbursement for patients.

Healthy adult female subjects who clinically present with vaginal laxity (it was only one inclusion criterion) and expressed interest in treatment were considered eligible for the study. The patients were offered participation in the study by their attending physicians.

Inclusion criteria for subject selection consisted of voluntarily signed informed consent, age at least 18 years, a negative pregnancy test, self-reported perceptions of vaginal laxity defined on the vaginal laxity questionnaire (VLQ), and Papanicolaou smear cytology showing no dysplasia within 36 months prior to the treatment.

Women with evidence of epilepsy, pregnancy, metal implants in the treated area, skin diseases or inflammations in the treatment area, urinary tract infection, collagen vascular disease, oncological disease in the vulvovaginal region, any untreated/badly treated disease in vulvovaginal region, birth defects of vagina, stenosis and strictures of the vagina, synechia of vulva, previous reconstructive vaginal surgery, vaginal lasers, or vaginal injections of fat or fillers within 6 months, BMI $\geq$ 35 were excluded.

Prior to the treatment and at the 1, 3, 6, and 12 month follow-up visits, participants completed a packet of self-report questionnaires to characterize and follow the effects of treatment. Five validated questionnaires were included in the packet: the vaginal laxity questionnaire (VLQ), the female sexual function index (FSFI), the sexual satisfaction questionnaire (SSQ), and short forms of the urogenital distress inventory (UDI-6) and the incontinence impact questionnaire (IIQ-7).

In 25 patients out of the total number of patients, vaginal mucosa biopsies were performed. The first biopsy took place before the treatment, and the second sample was taken 3 months after the third treatment.

Treatments were performed three times by the vaginal probe of Jett Plasma for Her II (Compex, Ltd., Brno, Czech Republic) with a time interval of 10 to 14 days. It uses an electric current of 2.8 mA with a voltage of 5 kV. It induces a maximum temperature of 45 °C in the tissue of the vagina wall. Each application takes approximately 7.5 min.

2.1. Questionnaires

The vaginal laxity questionnaire has seven-level ordered responses (1—very loose, 2—moderately loose, 3—slightly loose, 4—neither loose nor tight, 5—slightly tight, 6—moderately tight, or 7—very tight). For the primary efficacy endpoint, "no vaginal laxity" was classified as a VLQ score of at least 5 (i.e., $\geq$5) [12].

The incontinence impact questionnaire (IIQ-7) assesses the psychosocial impact of incontinence in women. It consists of 7 items: 1—household chores, 2—physical recreation, 3—entertainment activities, 4—travel > 30 min away from home, 5—social activities, 6—emotional health (nervousness, depression, etc.), and 7—feeling frustrated, which are subdivided into 4 domains: PA—physical activity (items 1 and 2), TR—travel (items 3 and 4), SA—social activities (item 5), and EH—emotional health (items 6 and 7). The total score is in the range of 0–100 [15].

The urogenital distress inventory (UDI-6) is a condensed version of a condition-specific quality of life instrument, UDI. Presently, UDI-6 is much more often used than its longer version. UDI-6 consists of 6 items: 1—frequent urination, 2—leakage related to the feeling of urgency, 3—leakage related to activity, coughing, or sneezing, 4—lesser amounts of leakage (drops), 5—difficulty emptying the bladder, and 6—pain or discomfort in the lower abdominal or genital area. Higher scores in UDI-6 indicate higher disability. The total score is from 0 to 100 [15].

The female sexual function index (FSFI) is a validated instrument for the assessment of sexual function, consisting of 19 questions. The questions are grouped for domains of libido, arousal, lubrication, orgasm, satisfaction, and pain; higher scores reflect better sexual function (maximum score 36). An FSFI total score less than or equal to 26.5 is recognized in the medical literature as indicating female sexual dysfunction (FSD) [16].

The sexual satisfaction questionnaire (SSQ) assesses sexual satisfaction from vaginal intercourse. SSQ has six-level ordered responses (1—excellent sexual satisfaction, 2—very

good sexual satisfaction, 3—good sexual satisfaction, 4—fair sexual satisfaction, 5—poor sexual satisfaction, and 6—no sexual satisfaction) [17].

Patients evaluated the pain of the procedure by a visual analogue scale of 0, painless, to 10, extremely painful [18].

2.2. Randomization

The assignment to the placebo or the active group was generated using the online tool https://www.random.org/lists/ (accessed on 1 November 2019), where numbers from 1 to 140 were randomly assigned to either the active treatment (90 occurrences) or the placebo treatment (50 occurrences). Physicians obtained a randomization letter for 10 patients. Each physician assigned a treatment to the patient according to the date of study entry, meaning that the first patient who entered the study was numbered 1, etc.

If a patient agreed to the biopsy, she was assigned a number 141–150, and all those patients underwent active treatment.

At the end of the study, each patient completed a blinding questionnaire to assess the blinding.

2.3. Statistical Analysis

All statistical analyses were performed using freely available software R, version 4.3.1. (https://mirrors.nic.cz/R/, CZ.NIC, Prague, Czech Republic) [19]. Package "ggplot2" [20] was used for creating advanced statistical graphs. To statistically evaluate several questionnaires (VLQ, FSFI, IIQ-7, UDI-6, and SSQ) with dependence on actively treated patients and the placebo group (or treatment time), a one-way analysis of variance (ANOVA) type I (sequential) sum of squares at a significance level of 0.05 was used [21].

To detect the difference among factor level means, Tukey's honestly significant difference (HSD) test and "treatment contrasts" for calculating the factor level means with 95% confidence intervals CI was used [22]. Pearson's chi-squared test of independence was used to check whether the observed frequency of vaginal laxity or sexual dysfunction occurrence (no or yes) differs significantly from the expected frequencies with dependence of method (actively treated patients and the placebo group). In the case of FSFI, a one-way analysis of covariance (ANCOVA) was performed to characterize the relationship between the age of the patient, treatment time, and type of method (actively treated patients and the placebo group). A coefficient of determination (R^2) was used to explain how well one can predict the variation in the values of FSFI questionnaire score using the age of patients [22]. After the analysis, the assumptions of all our statistical models were also checked at a significance level of 0.05 with the help of different statistical tests and several diagnostic plots [23].

