BASIC SCIENCE

Establishment of Rat Model of Female Genital Sexual Arousal Disorder

Guangyong Li, MD,[1] Puguang Yu, MM,[1] Yanan Hu, MMS,[3] Zhenxing Hu, MM,[2] Jian Li, MMS,[2] Xuekang Zhan, MM,[2] Yashan Su, MMS,[2] Chen Yu, MMS,[4] Jing Wen, MMS,[2] Hetao Liu, MMS,[2] and Rui He, PhD[2]

ABSTRACT

Introduction: Female Genital Sexual Arousal Disorder (FGSAD) seriously affects women's quality of life and Sexual life, but it still lacks ideal FGSAD animal models for further study.

Aim: To establish a specific model of female genital sexual arousal disorder and explore the mechanisms resulting in FGSAD.

Methods: After delivery, female rats were guided by expansions of the vagina and ovariectomy (VD+OVX, n = 10); in VD group female rats were just extended by the vagina (VD, n = 10), in OVX group female rats were treated with ovariectomy (OVX, n = 10);the remaining had 1 longitudinal incision as sham group(n = 10).

Outcomes: Vaginal dilatation combined with ovariectomy in rats may reflect female genital sexual arousal disorder with high reproducibility and stability.

Results: Vaginal tissue of female rats in OVX group and VD+OVX group showed an increase in blood flow, decrease in muscle content compared to the sham group. The proportion of collagen fiber I/III decreased and the elastic fiber showed significant rupture and fragmentation; Structural reticular integrity was also significantly separated and broken from the muscle fibers. However, there was no significant difference in vaginal blood flow, fibers and vascular between VD group and Sham group. The damage of vaginal tissue in VD+OVX group was more significant than that in OVX and VD groups.

Clinical Translation: We have constructed a specific animal model that can provide clinical insights into the mechanism of FGSAD and serves as a good avenue for further research of its treatment.

Strengths and Limitations: Vaginal dilatation combined with ovariectomy in rats is a specific animal model with high reproducibility and stability, but we do acknowledge the shortcomings and limitation present in our study. Since genital arousal disorder has many different etiologies that impact the vagina, the clitoris and surrounding tissues, there is no "gold standard" model that different models attempt to investigate different etiologies.

Conclusion: The female genital sexual arousal disorder model established by vaginal dilatation combined with ovariectomy is a novel rat model with simple induction conditions, which pathogenic mechanism of female genital sexual arousal disorders maybe connected with the change of VEGF and MMP-9 in vaginal fibromuscular system and microvascular. **Li G, Yu P, Hu Y, et al. Establishment of Rat Model of Female Genital Sexual Arousal Disorder. Sex Med 2022;10:100530.**

Key Words: Female Genital Sexual Arousal Disorder; Vaginal Blood Flow; Fibro-Muscular System; Microvascular

Received June 12, 2021. Accepted April 14, 2022.

[1]Department of Urology, General Hospital of Ningxia Medical University, Ningxia Medical University, Yinchuan, P.R. China;

[2]Key Laboratory of Fertility Preservation and Maintenance of Ministry of Education, School of Basic Medical Sciences, Ningxia Medical University, Yinchuan, P.R.China;

[3]Guangzhou First People's Hospital, Guangzhou, Guangdong, P.R. China;

[4]Department of Obstetrics, People's Hospital of Yichun City, Yichun City, P. R. China

https://doi.org/10.1016/j.esxm.2022.100530

INTRODUCTION

Female genital sexual arousal dysfunction (FGSAD) refers to the lack or damage to the genital sexual arousal caused by organic lesions of the female genitalia. It manifests as decreased sensitivity of the sexual organs, vaginal congestion, relaxation, smoothness, clitoris hyperemia and swelling disorders,[1−3] with vaginal lesions being the most common presentation. FGSAD is an unspoken sorrow of many postpartum women, which seriously affects women's quality of life and sexual life.[4] Vaginal arousal is related to neurovascular regulation, involving the interaction of neurotransmitters, vasoactive substances, and hormones with vascular smooth muscle and non-vascular smooth muscle of the vagina.[5] So the causative factors of female sexual arousal disorder are complicated, with menopause and labor injury being the high probably causes. The original animal model of FGSAD constructed by simple ovariectomy to simulate estrogen withdrawal,[6] but which cannot reflect the pathogenesis of human female genital sexual arousal disorder. Because not every estrogen deficient woman has sexual arousal disorders, including postmenopausal woman, which infers that the etiology of FGSAD is not solely due to a single lack of estrogen, and is the main reason why estrogen has been unsuccessful to treat FGSAD.[7,8]

Further research found that FGSAD was not only associated with decrease or loss of blood estrogen levels, but also with delivery injury, metabolic syndrome and inflammation of the female reproductive organs. Studies report that prolonged labor, delayed labor, and dystocia has a high risk of vaginal injuries (perineal laceration, lateral perineotomy or instrumental delivery)[9,10] and injuries of pelvic floor muscles, peripheral nerves, as well as spine and spinal cord as possible negative consequences of prolonged and/or delayed labor and dystocia which leading to the occurrence of FGSAD.[11] Stress urinary incontinence (SUI) also an important factor (Adjusted OR (95% CI):4.02 (2.75-5.89)), has a significant correlation with FGSAD.[12−14]

So, what is the pathogenic mechanism of female genital sexual arousal disorders? Is there any FGSAD animal model that may be established by combining the original female sexual dysfunction model with female SUI model to simulate the physiological process of labor injury and menopause? In this study, we tried to establish a specific animal model to answer these 2 questions. In our study, vaginal dilatation simulates the physiological process of vaginal birth injury caused by prolonged second stage of labor, while ovariectomy simulates the physiological process of hormone withdrawal. These modeling methods were used to simulate menopause and labor injury to construct a novel model of FGSAD.

