Established a comprehensive mice model of persistent genital arousal disorder and investigated the mechanism of VEGF-ERK signal pathing in vaginal microvascular injury

Puguang Yu
The General Hospital of Ningxia Medical University, Ningxia Medical University
Guangyong Li
The General Hospital of Ningxia Medical University, Ningxia Medical University
Xiaojiang Chen
Ningxia Medical University
Shuai Ren
The General Hospital of Ningxia Medical University, Ningxia Medical University
Qiangqiang Wang
The General Hospital of Ningxia Medical University, Ningxia Medical University
Yashan Su
The General Hospital of Ningxia Medical University, Ningxia Medical University
Keming Chen
The General Hospital of Ningxia Medical University, Ningxia Medical University
Jiajin Feng
The General Hospital of Ningxia Medical University, Ningxia Medical University
Hetao Liu
Ningxia Medical University
Huaiqin Han
Ningxia Medical University
Rui He (ruihe515@163.com)
Ningxia Medical University

Article

Keywords: persistent genital arousal disorder, mice model, sexual behavior, microvascular damage, VEGF-ERK signal pathing

Posted Date: July 5th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1773638/v1

Abstract

Aim: (1) Simulating vaginal delivery and menopause to establish a mouse model of PGAD; (2) screen out a set of detection methods for quantifying and evaluating mouse sexual function; (3) further explore the relationship and possible mechanism of vaginal microvascular injury and PGAD.

Methods: Animal study about established a PGAD mouse model with vaginal dilation and ovariectomized, and ligated vaginal microvascular to compared effects of blood flow on sexual behavior with sham. Animals: C57/B6J mice. Intervention: 8-week-old post-childbirth mice were vaginal dilation and ovariectomy and reared for 1 or 2 months. In addition, ligated microvascular around the vagina to build a vaginal ischemia model.

Main Outcome Measures: Video cameras continuously recorded the mice's sexual behavior, vaginal temperature and lubricating were observed by thermometer and water absorption test paper, vagina surface tension was observed by multi-channel physiological recorder. Various stains were used to observe the vaginal morphology, blood vessels, nerves, muscles and fibers of vaginal tissue.

Results: (1) Different modeling methods and time will cause atrophy in the vagina and uterus in different groups ($F=169.4$, $p<0.01$; $F=114.3$, $p<0.01$), among which VD2H has less effect, while OVX and VD4H have more. The influence of the two methods combination makes the atrophy more obvious. (2) Vaginal dilation had no significant effect on E2, T and DA ($F=147.6$, $p>0.05$), but the contents of these three hormones decreased after ovariectomy ($F=480.9$, $p<0.01$). (3) Except for VD2H, the acceptance of sexual response in other groups were decreased ($F=58.4$, $p<0.01$), the aggressive was increased ($F=121.1$, $p<0.01$), and the lordosis were also decreased ($F=67.9$, $p<0.01$). Besides, the vaginal lubrication and vaginal contractions were decreased ($F=61.3$, $p<0.01$), but the vaginal temperature did not change significantly in each group ($F=38.4$, $p>0.05$). (4) Different modeling methods caused different damage and atrophy in the vaginal tissue, which showed that the thickness of epithelial and muscle layer was reduced, the fibrosis was deepened, the content of muscle, nerve fibers and glycogen were reduced. The above phenomenon was most obvious in the OVX&VD4H group, and the VD2H group was the least. Compared with 1 month, the vaginal tissue had restored at 2 months in most groups. (5) Ovariectomy and vaginal dilation both decreased the number of vaginal microvascular ($F=5.4$, $p<0.05$), and the contents of VEGF and MMP-9 decreased ($F=367.9$, $p<0.01$; $F=121.3$, $p<0.01$), particularly, VD4H decreased more than VD2H ($F=117.3$, $p<0.01$), OVX&VD4H was more than OVX&VD2H ($F=413.3$, $p<0.01$), and in 2 months, their levels all increased to varying degrees. (6) Vaginal atrophy occurred after blood flow occlusion, manifested as uniform atrophy of the entire vaginal thickness and aggravated fibrosis. The weights of vagina and uterus were both lower than sham group ($F=13.7$, $p<0.01$; $F= 12.1$, $p<0.01$), the estrous cycle was disturbed, and these manifestations were more obvious in bilateral blood flow occlusion than unilateral occlusion. (7) The number of microvascular in the vaginal tissue were decreased after blood flow occlusion ($F=16.4$, $p<0.01$), and the contents of VEGF and MMP-9 also decreased ($F=669.1$, $p<0.01$; $F=793.1$, $p<0.01$), in which bilateral blockade was more obvious than unilateral blockade. After WB experiment, it was found that the contents of VEGF and ERK decreased after blood flow blockade, and the decrease in bilateral blockade group was more than that in unilateral blockade group.