3. Results

A total of 91 patients (aged 26 to 77, median 48.6 years) were involved in this trial with average age of 48.6 (SD = 10.8) years. The average age in the group of actively treated patients was 47.7 (95% CI: 45.4, 50.1; $n = 82$) and 56.5 (95% CI: 49.6, 63.5; $n = 9$) in the case of the placebo group. This difference ($F_{1,87} = 5.68$; $p = 0.02$) was statistically significant only at 5% significance level.

Due to the results of the one-way analysis of variance, it can be concluded that in the case of the vaginal laxity questionnaire (VLQ), there is a statistically significant difference between the actively treated patients and the placebo group ($F_{1,574} = 46.91$; $p < 0.001$). The average value of the VLQ in the case of actively treated patients was 3.98 (95% CI: 3.86, 4.11) and in the case of the placebo group, 2.68 (95% CI: 2.33, 3.03), which is 32.6% less. The average improvement between the first treatment and the last follow-up was 1.22 points from 3.15 (SD = 10.8) to 4.27 (SD = 1.37). The 28.6% increase is statistically significant ($F_{6,506} = 12.37$; $p < 0.001$). However, the largest difference was found between the first treatment and the second control (diff: −1.43, $p < 0.001$). The highest VLQ value was observed in the case of the second control with an average of 4.57 (95% CI: 4.25, 4.89).

The placebo group improved by 0.22 points from 2.56 (SD = 1.24) to 2.78 (SD = 1.09), i.e., 4%, which is not statistically significant ($F_{6,56}$ = 0.09; p = 1). The differences between the active treatment and the placebo group were also statistically significant (except for the first treatment) in all follow-up controls ($p < 0.05$). The primary efficacy analysis of the VLQ is presented in Table 1 and Figure 1. The percentage of subjects in the actively treated patients reporting no vaginal laxity (VLQ score $\geq$ 5) at the first treatment was 15.6% (13 of 82) compared with 0% (0 of 9) in the placebo group, which should not be statistically different ($\chi^2_{(1, n=89)}$ = 1.41, p = 0.24). The largest percentage was achieved in the case of the second control, where actively treated patients reported no vaginal laxity (VLQ score $\geq$ 5) with a percentage of 45.7% (32 of 70) compared with 0% (0 of 9) in the placebo group. This difference showed borderline significance ($\chi^2_{(1, n=79)}$ = 3.97, p = 0.05).

Table 1. Results of analysis of contingence table with the help of Pearson's Chi-squared test of independence in the case of the vaginal laxity questionnaire (VLQ). A—active, P—placebo.

	Method	Total	No Laxity	No Laxity (%)	Chisq Test	*p*-Value
1st treatment	A	82	13	15.9	1.41	0.24
	P	9	0	-		
2nd treatment	A	81	14	17.3	1.53	0.22
	P	9	0	-		
3rd treatment	A	80	24	30	2.64	0.10
	P	9	0	-		
1st control	A	78	34	43.6	3.80	0.05
	P	9	0	-		
2nd control	A	70	32	45.7	3.97	0.05
	P	9	0	-		
3rd control	A	66	27	40.9	3.55	0.06
	P	9	0	-		
4th control	A	56	22	39.3	3.40	0.07
	P	9	0	-		

In the case of the female sexual function index (FSFI), the one-way ANOVA also showed a significant difference between actively treated patients and the placebo group ($F_{1,278}$ = 7.97; p = 0.005). The average value of FSFI in actively treated patients was 28.6 (95% CI: 27.9, 29.2) and in the placebo group, 26.0 (95% CI: 24.3, 27.7), which is 2.6% less. The average improvement between the first treatment and the last follow-up was 3.81 points from 25.6 (SD = 6.66) to 29.4 (SD = 5.4). The 12.9% increase is significant ($F_{4,235}$ = 6.68; $p < 0.001$). However, the largest difference was found between the first treatment and the third control (diff: −4.32, $p < 0.001$). The largest FSFI value was observed in the case of the third control on average, with 29.9 (95% CI: 28.4, 31.4). The placebo group improved by 1.4 points from 25.1 (SD = 4.81) to 26.5 (SD = 5.39), i.e., 5.3%, which is not statistically significant ($F_{4,35}$ = 0.11; p = 0.98). The differences between the active treatment and the placebo group were only statistically significant in the case of the first and second controls ($p < 0.05$). The primary efficacy analysis of FSFI is presented in Table 2. The percentage of subjects in the actively treated patients reporting no sexual dysfunction (FSFI score $\geq$ 26.5) at the first treatment was 46.6% (27 of 58) compared with 37.5% (3 of 8) in the placebo group, which is not different ($\chi^2_{(1, n = 66)}$ = 0.09, p = 0.76). The largest percentage was achieved in the case of the third control, where 84.1% (37 of 44) of the actively treated patients reported no sexual dysfunction (FSFI score $\geq$ 26.5) compared to 50% (4 of 8) in the placebo group. This difference is not statistically significant ($\chi 2_{(1, n = 52)}$ = 0.64, p = 0.42) either.