METHODS

Model Building

The study was performed according to the Care and Use of Laboratory Animals protocol of the National Research Council of China and was approved by the Ningxia Medical University Ethics Committee (Ethical code:2018-180). The experimental design, implementation and outcome index evaluation methods and results of this study are in accordance with the ARRIVE guideline. Forty eight-week-old female rats were caged with male rats with a 4:1 ratio for 1 month. After successful copulation awaiting delivery, female rats were divided into 4 groups: sham operation group (Sham, n = 10), vaginal dilatation + ovariectomized group (VD+OVX, n = 10), vaginal dilatation group (VD, n = 10) and ovariectomized group (OVX, n = 10).[15,16]

The rats were anesthetized with 5% pentobarbital sodium at a dose of 0.1ml/100g. The vagina of rats in the VD group and the VD+OVX group prior to surgery. 2cm of the front end of a 22FR foley catheter balloon was removed, and then inserted 2cm∼3cm into the vagina with iodophor. The skin around the vagina was tightly cross-sutured with 4-0 silk thread to ensure that the urethral catheter was fixed (1 suture line was placed 0.5 cm above the urethra and 0.5 cm below the vagina. The 2 sutures were fixed in the urethra by passing 1 thread above the urethra and bypassed under the urethra for re-fixation. The urethral balloon was then filled with 5 ml of warm saline to emulate the second stage of labor and to prevent compression of the uterus during the modeling process. In the sham operation group, the skin around the vagina was also tightly cross-sutured with 4-0 silk thread to maintain the consistency of the experiment. The pubic symphysis of the rats was placed on the edge of an operating table, and the rats were properly fixed to ensure that the rats were in a comfortable position. The catheter is lowered and a 170 g water bag is added to apply gravity, as showed in the supplement (Supplement Figure1B). After 4 hours, the rats were released from the fixation and returned to the cage. During the operation and after the attention to keep room temperature, pay attention to observe the rat condition, especially to do a good job of postoperative care. The balloon catheter was loaded to simulate the second stage of labor and to prevent compression of the uterus during the modeling process.

After 1 week, rats of the VD+OVX group and OVX group were anesthetized and a lower midline abdominal incision was made to excise both ovaries, as showed in supplement figure (supplement Figure1 C). Sham group rats only underwent midline abdominal incision without ovariectomy.

Measurement of Vaginal Blood Flow by Laser Doppler and Weighing

Four weeks later after ovariectomy, changes in vaginal blood flow in response to pelvic nerve stimulation were detected by laser doppler flowmetry[17] under anesthesia using chloral hydrate (10% chloral hydrate 0.3 ml/100g). A total of 10 vaginal tissue was collected from each group (n = 10).

To expose the vagina, the rats were positioned in a supine position, exploring the pelvic nerves fanning out next to the

vagina near the rectum, the urethra along the bladder. The bladder and vagina were positioned to 1 side is in order to expose the vaginal branch of the pelvic nerve on the lateral wall of the vagina, extending to the inguinal ligament along the lateral wall of the vagina, as showed in supplement figure (supplement Figure1 D). The starting point of the stimulator and the pelvic nerve vaginal branch was connected to both ends of the bipolar concentric circle electrode. The vaginal branches were stimulated at a stimulation frequency of 10 Hz and a voltage of 6 V an interval pulse of 0.8 ms, a stimulation interval of 30 second, and the interval between stimulations was typically 5 minutes. Vaginal blood flow has been measured by others in multiple studies, and this paper follow the same stimulation parameters and nerve isolation. After the stimulator was adjusted, a laser doppler probe was placed on the distal 1 of 3 of the anterior vaginal wall of the vagina to measure the blood flow to the vaginal wall after electrical stimulation. The BPU is Blood Perfusion Units. The blood flow were recorded throughout the experiment using a MP150 System (Biopac Systens, Inc.), as showed in supplement figure (supplement Figure1 E). When $P < .05$, the difference was statistically significant.[6]

After measuring vaginal blood flow quantity, all of the rats were euthanized and the uterus and vagina were weighted. Each vaginal tissue is divided lengthwise into 2 parts. Half vaginal tissue in each group were fixed in 4% paraformaldehyde for 24 hours and then used to make paraffin sections. The other half of the vagina tissue was frozen at -80 °C for protein extraction.

Staining of Muscle Fibers, Collagen Fibers, Elastic Fibers, and Reticular Fibers

About 4 um thickness paraffin sections of vaginal tissue were prepared then performed strict xylene dewaxing and gradient alcohol hydration. The sections were stained with standard procedures of Masson's trichrome stain (× 10), picrosirius red stain(× 400), Hart's elastin stain (× 400) and Gordon & Sweet's stain (× 1000).[18] The morphological changes of vaginal tissue were observed under the microscope and photographed (German Leica binocular microscope DM500). The staining in Figure 2 and Figure 3 were quantified by using Image-J software. In our study, ten sections were selected from each group and 2 of each were placed onto a single slide. We stained the slides from each group together with same conditions and 10 fields were selected from each section for analysis using a double-blind detection method. The vaginal tissue in each group is 10, that is, (n = 10).

Immunohistochemistry

A total of 10 sections from each group were incubated with antibodies to Von Willebrand Factor (1:100) (Abcam, Cambridge, MA, USA), VEGF (1:100) (Abcam, Cambridge, MA, USA) and MMP-9 (1:200) (Cell Signaling, Beverly, MA, USA) overnight at 4 degrees Celsius. Slides were rinsed in PBS, followed by treatment using the second antibody using the Max Vision HRP-Polymer anti-Rabbit IHC Kit, (Maxim Co. China). The morphological changes of vaginal tissue were observed under the microscope and photographed (German Leica binocular microscope DM500, × 400). The vaginal tissue in each group is 10, that is, (n = 10). When $P < .05$, the difference was statistically significant.

Number of Blood Vessels

The blood vessels were quantified using light microscopy (German Leica binocular microscope DM500) in 5 randomly selected fields from 5 nonconsecutive sections of the vagina for each rat according to the immunohistochemistry of vWF (1:100) under an image magnification of × 400 (× 40 objective and × 10 eyepiece). The results are expressed as the mean number of vessels per high power field (× 400) per rat. The vaginal tissue in each group is 10, that is, (n = s10).

Western Blot

Anterior vagina tissue protein samples were prepared by homogenizing in RIPA lysis buffer. Equal protein (20 μg/lane) was electrophoresed on 10% SDS-PAGE and then transferred to polyvinylidene fluoride membrane (Millipore Corp, Bedford, MA, USA). Western blot was performed with antibodies against MMP-9 (1:400) (Cell Signaling, Beverly, MA, USA), VEGF (1:800) (Abcam, Cambridge, MA, USA), SMA (1:1000) (Santa Cruz Biotechnology, CA, USA) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (1:1000) (Santa Cruz Biotechnology, CA, USA).The tissue from each animal kept separate, and 1 sample from each treatment group was run on each gel, so 4 lanes were run per gel. More than 3 times were this repeated on different gels.

Statistical Method

Statistical analysis was carried out by using SPSS19.9 software, and measurement data are expressed as mean ± standard deviation ($x \pm s$). One-Way ANOWA analysis of variance was used for comparison between groups and Tukey Post Hoc test is used to perform multiple comparisons. A P value lower than .05 was regarded as statistically significant.

RESULTS

Comparison of Value of Vaginal Blood Flow and Weight of Vagina and Uterus

Compared to the sham group, the vaginal blood flow in VD +OVX group and OVX group were decreased significantly, and the value in VD+OVX group was lower (Figure 1A and B). Vaginal weight in VD+OVX group, OVX group, and VD group was significantly lower than that in sham group ($P < .05$), and the decrease of VD+OVX group and OVX group was lower than

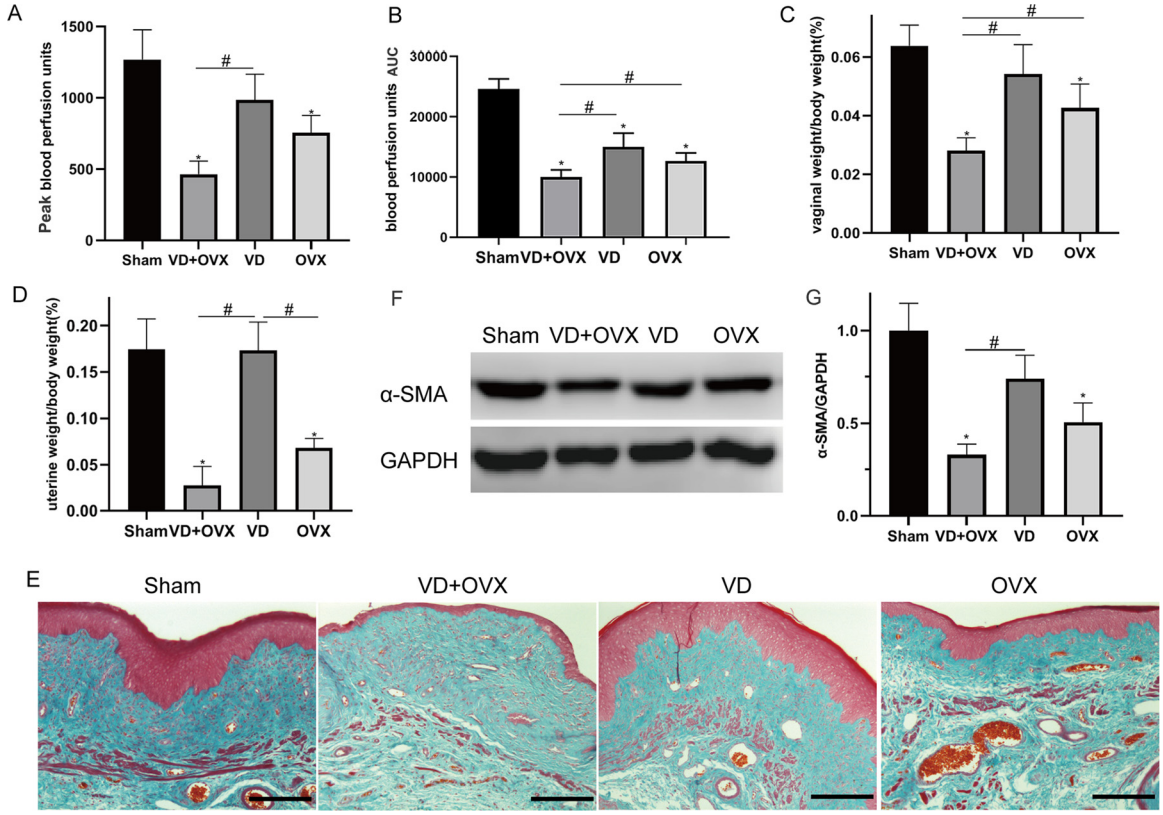

Figure 1. (A and B) Vaginal blood flow peak value (Peak BPU) and vaginal blood flow area under curve (AUC BPU), vaginal blood flow decreased significantly in VD+OVX group. (C) The Vaginal weight of rats in VD+OVX group decreased significantly. (D) The uterine weight of rats in VD+OVX group decreased significantly, but there was no significant difference compared with the OVX group. (E) Masson staining showed that the vaginal muscle tissue of rats in the VD+OVX group decreased significantly. Scale bars: 100 um. (F and G) The vaginal muscles of rats in α-SMA western blot, VD+OVX group decreased significantly. The vaginal tissue in each group is 10, that is, (n = 10). * vs Sham, $P < .05$; # vs Model group, $P < .05$.

that in VD group. The differences was statistically significant (Figure 1C). The uterine weight of the VD+OVX group and OVX group were significantly lower than that of sham group ($P < .05$) (Figure 1D).

Changes of Muscle Tissue

Masson's trichrome stains cells red and extracellular matrix blue and/or green and/or purple. Trichrome staining demonstrated that the vaginal muscle was well organized, clearly defined layer in which numerous bundles of longitudinally and circularly oriented smooth muscle fibers are interspersed with fine trabeculae of loose connective tissue in sham rats. However, the muscle underwent significant attrition in the other 3 groups. As can be seen in Figure 1E, the bundles of smooth muscle were fewer, smaller, and more condensed and in many places the layer could scarcely be recognized as such in different groups. These performances are more obvious in VD+OVX group, as showed in supplement figure (supplement Figure1 A). Western blot detected the expression of α-SMA in vaginal tissue in Figure 1F, G, which was a significant decrease in VD+OVX group, consistent with our masson staining results.

Changes of Vaginal Epithelium

In sham group, the vaginal epithelium ranged from 8 to 13 layers of cells in the thickness which located along the basement membrane were low columnar or cuboidal in appearance. Cells displaced toward the lumen took on an increasingly flattened appearance. In the OVX and VD+OVX groups, vaginal epithelium were atrophic. These performances are more apparent in VD+OVX group. As evidenced by Figure 1E, the epithelial thickness did not exceed 5 cells deep and appeared squamous and keratinized in the VD+OVX animals.