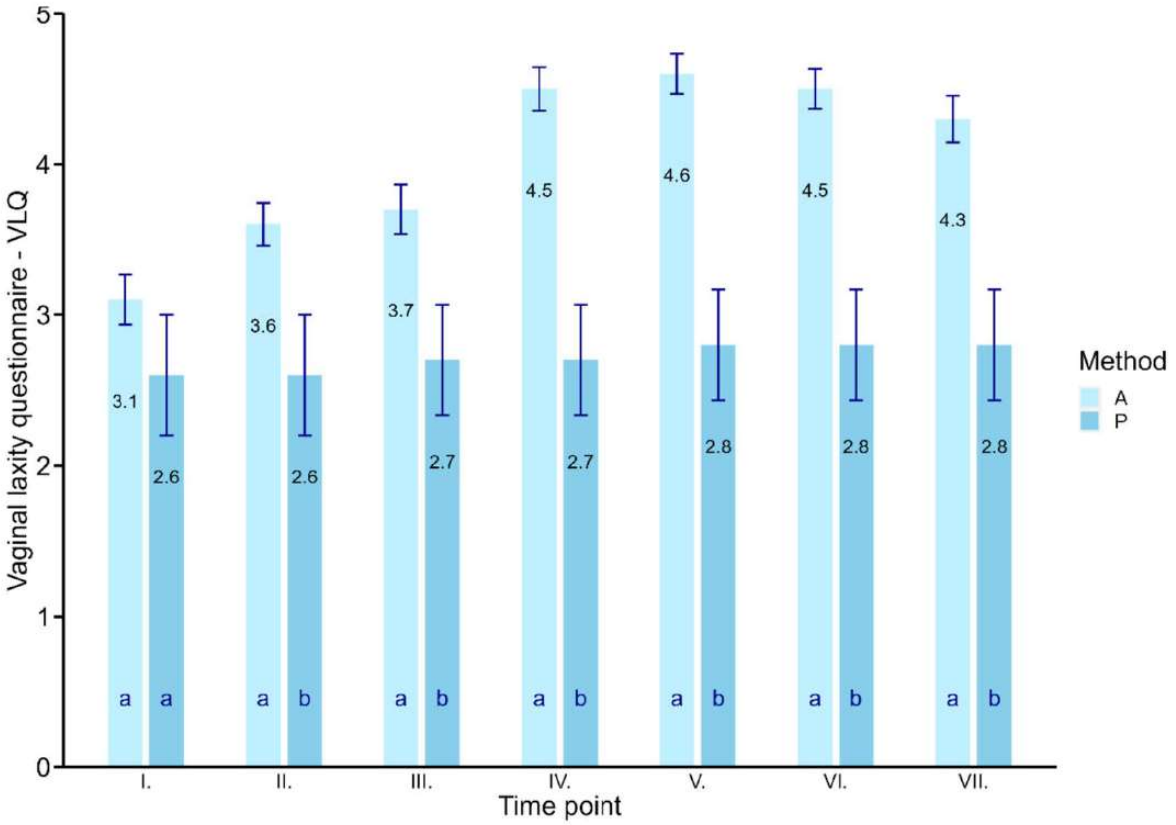

Figure 1. Mean values with standard error of the mean (SEM) for the vaginal laxity questionnaire VLQ with the dependence of "method" and "time point" (I.—1st treatment, II.—2nd treatment, III.—3rd treatment, IV.—1st control, V.—2nd control, VI.—3rd control, VII.—4th control). A—active, P—placebo. (Letters show statistically significant difference between actively treated patients and placebo group at significance level of 0.05).

Table 2. Results of the analysis of the contingence table with the help of Pearson's Chi-squared test of independence in the case of the female sexual function index (FSFI). A—active, P—placebo.

Treatment	Method	Total	No Dysfunction	No Dysfunction (%)	Chisq Test	*p*-Value
1st treatment	A	58	27	46.6	0.09	0.76
	P	8	3	37.5		
1st control	A	56	42	75.0	0.99	0.32
	P	8	3	37.5		
2nd control	A	48	38	79.2	0.51	0.48
	P	8	4	50.0		
3rd control	A	44	37	84.1	0.64	0.42
	P	8	4	50.0		
4th control	A	34	26	76.8	0.41	0.52
	P	8	4	50.0		

The IIQ-7 and UDI-6 questionnaires confirm that improvement of vaginal laxity is conjoined with benefits in symptoms of urinary incontinence. In the case of the incontinence impact questionnaire-7 (IIQ-7), a one-way ANOVA also showed a significant difference between the actively treated patients and the placebo group ($F_{1,384} = 15.51$; $p < 0.001$). The average value of IIQ-7 in actively treated patients was 12.0 (95% CI: 9.94, 14.06) and in the placebo group, 24.1 (95% CI: 18.42, 29.77), which is 50% higher. The average change between the first treatment and the last follow-up was 20.64 points from 26.05 (SD = 22.03) to 5.41 (SD = 14.08). Such a 79.2% decrease is statistically significant ($F_{4,336} = 17.0$; $p < 0.001$). However, the largest difference was registered between the first treatment and the first control (diff: −16.92, $p < 0.001$). The largest IIQ-7 value was observed in the case of the first treatment on an average of 26.05 (95% CI: 22.19, 29.91). The placebo group improved by 2.62 points from 26.19 (SD = 22.87) to 23.57 (SD = 24.04); this 10% decrease is not significant ($F_{4,40} = 0.02$; $p = 0.99$). The differences between the active treatment and the placebo group were statistically significant in all follow-up controls ($p < 0.05$).