Changes of Various Fibers

We used 3 staining methods to compare fibers changes: Sirius red staining, Hart's elastic fiber staining and Gordon & Sweet's staining. In sham group, type I collagen fiber and type III collagen fibers were closely arranged, and the ratio of collagen fibers I/III was 13.1 ± 2.9 (Figure 2A and C), the elastic fibers were continuous and could extend from epithelium to muscular layer (Figure 2B and D), and the reticular fibers were closely connected with muscle fibers and the fibers were continuous in smooth muscle layer of the vagina (Figure 3A and C) .Compared with Sham group, the collagen fiber I/III, elastic fibers and

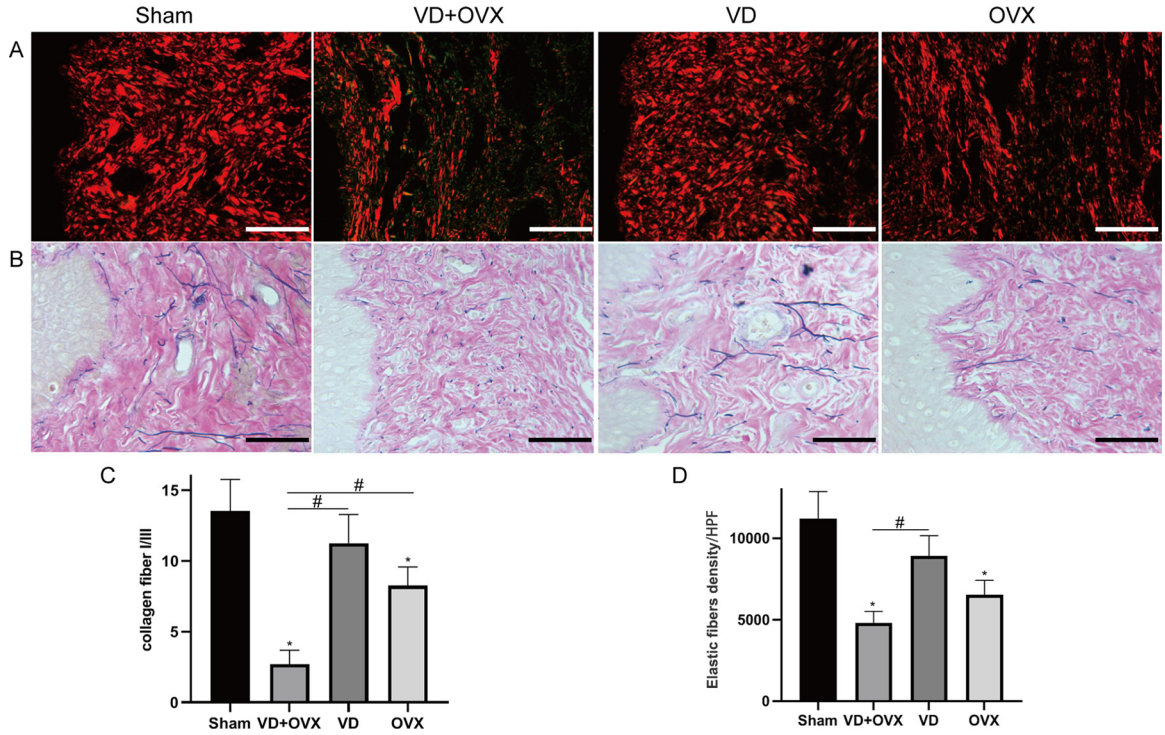

Figure 2. (A, C) Sirius red staining, in which type I colllgen fibers are red, type III collagen fibers are green thin fibers, showed that the vaginal tissue fibers and ratio of collagen fibers I/III decreased significantly in VD+OVX group. Scale bars: 25 um. (B, D) Hart's staining, in which showed that the elastic fibers of rats in Sham group were continuous and could extend from epithelium to muscular layer. The elastic fibers in vaginal tissue of rats in VD+OVX group were significantly broken and fragmented. Scale bars: 25 um. The vaginal tissue in each group is 10, that is (n = 10). * vs Sham, $P < .05$; # vs Model group, $P < .05$.

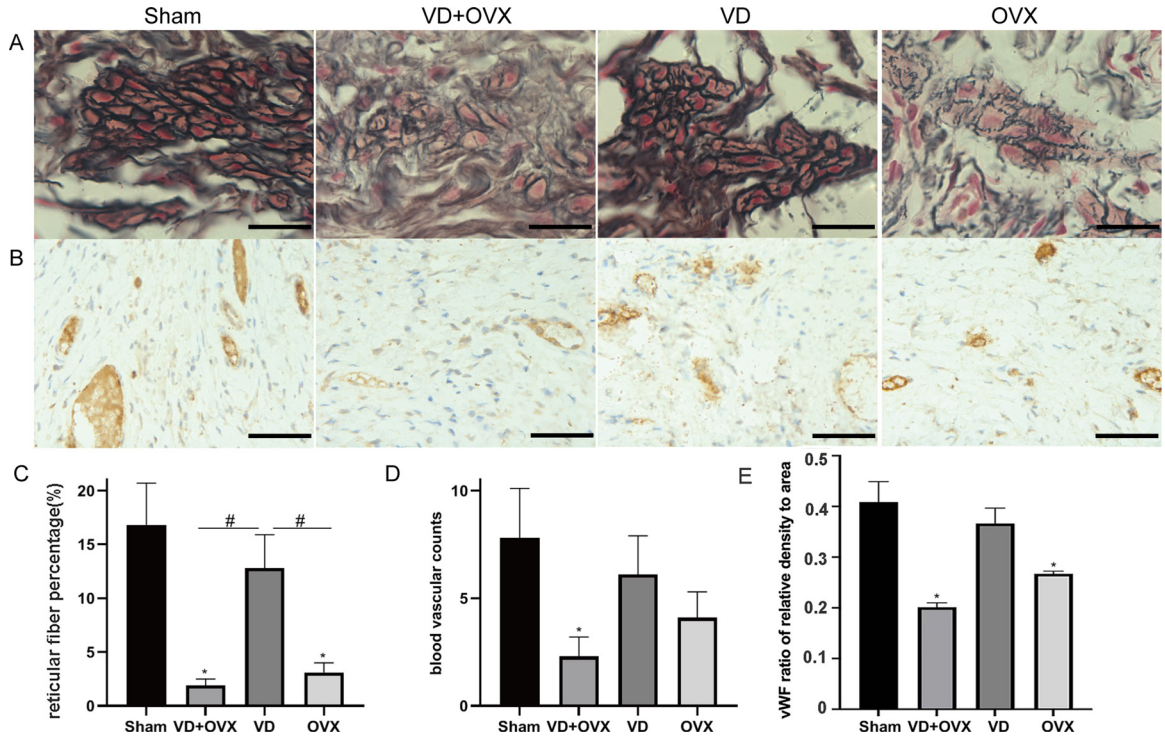

Figure 3. (A, C) Reticular fibers are black, collagen fibers are grayish red, and nucleus is red. The reticular fibers in smooth muscle layer of rats in Sham group were closely connected with muscle fibers and the fibers were continuous, while which in VD+OVX group were significantly separated from muscle fibers and fragmented. Scale bars: 10 um. (B, D, E) vWF immunohistochemical staining showed that vaginal submucosal vessels decreased significantly in VD+OVX group. Scale bars: 25 um. The vaginal tissue in each group is 10, that is (n = 10). * vs Sham, $P < .05$; # vs Model group, $P < .05$.

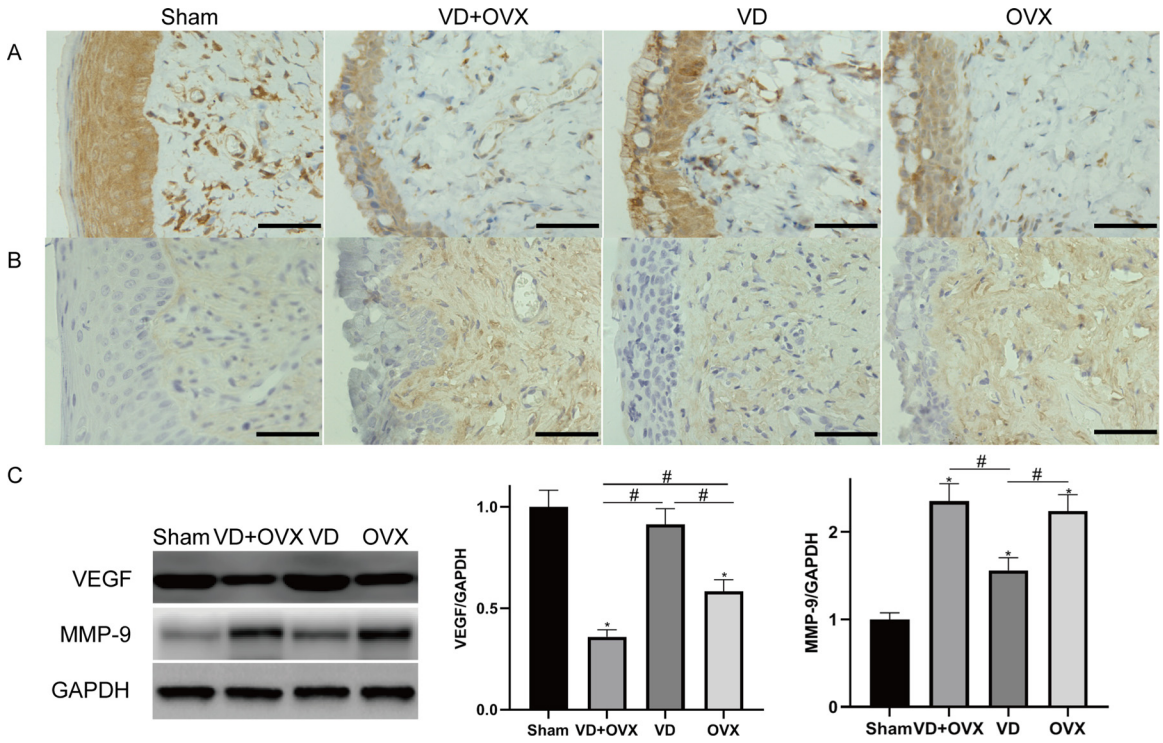

Figure 4. (A) VEGF immunohistochemical staining. Scale bars: 25 um. (B) MMP-9 immunohistochemical staining. Scale bars: 25 um. (C) VEGF and MMP-9 Westernblot detection. The expression of VEGF in vaginal tissue of rats in the VD+OVX group decreased significantly, while the expression of MMP-9 increased significantly. The vaginal tissue in each group is 10, that is (n = 10). * vs Sham, $P < .05$; # vs Model group, $P < .05$.

reticular fibers of vaginal tissue in VD+OVX group and OVX group were decreased, and shown to be significantly broken and fragmented ($P < .05$). Damages of vaginal tissue in VD+OVX group were much more prominent than that in VD group and OVX group.

Changes of Microvascular System

vWF immunohistochemical staining were in Figure 3B and D. vWF mainly expresses in lamina propria of vaginal tissue, which signs injury or activation of vascular endothelial cells. Compared with sham group, in VD+OVX group OVX group, the number of vaginal submucosal vessels and the expression of vWF significantly decreased, and vascular endothelial cells were seriously injured and activated less. The damage of vaginal submucosal vessels and vWF expression in VD+OVX group were lower than that in OVX group.

Immunohistochemical staining and western blot results are shown in Figure 4A and C. VEGF is mainly distributed in whole layer of vaginal tissue and play an important role in repairment of vascular endothelial injury. The expression of VEGF was decreased consistent with the change of results of vascular number that the changes in VD+OVX group were the most significantly.

Changes of Fibromuscular System

MMP-9, which mainly present in extracellular matrix of vaginal tissues, was detected by immunohistochemical staining and western blot in Figure 4B and C. The expression of MMP-9 in VD group, VD+OVX group and OVX group were significantly increased than that in sham group, indicating that there is an increased degradation of extracellular matrix in the vaginal tissues. The degradation of collagen fibers, elastic fibers and other connective tissues in vaginal tissues higher than that of the sham group.

DISCUSSION

Similar to the urethra and pelvic floor tissue,[18,19] there is also a fibromuscular system in vaginal tissue, which is composed of collagen fibers, elastic fibers, reticular fibers and muscle tissue. We inferred that the fibromuscular system is closely related to vaginal congestion and relaxation. The vaginal tissue is sinus-like tissue,[2,20] and micro-vasodilation in vaginal tissue during sexual arousal is the basis of vaginal relaxation, congestion, and lubrication.[21] We theorized that FGSAD damage in animal models might be caused by microvascular damage in vaginal tissue.