Due to the result of the one-way ANOVA, it can be concluded that in the case of the urogenital distress inventory (UDI-6), there is a statistically significant difference between the actively treated patients and the placebo group ($F_{1,385} = 9.22$; $p = 0.003$). The average value of UDI-6 in the case of an actively treated patient was 13.5 (95% CI: 11.91, 15.07) and in the case of the placebo, 20.7 (95% CI: 16.30, 25.01), which is 34.8% higher. The average change between the first treatment and the last follow-up was 21.3 points from 27.8 (SD = 17.1) to 6.52 (SD = 9.8). This 76.6% decrease is statistically significant ($F_{4,337} = 33.15$; $p < 0.001$). However, the largest difference was found between the first treatment and the first control (diff: −16.21, $p < 0.001$). The largest UDI-6 value was observed in the case of the first treatment average of 27.8 (95% CI: 24.94, 30.62). The placebo group improved by 2.47 points from 22.61 (SD = 12.43) to 20.17 (SD = 14.55); this 11% decrease is not statistically significant ($F_{4,40} = 0.05$; $p = 0.99$). The differences between the active treatment and the placebo group were statistically significant (except for the first treatment) in all follow-up controls ($p < 0.05$).

Different results were observed in the case of the sexual satisfaction questionnaire (SSQ) because, in this case, a one-way ANOVA did not detect a statistically significant difference between the actively treated patients and the placebo group ($F_{1,315} = 0.80$; $p = 0.37$). The average value of SSQ in the case of an actively treated patient was 2.93 (95% CI: 2.79, 3.10) and in the case of the placebo group, 3.10 (95% CI: 2.75, 3.45), which is 5% higher compared to the actively treated patients. The average change between the first treatment and the last follow-up was 0.92 points from 3.54 (SD = 1.28) to 2.62 (SD = 0.9). This 26% decrease is statistically significant ($F_{4,272} = 6.78$; $p < 0.001$). However, the largest difference was found between the first treatment and the fourth control (diff: 0.91, $p < 0.001$). The largest SSQ value was observed in the case of the first treatment average of 3.54 (95% CI: 3.27, 3.81). The placebo group improved by 0.25 points from 3.25 (SD = 1.04) to 3 (SD = 0.93); this 7.7% decrease is not statistically significant ($F_{4,35} = 0.08$; $p = 0.99$). The differences between the active treatment and the placebo group were not statistically significant either in all follow-up controls ($p > 0.05$) (Figure 2).

A one-way ANOVA showed a statistically significant difference in vaginal mucosa thickness in samples before and after treatment ($F_{1,48} = 26.57$; $p < 0.001$). The average value of vaginal mucosa thickness in the first treatment was 241.4 (95% CI: 173.6, 309.1), and in the second control, 486.8 (95% CI: 419.1, 554.6), which is 50% higher compared to the first treatment. Results of the analysis of covariance also detected not only the impact of the treatment duration ($F_{1,45} = 44.1$; $p < 0.001$) but also the role of age ($F_{1,45} = 39.18$; $p < 0.001$). This relationship is medium-strong, assessed by the coefficient of determination ($r^2 = 52$) in both treatments. A negative correlation was also found between age and vaginal mucosa thickness; the older the patient, the lower the value of vaginal mucosa thickness (Figure 3). Biopsy performed after the end of the treatment showed an increase in the vaginal mucosa thickness by an average of 112.6% (SD = 75.4) in the case of actively treated patients.