The sexual arousal phase of the female sexual response involves an increase in genital blood flow to allow lubrication and swelling, a process that can be altered by insufficient or reduced vasodilation of the arteries supplying female genitalia.[22] The physiological markers of sexual arousal were modeled by stimulating the pudendal, clitoral and pelvic nerve in a few previous anesthetized rats.[23,24] In these experiments, stimulation-

driven genital arousal was tested by vaginal hyperemia or vaginal perfusion and measured by laser Doppler flow meter (LDF). The experiment used nerve stimulation to observe changes in vaginal blood flow to reflect sexual arousal. It was reported that vaginal dilatation can lead to a decrease in vaginal blood flow,[25] with estradiol regulating of vaginal blood flow. Hormone withdrawal caused by ovariectomy similarly can also lead to a decrease in vaginal blood flow.[26] Studies have shown that hormonal disorder caused by ovariectomy can lead to a reduction in the weight of the uterus and vagina.[26] In this study, the electrophysiological model of sexual arousal measured the vaginal peak blood flow in female rats.

The measurement curve of VBF is shown in the supplement Figure 1 E. The neutral stimulus response time in our experiment was 30 seconds interval of 5 minutes. Each measurement varies, and we measured the distal part of the vagina at 3 different intervals. Because the changes of female blood flow are mainly vaginal micro vessels, we can only use laser doppler to detect blood flow. Due to the limitations of our experimental conditions, we cannot detect carotid pressure at the same time. It was found that the vaginal blood flow, weight of vagina and uterus decreased in treatment groups, which was consistent with the results reported in previous studies, but the most obvious changes was signified by the VD+OVX group, and there was no statistical significance in VD group and sham group. The results showed that FGSAD was accompanied by the change of uterine weight, which may be caused by the decrease of estrogen in premature ovarian failure,[27] which is consistent with the results of previous studies.

By measuring the changes of vaginal temperature, we found that different modeling methods had little effect on the vaginal temperature of rats, and there was no significant difference in vaginal temperature between rats in each group ($P > .05$). Significant differences were shown in rats: compared with the sham group, the degree of vaginal lubrication in rats was reduced after vaginal dilation and oophorectomy ($P < .05$), and after simultaneous vaginal dilation and oophorectomy, the degree of vaginal lubrication was reduced. In terms of vaginal surface tension, we found that when the nerves innervating vaginal muscles were stimulated, the vaginal surface tension of rats in the sham group would contract regularly, and regular tension peaks could be detected. This contraction was gentle and regular, but in rats with ovariectomized or vaginal dilation, we found that the contraction became irregular, and the peak of contraction was also reduced, and it was more pronounced in rats with vaginal dilation combined with oophorectomy, It is manifested as spastic contractions without the appearance of systolic peaks.

Some studies have shown that the sexual arousal function of patients with premature ovarian failure is impaired,[28] and Masson staining showed significant vaginal mucosal atrophy and a decrease in muscle layer. As shown in Figure 1D, smooth muscle fibers run longitudinally, and in circular or oblique layers. Our research showed that in the VD group, VD+OVX group and OVX group, the stratified squamous epithelium of vaginal wall became thinner, the number of mucosal layers decreased, mucous membrane atrophied, blood vessels in lamina propria were tortuous, vaginal muscle atrophy and content decreased, breakage and arrangement disorder appeared, which were consistent with the previous research results. α-SMA is the main structural and functional protein of fine myofilament, which is directly related to myofilament sliding. α-SMA is mainly expressed in the muscle fiber system. Compared with the muscle fiber system, the microvascular muscle content in the vaginal tissue is miniscule and so the expression level of α-SMA is taken as an indicator of the change in the content of muscle tissue. The decrease of α-SMA marks atrophy and degeneration of muscle cells. After ovariectomy, the vaginal contractility and muscle tissue content decreased in model group, especially in the VD +OVX group, which accorded with the pathophysiological mechanism of FGSAD.

The fascia of vaginal tissue are responsible for the secretion of collagen fibers I and III. The ratio of collagen fiber I/III determines the overall thickness of the structural collagen integrity. It has been reported that the total collagen content of vaginal fascia in patients with SUI is severely compromised due to a decrease in the amount of collagen fibers I and III.[29] Our results of Sirius red staining showed that collagen fiber I was significantly decreased and collagen fiber III decreased slightly in vaginal tissue of VD+OVX group and OVX group. At the same time, the ratio of collagen fiber I/III decreased significantly, which led to the thinning of collagen fiber and the decrease of tension ability, which was consistent with the previous study.

The externally supportive fibrous network composed of elastin and collagen fibers expands and provides structural support during female sexual arousal. Collagen fibers have high toughness, strong tensile force, elastic fibers are rich in elasticity but poor toughness, the 2 are intertwined together, so that the pelvic floor tissue is both elastic and tough, which is beneficial for organs and tissues to maintain a relatively constant morphological position. In this experiment, Hart's elastic fiber staining showed that the elastic fibers of VD group, OVX group, especially VD+OVX group were broken compared with sham group, and no longer extended from epithelium to muscle layer.

Reticular fibers are widely distributed in tissues and organs throughout the body in 2 forms. One main distribution of reticular fibers are in scaffolds for certain organs such as the bone marrow, thymus, and tonsils. The second form exists in basal membrane of the epithelium, smooth muscle, fat cells, capillaries, and nerve fibers are all covered by reticular fibers. To use reticular fiber staining to observe the morphological changes of tissue reticular fibers, such as number, thickness, density, fracture and collapse, can find that is of great significance for judging the nature, degree, development and outcome of diseases.[30–32] In our experiment, Gordon & Sweet's staining showed that the reticular fibers of vaginal tissue in VD+OVX group and OVX group were significantly separated from muscle fibers and obviously fractured.

In our study, ovarian resection was performed on the seventh day after vaginal dilation, and further sampling and subsequent experiments were carried out after 4 weeks, reflecting a chronic injury in model. It is consistent with female sexual genital arousal syndrome as it properly establishes a chronic environment or a late-onset condition. Studies have reported vascular destruction in human female sexual arousal disorders, which was consistent with our model. We chose VEGF and MMP9 which are sensitive to the onset of angiogenesis in our study.