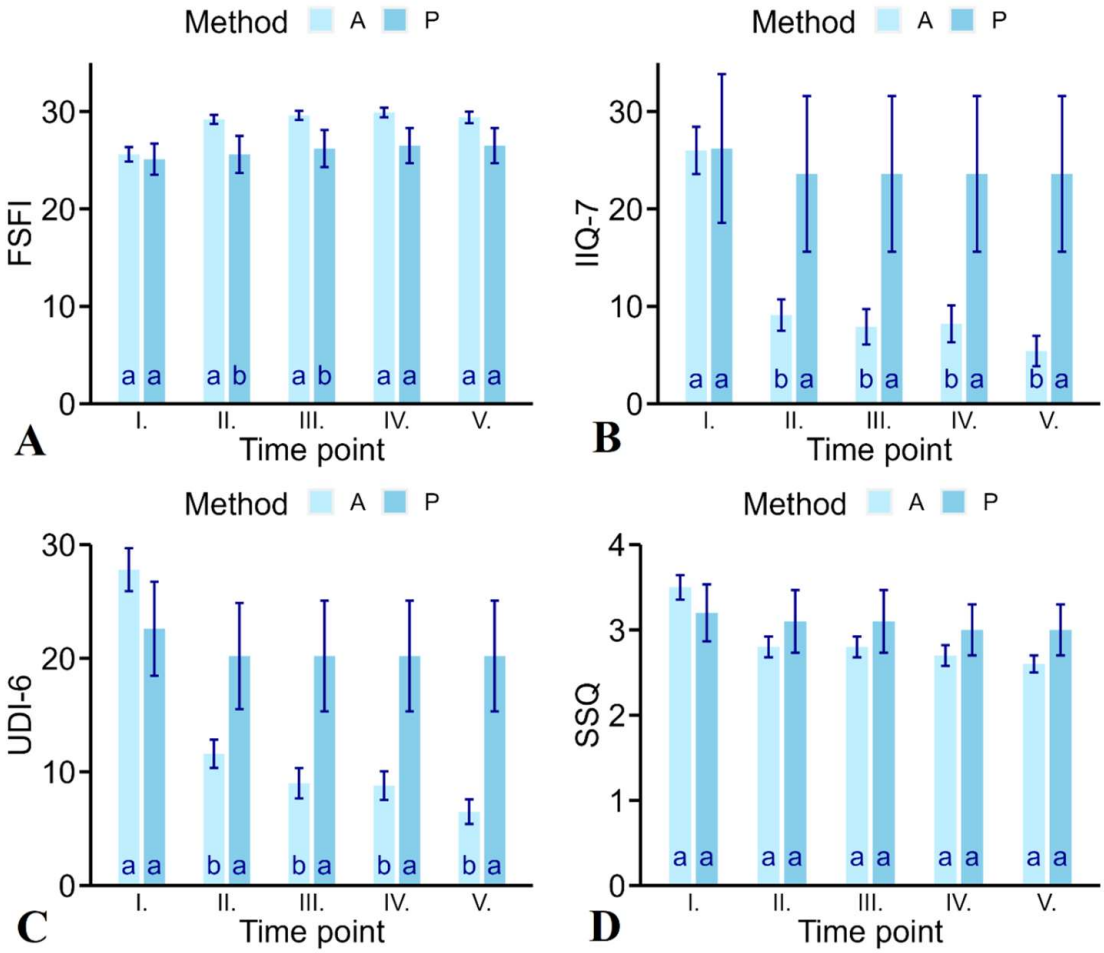

Figure 2. Mean values with standard error of the mean (SEM) for female sexual function index FSFI (**A**), incontinence impact questionnaire-7 IIQ-7 (**B**), urogenital distress inventory UDI-6 (**C**), and sexual satisfaction questionnaire SSQ (**D**) with the dependence of "method" and "time point" (I. –1st treatment, II.—1st control, III.—2nd control, IV.—3rd control, V.—4th control). A—active, P—placebo. (Letters show statistically significant difference between actively treated patients and placebo group at significance level of 0.05).

On average, a total of 80.6% felt no pain during the procedure. Only one patient rated the treatment as 5. In the case of the actively treated patients, this ratio was 70.1%. In the case of the first treatment, painlessness was 76.9% (74.3% in actively treated patients); in the case of the second treatment, it was 79.1% (76.8% in actively treated patients), and in the case of the third treatment it was 85.7% (84.1% in actively treated patients). The differences between the actively treated patients and the placebo group were significant only at a 5% significance level in all cases: first treatment ($\chi^2_{(1,\, n=91)} = 6.41$, $p = 0.01$), second treatment ($\chi^2_{(1,\, n=91)} = 6.62$, $p = 0.01$), and third treatment ($\chi^2_{(1,\, n=91)} = 7.23$, $p = 0.01$). In the first treatment, the pain was 0.33 (95% CI: 0.19, 0.48; $n = 91$) on a visual analogue scale, 0.26 (95% CI: 0.12, 0.41; $n = 91$) in the second treatment, and 0.16 (95% CI: 0.01, 0.31; $n = 91$) in the third treatment. There was no statistically significant difference among the treatments ($F_{2,257} = 1.30$; $p = 0.27$).

After the fourth follow-up control, 90% of women were satisfied with the treatment, and the average value of the satisfaction scale was 4.5 (SD = 0.68).

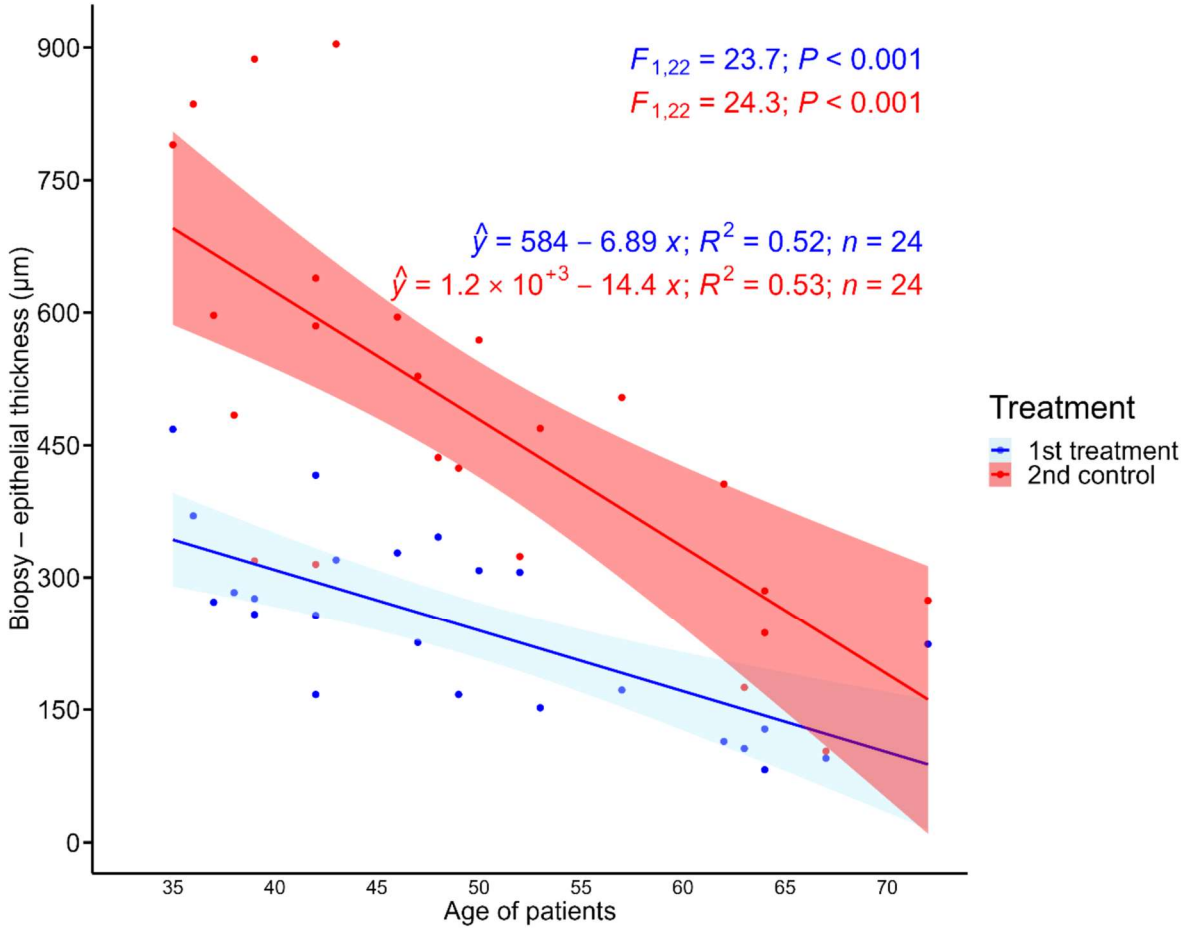

Figure 3. Negative linear relationship between the biopsy: epithelial thickness (µm) and the age of patients with dependence on "Treatment".