Von Willebrand factor (vWF) is a sign of injury or activation of vascular endothelial cells. In this study, vWF was used to mark vaginal blood vessels and count the number of vaginal blood vessels. The results showed that the number of micro-vessels in OVX+VD group decreased significantly. Vascular endothelial growth factor (VEGF) plays important role in repair of vascular endothelial injury. Its immunohistochemical results were consistent with the results of vascular counting. In our research, the expressions of vWF and VEGF were decreased in vagina, especially in VD+OVX group, that mean, the submucosal vessels decreased significantly, which showed that the ability of vascular endothelium formation was weakened and vascular endothelial cells were injured. It was also supported by the decrease of vaginal blood flow measured by Doppler in the electrophysiological model, which explained the decrease of vaginal blood vessels and blood flow. Our results further prove that vaginal microvascular injury plays an important role in genital arousal disorder in female rats.

Matrix metalloproteinase-9 (MMP-9), which belongs to the matrix metalloproteinase (MMP) family, is a major degrading enzyme in process of extracellular matrix degradation. Studies have reported that MMP9 can be activated when the pelvic floor connective tissue is damaged, thus affecting the dynamic balance between the synthesis and degradation of extracellular matrix such as collagen and elastin in pelvic support tissues.[33,34] In our study, the expression of MMP-9 increased significantly in VD group, VD+OVX group and OVX group, especially VD+OVX group, showing that connective tissue such as collagen fibers in muscle fiber system of vagina degrades. It further illustrated the injury of vaginal connective tissue, such as elastic fiber, collagen fiber and reticular fiber. It was proved that the destruction of fibrous connective tissue is involved in the occurrence of genital arousal disorder in female rats, which is consistent with the previous study.

Compared with other groups, the VD+OVX group shows obvious damage in all aspects of vaginal tissue, which has research significance, and accord with the pathophysiological mechanism of female genital arousal disorder. When the mechanical injury such as the prolongation of the second stage of labor exceeds the bearing scope of the vaginal tissue or the biomechanical properties of vaginal tissue decreases,[35] hormone withdrawal and damage of the fibromuscular system in vaginal tissue are combined,[36] pathological damage occurs in vaginal tissue, such as vaginal microvascular damage, muscle atrophy, etc., and finally causes FSGAD lesions. To sum up, our hypothesis was verified by these experiments, that the vagina contains muscle fiber systems, and it interact with vaginal capillaries, jointly maintain vaginal expansion and flexibility of the normal physiological function, such as any part of muscle fiber system and microvascular damage will affect the function of the vagina, then female genital sex sexual arousal disorder occurs.

We do acknowledge the shortcomings present in our study. Firstly, the pathogenic factors of FGSAD are complex, and the incidence of FGSAD is the highest in postmenopausal women whereas our study mainly discusses the most common FGSAD caused by vaginal lesions caused by the interaction of labor injury and estrogen reduction factors. Other important aspects of sexual arousal are beyond the scope of discussion in this paper. Secondly, the evaluation of FGSAD parameters is still subjective and difficult to be quantified. Questionnaires used in the clinical setting such as the female sexual function indicator scale or vaginal plethysmography are often used for evaluation, but qualitative in nature. In the experimental model, no scale evaluation can be properly established and only functional indicators can be used for evaluation. At present, the most recognized quantitative index to evaluate the success of FGSAD modeling is electrophysiological model combined with vaginal laser Doppler blood flow measurement. There is no unified international quantitative index for the evaluation of vaginal temperature and vaginal lubrication at present. Thirdly, due to the difficulty in obtaining FGSAD samples for clinical research, this study only conducted basic research and was limited to the theoretical level, without combining with clinically relevant cases.

According to the results of our study, vaginal dilatation combined with ovariectomy can successfully establish an animal model of FSGAD with a high success rate and good stability. It is more comprehensive to simulate the occurrence of FSGAD under the action of multiple factors, which is more consistent with the mechanism of human FSGAD.

CONCLUSION

Our results show that our animal model of FSGAD through vaginal dilatation combined with ovariectomy does not only has more scientific research value but also can reflect the occurrence mechanism of human FSGAD from the pathophysiological changes. We also simulated the 2 important etiologies of hormone withdraw and prolonged second stage of labor. Utilizing this FSGAD model, further research involving drug treatments may be possible to explore the possibility of medicinal treatment of FSGAD.

ACKNOWLEDGMENTS

This work is supported by National Natural Science Foundation of China (81860268), Ningxia Natural Science Foundation (2021AAC02025), Key R & D projects in Ningxia(2020BFG02010), Ningxia science and technology innovation leading talent training project (2020GKLRLX06, 2020GKLRLX11).

Corresponding Author: Rui He, PhD, Key Laboratory of Fertility Preservation and Maintenance of Ministry of Education, School of Basic Medical Sciences, Ningxia Medical University, No.1160, Shengli Street, Xingqing District, Yinchuan, Ningxia Hui Autonomous Region, Yinchuan 750004, China. E-mail: ruihe515@163.com; Hetao Liu, MMS, Key Laboratory of Fertility Preservation and Maintenance of Ministry of Education, School of Basic Medical Sciences, Ningxia Medical University, Yinchuan, P.R.China; E-mail: lht830116@163.com

Guangyong Li, Puguang Yu, and Yanan Hu contributed equally a first authors.
Conflict of Interest: The authors report no conflicts of interest.

Funding: National Natural Science Foundation of China (81860268), Ningxia Natural Science Foundation (2021AAC02025), Key R & D projects in Ningxia (2020BFG02010), Ningxia science and technology innovation leading talent training project (2020GKLRLX06, 2020GKLRLX11).

STATEMENT OF AUTHORSHIP

Original: R.H,GY.L. conceived and performed experiments, wrote the manuscript, and secured funding. GY.L., PG.Y, YN. H., ZX.H. and J.L. performed experiments. YS.S. ,J.W. and XK. Z. provided reagents. GY.L. ,HT.L, YN.H. and R.H.provided expertise and feedback.