4. Discussion

The main advantage of our study is that it is the first study using electroporation for the treatment of vaginal laxity. Additionally, the use of a control (placebo) subgroup is not common in other vaginal laxity treatment studies. On the other side, the size of the control group and the 26.3% drop-out of patients not during the therapy but during the follow-up may be considered weaknesses. The weaknesses of the study are also unknown hormonal status and sexual partnership. Another weakness could be missing data such as parity status and birth weight of patients' children, but we believe that it is important for the cause of vaginal laxity, not for its therapy.

Vaginal laxity is a topic that has generated significant attention in recent years, with many women expressing concerns about the perceived looseness of their vaginal muscles. While some people may view this as a purely cosmetic concern, it can have a real impact on a woman's sexual health and overall well-being.

The symptoms of vaginal laxity can include decreased sexual sensation, reduced sexual satisfaction, difficulty achieving orgasm, and urinary incontinence. While these symptoms can have a significant impact on a woman's quality of life, they can often be effectively treated using a variety of medical interventions.

To treat a wide vagina sensation, perineoplasty can be successfully used with low complication rates. Patients report high satisfaction and anatomical repair [24,25]. Perineoplasty is surgery removing excess or ruined skin and mucosa of the vaginal entrance. The repairing of supporting muscles is conjoined. It takes approximately 30 to 45 min in local or general anaesthesia. In a retrospective study of 38 women with a 6-month follow-up, the success rate of the perineoplasty procedure was 87.9%; according to a visual analogue scale,

the partner satisfaction rate was 92.6%. Ten percent of patients said they had experienced dyspareunia at the introitus of the vagina during sexual intercourse [26].

There is no scientific evidence to prove the efficacy of over-the-counter vaginal tightening products, injectable volumizers, and physical devices such as silicon threads [27–29]. Pelvic physiotherapy is an accepted intervention for pelvic organ prolapse as well as vaginal laxity in the form of pelvic floor muscle training (PFMT) and Kegel exercises [30,31].

Energy-based devices (EBD) attempt to induce favorable changes in tissue using heat in the range of 40–42 °C. These devices restore the elasticity of connective tissue of the vaginal wall. At the same time, they improve vaginal lubrication and humidity of the vaginal mucosa. The procedures of energy-based vaginal rejuvenation are non-invasive and take from 8 to 30 min. The procedure is painless. Two or three sittings are recommended, spaced a month apart. A touch-up sitting is usually performed after 12–18 months. Indication for these procedures is vaginal laxity, vaginal dryness, mild symptoms of urinary incontinence, overactive bladder, low-grade prolapse, and orgasmic dysfunction [32].

Energy-based therapy could be based on LASER or radiofrequency. In a recent review, 59 studies were evaluated with 3609 women [33]. Minimally ablative fractional laser therapy was recognized as a safe, accurate, and efficient approach for resurfacing and regeneration of the skin. The most widely used lasers in vaginal tissues are the CO_2 (10,600 nm) and the erbium: yttrium-aluminium-garnet (Er: YAG) laser. In 2013, an EBD was validated by the North American Menopause Society. The North American Menopause Society acknowledged the use of lasers for therapy of the genitourinary syndrome of menopause in 2013. However, results are frequently obtained from small, short-term studies without randomization [34,35].

For example, Gao et al. published a descriptive study without controls. A total of 29 patients were enrolled and treated with two sessions of FemTouch vaginal fractional CO_2 laser, with a one-month interval between the sessions. Both subjective and objective measurements, including female sexual function index (FSFI), vaginal health index score (VHIS), vaginal tactile imaging (VTI), and histology, were used to validate the clinical efficacy and biophysical benefits after treatment. Results: The overall FSFI scores and VHIS scores after the first and second treatment sessions were significantly higher than the baseline scores ($p < 0.01$, $n = 29$). VTI measurements showed a significant increase in maximal pressure resistance (kPa) of both the anterior and posterior vaginal walls at a 10–12-month post-treatment visit compared with pre-treatment controls ($p < 0.001$; $n = 16$). Histological examination showed that laser treatment led to increases in the thickness of the stratified squamous epithelium layer and the density of connective tissues in the lamina propria [36].

Monopolar radiofrequency (RF) treatment with cryogenic surface cooling offers another less-invasive ambulatory therapy for vaginal laxity. The density of small nerve fibers in the papillary dermis increased after the application of RF. The biopsy test has shown neocollagenesis and neoelastogenesis in the submucosa and the development of new elastin. The Vaginal Introitus's Viveve Treatment to Evaluate Effectiveness (VIVEVE I) trial was the first randomized control trial with radiofrequency in the therapy of vaginal laxity. A single RF treatment was proven to be safe and was associated with the improvement of vaginal laxity and sexual function [37].

The treatment relies on the concept that carefully controlled heat energy can be used to reach deeper submucosal tissue. The therapeutic goal is to stimulate connective tissue activation with subsequent tissue revitalization. It is the same situation as for the skin in beauty treatment. A similar process of neoelastogenesis and neocollagenesis might also occur in vaginal tissue.

Some studies used a sheep vagina as an animal model. RF treatment procedures identical to those used in this human report were evaluated in serial tissue biopsies. Stromal remodeling with fibroblast activation in soft tissue was identified between one week and one month after the treatment. The increased submucosal and/or muscularis collagen was focally present over 6 months after treatment. The absence of ulceration, regional

necrosis, and effacing dense collagen scarring over the 6-month follow-up period supports an acceptable safety profile for this treatment regimen [38].

5. Conclusions

This study confirms that a nonsurgical, nonablative treatment with Jett Plasma Medical for Her II, a new type of energy-based device working with electroporation, is a well-tolerated and safe procedure shown to produce statistically significant and clinically important improvements in vaginal laxity, incontinence, and improved sexual satisfaction in women in the active group compared to placebo treatment. This effect was prolonged until 12 months after the treatment.

Author Contributions: Formal analysis, T.B.; Investigation, J.K., M.S. and M.M.; Writing—original draft, T.F.; Writing—review & editing, L.B.; Supervision, P.T. All authors have read and agreed to the published version of the manuscript.

Funding: Publication was supported by Educational grant of Compex Ltd., Brno, Czech Republic.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of University Hospital Ostrava, code number 16.10-TF-JPH II.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data is unavailable due to ethical restrictions. Anonymized data are accessible on demand on authors mail address.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Pauls, R.N.; Fellner, A.N.; Davila, G.W. Vaginal laxity: A poorly understood quality of life problem; a survey of physician members of the International Urogynecological Association (IUGA). *Int. Urogynecol. J.* **2012**, *23*, 1435–1448. [CrossRef] [PubMed]
2. Thibault-Gagnon, S.; Yusuf, S.; Langer, S.; Wong, V.; Shek, K.; Dietz, H. Do women notice the impact of childbirth-related levator trauma on pelvic floor and sexual function? *Int. Urogynecol. J.* **2014**, *25*, 1389–1398. [CrossRef] [PubMed]
3. Hustak, K.; Kumbla, P.A.; Liu, S. Anatomical changes of the vulva due to childbirth and aging. *Clin. Plast. Surg.* **2022**, *49*, 429–433. [CrossRef] [PubMed]
4. Kamisan Atan, I.; Gerges, B.; Shek, K.; Dietz, H.P. The association between vaginal childbirth and hiatal dimensions: A retrospective observational study in a tertiary urogynaecological centre. *Br. J. Obstet. Gynaecol.* **2015**, *122*, 867–872. [CrossRef]
5. Toozs-Hobson, P.; Athanasiou, S.; Khullar, V.; Boos, K.; Anders, K.; Cardozo, L.D. Does vaginal delivery damage the pelvic floor? *Neurourol. Urodyn.* **1997**, *16*, 385–386.
6. Talab, S.; Al-Badr, A.; AlKusayer, G.M.; Dawood, A.; Bazi, T. Correlates of vaginal laxity symptoms in women attending urogynecology clinic in Saudi Arabia. *Int. J. Gynecol. Obstet.* **2016**, *145*, 278–282. [CrossRef]
7. Dietz, H.P.; Stankiewicz, M.; Atan, I.K.; Ferreira, C.W.; Socha, M. Vaginal laxity: What does this symptom mean? *Int. Urogyn. J.* **2018**, *29*, 123–128. [CrossRef]
8. Alexander, J.W.; Gillor, M.; Diet, H.P. Is vaginal laxity and early symptom of pelvic organ prolapsing? *Int. Urogyn. J.* **2022**, *33*, 1927–1931. [CrossRef]
9. Polland, A.; Duong, V.; Furuya, R.; Fitzgerald, J.J.; Wang, H.; Iwamoto, A.; Bradley, S.; Iglesia, C.B. Description of vaginal laxity and prolapse and correlation with sexual function (DeVeLoPS). *Sex. Med.* **2021**, *9*, 100443. [CrossRef]
10. Pomian, A.; Lisik, W.; Kosieradzki, M.; Barcz, E. Obesity and pelvic floor disorders: A review of the literature. *Med. Sci. Monit.* **2016**, *22*, 1880–1886. [CrossRef]
11. Hamori, C. Aesthetic surgery of the female genitalia: Labiaplasty and beyond. *Plast. Reconstr. Surg.* **2014**, *134*, 661–673. [CrossRef] [PubMed]
12. Millheiser, L.S.; Pauls, R.N.; Herbst, S.J.; Chen, B.H. Radiofrequency treatment of vaginal laxity after vaginal delivery: Nonsurgical vaginal tightening. *J. Sex Med.* **2010**, *7*, 3088–3095. [CrossRef] [PubMed]
13. Gehl, J. Electroporation: Theory and methods, perspectives for drug delivery, gene therapy and research. *Acta Physiol. Scand.* **2003**, *177*, 437–447. [CrossRef] [PubMed]
14. Blantic, K.; Miklavcic, D.; Krizaj, I.; Kramar, P. The good and the bad of cell membrane electroporation. *Acta Chim. Slov.* **2021**, *68*, 753–764. [CrossRef] [PubMed]
15. Uebersax, J.S.; Wyman, J.F.; Shumaker, S.A.; McClish, D.K.; Continence Program for Women Research Group. Short forms to assess life quality and symptom distress for urinary incontinence in women: The incontinence impact questionnaire and urogenital distress inventory. *Neurourol. Urodyn.* **1995**, *14*, 131. [CrossRef]