REFERENCES

1. Moore RD, Miklos JR, Chinthakanan O. Vaginal reconstruction/rejuvenation: is there data to support improved sexual function? An update and review of the literature. **Surg Technol Int 2014;25:179–190.**
2. Lalumière ML, Sawatsky ML, Dawson SJ, et al. The empirical status of the preparation hypothesis: explicating women's genital responses to sexual stimuli in the laboratory. **Arch Sex Behav 2020:1–20.**
3. Wallen K. Protection or pleasure: the role of genital arousal in sexual intercourse. **Arch Sex Behav 2020:1–5.**
4. Handy AB, Stanton AM, Pulverman CS, et al. Differences in perceived and physiologic genital arousal between women with and without sexual dysfunction. **J Sex Med 2018;15:52–63.**
5. Pessina MA, Hoyt RF, Goldstein I, et al. Differential effects of estradiol, progesterone, and testosterone on vaginal structural integrity. **Endocrinology 2006;147:61–69.**
6. Kim SW, Kim NN, Jeong SJ, et al. Modulation of rat vaginal blood flow and estrogen receptor by estradiol. **J Urol 2004;172:1538–1543.**
7. Ågmo A. Animal models of female sexual dysfunction: basic considerations on drugs, arousal, motivation and behavior. **Pharmacol Biochem Behav 2014;121:3–15.**
8. Nastri CO, Lara LA, Ferriani RA, et al. Hormone therapy for sexual function in perimenopausal and postmenopausal women. **Cochrane Database Syst Rev 2013;6:CD009672.**
9. Rathfisch G, Dikencik BK, Kizilkaya Beji N, et al. Effects of perineal trauma on postpartum sexual function. **J Adv Nurs 2010;66:2640–2649.**
10. Abdool Z, Thakar R, Sultan AH. Postpartum female sexual function. **Eur J Obstet Gynecol Reprod Biol 2009;145:133–137.**
11. Verit FF, Verit A, Billurcu N. Low sexual function and its associated risk factors in pre- and postmenopausal women without clinically significant depression. **Maturitas 2009;64:38–42.**
12. Lowenstein L, Gruenwald I, Itskovitz-Eldor J, et al. Is there an association between female urinary incontinence and decreased genital sensation? **Neurourol Urodyn 2011;30:1291–1294.**
13. Coksuer H, Ercan CM, Haliloğlu B, et al. Does urinary incontinence subtype affect sexual function? **Eur J Obstet Gynecol Reprod Biol 2011;159:213–217.**
14. Sacomori C, Cardoso FL. Predictors of improvement in sexual function of women with urinary incontinence after treatment with pelvic floor exercises: a secondary analysis. **J Sex Med 2015;12:746–755.**
15. Li GY, Zhou F, Gong YQ, et al. Activation of VEGF and ERK1/2 and improvement of urethral function by adipose-derived stem cells in a rat stress urinary incontinence model. **Urology 2012;80:953. e1-8.**
16. Lin G, Shindel AW, Banie L, et al. Molecular mechanisms related to parturition-induced stress urinary incontinence. **Eur Urol 2009;55:1213–1222.**
17. Jeong H, Seong M, Lee HS, et al. Design of an optical probe to monitor vaginal hemodynamics during sexual arousal. **Sensors (Basel) 2019;19:2129.**
18. Li GY, Cui WS, Zhou F, et al. Pathology of urethral fibromuscular system related to parturition-induced stress urinary incontinence and TGF-β1/Smad pathway. **Mol Cell Biochem 2012;364:329–335.**
19. Goepel C, Thomssen C. Changes in the extracellular matrix in periurethral tissue of women with stress urinary incontinence. **Acta Histochem 2006;108:441–445.**
20. Oh SJ, Hong SK, Kim SW, et al. Histological and functional aspects of different regions of the rabbit vagina. **Int J Impot Res 2003;15:142–150.**
21. Handy AB, Meston CM. An objective measure of vaginal lubrication in women with and without sexual arousal concerns. **J Sex Marital Ther 2021;47:32–42.**
22. Angulo J. Neutral endopeptidase inhibition: Could it have a role in the treatment of female sexual arousal disorder? **Br J Pharmacol 2010;160:48–50.**
23. Cai RS, Alexander MS, Marson L. Activation of somatosensory afferents elicit changes in vaginal blood flow and the urethrogenital reflex via autonomic efferents. **J Urol 2008;180:1167–1172.**
24. Kim SW, Jeong SJ, Munarriz R, et al. An in vivo rat model to investigate female vaginal arousal response. **J Urol 2004;171:1357–1361.**

25. Damaser MS, Whitbeck C, Chichester P, et al. Effect of vaginal distension on blood flow and hypoxia of urogenital organs of the female rat. **J Appl Physiol (1985) 2005;98:1884–1890.**
26. Kim SW, Kim NN, Jeong SJ, et al. Modulation of rat vaginal blood flow and estrogen receptor by estradiol. **J Urol 2004;172:1538–1543.**
27. Heidari M, Ghodusi M, Rezaei P, et al. Sexual function and factors affecting menopause: a systematic review. **J Menopausal Med 2019;25:15–27.**
28. Lindau ST, Abramsohn EM, Matthews AC. A manifesto on the preservation of sexual function in women and girls with cancer. **Am J Obstet Gynecol 2015;213:166–174.**
29. Bakas P, Liapis A, Salamalekis E, et al. Tension-free vaginal tape efficacy in relation to collagen quantity of pubocervical fascia. **Gynecol Obstet Invest 2004;57:149–152.**
30. Papotti M, Libè R, Duregon E, et al. The Weiss score and beyond—histopathology for adrenocortical carcinoma. **Horm Cancer 2011;2:333–340.**
31. Duregon E, Fassina A, Volante M, et al. The reticulin algorithm for adrenocortical tumor diagnosis: a multicentric validation study on 245 unpublished cases. **Am J Surg Pathol 2013;37:1433–1440.**
32. Wanis KN, Kanthan R. Diagnostic and prognostic features in adrenocortical carcinoma: a single institution case series and review of the literature. **World J Surg Oncol 2015;13:117.**
33. Edwall L, Carlström K, Jonasson AF. Markers of collagen synthesis and degradation in urogenital tissue from women with and without stress urinary incontinence. **Neurourol Urodyn 2005;24:319–324.**
34. Moalli PA, Shand SH, Zyczynski HM, et al. Remodeling of vaginal connective tissue in patients with prolapse. **Obstet Gynecol 2005;106:953–963.**
35. Lei L, Song Y, Chen R. Biomechanical properties of prolapsed vaginal tissue in pre- and postmenopausal women. **Int Urogynecol J Pelvic Floor Dysfunct 2007;18:603–607.**
36. Goepel C. Differential elastin and tenascin immunolabeling in the uterosacral ligaments in postmenopausal women with and without pelvic organ prolapse. **Acta Histochem 2008;110:204–209.**

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.esxm.2022.100530.