16. Rosen, R.; Brown, C.; Heiman, J.; Leiblum, S.; Meston, C.; Shabsigh, R.; Ferguson, D.; D'Agostino, R., Jr. The Female Sexual Function Index (FSFI): A Multidimensional Self-Report Instrument for the Assessment of Female Sexual Function. *J. Sex Marit. Ther.* **2000**, *26*, 191–208. [CrossRef]
17. Nomejko, A.; Dolinska-Zygmunt, A. The sexual satisfaction questionnaire–psychometric propertie. *Pol. J. Appl. Psychol.* **2014**, *12*, 105–112. [CrossRef]
18. Scott, J.; Huskisson, E.C. Graphic representation of pain. *Pian* **1976**, *2*, 175–184. [CrossRef]
19. *R Core Team: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2019; Available online: http://www.R-project.org/ (accessed on 15 May 2023).
20. Wickham, H. *ggplot2: Elegant999999 Graphics for Data Analysis*; Springer: New York, NY, USA, 2016; 182p.
21. Zar, J.H. *Biostatistical Analysis*, 2nd ed.; Prentice-Hall Internat.: London, UK, 1984; 718p.
22. Faraway, J.J. *Linear Models with R*; Chapman & Hall/CRC: Boca Raton, FL, USA, 2005; 229p.
23. Pekár, S.; Brabenec, M. *Modern Analysis of Biological Data. Generalized Linear Models in R*; Masaryk University Press: Brno, Czech Republic, 2016; 226p.
24. Pardo, J.S.; Solà, V.D.; Ricci, P.A.; Guiloff, E.F.; Freundlich, O.K. Colpoperineoplasty in women with sensation of a wide vagina. *Acta Obstet. Gynecol. Scand.* **2006**, *85*, 1125–1127. [CrossRef]
25. Jamali, S.; Abedi, P.; Rasekh, A.; Mohammadjafari, R. The Long-Term Effect of Elective Colpoperineoplasty on Sexual Function in the Reproductive Aged Women in Iran. *Int. Sch. Res. Not.* **2014**, 912786. [CrossRef]
26. Ulubay, M.; Keskin, U.; Fidan, U.; Ozturk, M.; Bodur, S.; Yılmaz, A.; Kinci, M.F.; Yenen, M.C. Safety, Efficiency, and Outcomes of Perineoplasty: Treatment of the Sensation of a Wide Vagina. *Biomed. Res. Int.* **2016**, 2495105. [CrossRef] [PubMed]
27. Aguilar, P.; Hersant, B.; SidAhmed-Mezi, M.; Bosc, R.; Vidal, L.; Meningaud, J.P. Novel technique of vulvo-vaginal rejuvenation by lipofilling and injection of combined platelet-rich-plasma and hyaluronic acid: A case-report. *Springerplus* **2016**, *5*, 1184. [CrossRef] [PubMed]
28. Park, T.H.; Whang, K.W. Vaginal rejuvenation with Gore-Mycromesh. *Aesthet. Plast. Surg.* **2015**, *39*, 491–494. [CrossRef] [PubMed]
29. Park, T.H.; Park, H.J.; Whang, K.W. Functional vaginal rejuvenation with elastic silikone threads: A 4year experience with 180 patients. *J. Plast. Surg. Hand. Surg.* **2015**, *49*, 36–39. [CrossRef]
30. Kolberg Tennfjord, M.; Hilde, G.; Staer-Jensen, J.; Siafarikas, F.; Engh, M.E.; Bø, K. Effect of postpartum pelvic floor muscle training on vaginal symptoms and sexual dysfunction-secondary analysis of a randomised trial. *Brit. J. Obstet. Gynecol.* **2016**, *123*, 634–642. [CrossRef]
31. Kegel, A.H. Progressive resistance exercise in the functional restoration of the perineal muscles. *Am. J. Obstet. Gynecol.* **1948**, *56*, 238–248. [CrossRef]
32. Gold, M.; Andriessen, A.; Bader, A.; Alinsod, R.; French, E.S.; Guerette, N.; Kolodchenko, Y.; Krychman, M.; Murrmann, S.; Samuels, J. Review and clinical experience exploring evidence, clinical efficacy, and safety regarding nonsurgical treatment of feminine rejuvenation. *J. Cosmet. Dermatol.* **2018**, *17*, 289–297. [CrossRef]
33. Juhász, M.L.W.; Korta, D.Z.; Mesinkovska, N.A. Vaginal rejuvenation: A retrospective review of lasers and radiofrequency devices. *Dermatol. Surg.* **2021**, *42*, 489–494. [CrossRef]
34. Kwon, T.R.; Kim, J.H.; Seok, J.; Kim, J.M.; Bak, D.-H.; Choi, M.J.; Mun, S.K.; Kim, C.W.; Ahn, S.; Kim, B.J. Fractional CO_2 laser treatment for vaginal laxity: A preclinical study. *Lasers Surg. Med.* **2018**, *50*, 940–947. [CrossRef]
35. Lee, M.S. Treatment of Vaginal Relaxation Syndrome switch an Erbium: YAG Laser Using 90° and 360° Scanning Scopes: A Pilot Study & Short-term Results. *Laser Ther.* **2014**, *23*, 129–138.
36. Sekiguchi, Y.; Utsugisawa, Y.; Azekosi, Y.; Kinjo, M.; Song, M.; Kubota, Y.; Kingsberg, S.A.; Krychman, M.L. Laxity of the vaginal introitus after childbirth: Nonsurgical outpatient procedure for vaginal tissue restoration and improved sexual satisfaction using low-energy radiofrequency thermal therapy. *J. Womens Health* **2013**, *22*, 775–781. [CrossRef] [PubMed]
37. Gao, L.; Wen, W.; Wang, Y.; Li, Z.; Dang, E.; Yu, L.; Zhou, C.; Lu, M.; Wang, G. Fractional Carbon Dioxide Laser Improves Vaginal Laxity via Remodeling of Vaginal Tissues in Asian Women. *J. Clin. Med.* **2022**, *11*, 5201. [CrossRef] [PubMed]
38. Coad, J.; Vos, J.; Curtis, A. Safety and mechanisms of action supporting nonablative radiofrequency thermal therapy for vaginal introitus laxity occurring in women after childbirth: Histological study in the sheep vaginal model. *J. Sex Med.* **2013**, *10*, 175.

SANTEC
Gynecology Energy Company

SCARICA
LA VERSIONE
PDF

SCOPRI

SANTEC srl
Reggio Emilia | Livorno
tel. 0586444539
e-mail: info@santecsrl.it
P IVA / C.F. 0210578035