Conclusion: By simulating different modeling methods and time, we believe that a stable and novel PGAD mouse model can be established after ovariectomy combined with vaginal dilation for 2 hours (OVX&VD2H) and rearing for 1 month. In addition, through a large number of experiments, we screened that sexual behavior, vaginal lubricating, vagina surface tension can be used to detect sexual function in mice objectively and accurately, but vaginal temperature not suitable for the detection of mouse sexual function. Finally, we further verified that microvascular injury can significantly appeared in the model, and the VEGF-ERK signaling pathway is a possible mechanism involved in microvascular injury.

Introduction

Persistent genital arousal disorder (PGAD), a condition of unwanted, unremitting sensations of genital arousal, is associated with a significant, negative psychosocial impact that may include emotional lability, catastrophizing, and suicidal ideation[1]. Although PGAD is not an pressing disease, it will bring extreme discomfort to the patient, and will seriously threaten the sexual health, even 10.4% of patients will be accompanied by these symptoms lifelong[2]. Previous studies generally believed that PGAD may only be a mental and psychological disease without genital damage[3], but with the deepening of research, we found that central and peripheral neuropathy, vascular damage, abnormal sex hormones, and the related lesions caused by end-sex-organ damage are all involved in the occurrence of PGAD, especially, the events related to vaginal vascular damage may be the most relevant one[4–9]. However, the current research on the role of blood flow in PGAD is analogous to the mechanism of male penile erection[10,11], the relationship between blood flow and PGAD is currently unclear, so we speculate whether the vaginal microvascular damage caused the occurrence of PGAD, or the PGAD affects vaginal blood perfusion, these requires us to further explore.

Animal models are important tools in medical research. Female sexual response is a complex process, which involving various mechanisms such as blood vessels, nerves, endocrine and psychological[12,13]. Previous researchers have established many PGAD models through continuous exploration, such as behavioral models of sexual desire and motivation, sexual arousal models, orgasm models(genital strip organ bath) and even cell model(genital smooth muscle cell culture)[14–16]. These models are all for a single type of sexual disorder, which lack of integrity and comprehensiveness, cannot represent the actual clinical manifestations of PGAD. In previous clinical practice, we found that the decline of sex hormones and vaginal damage during childbirth are closely related to the PGAD[17–21]. Therefore, if an animal model can be established to simulate vaginal injury during childbirth and menopause, it will be the closest to the pathophysiology and clinical characteristics of PGAD. In addition, the above models also lack a set of objective and novel detection methods to evaluate sexual dysfunction, based on these reasons, establishing a reliable animal model and screening out the comprehensively detection indicators that can evaluate the characteristics of sexual response is a scientific problem that needs to be studied urgently.

To summarize, the aim of the present work was to establish a comprehensive female mice model of PGAD by vaginal dilation and ovariectomized, and further to screen out a comprehensively detection methods for quantifying and evaluating mouse sexual function. Besides, we also intended to investigate the relationship and possible mechanism of vaginal microvascular injury and PGAD.

Materials And Methods

The experiment is divided into two parts. As shown in Fig. 1, in the first place, we established a novel PGAD mouse model through vaginal dilation and ovariectomized. Then, screened out objective indicators for quantifying mouse sexual behavior and other parameters through a variety of detection methods. Finally, we used this novel model to verify the causal relationship between microvascular injury and PGAD.

Animal recruitment

Experiments were conducted in 118 gravid female C57BL6J mice (Laboratory Animal Center of Ningxia Medical University, Yinchuan, Ningxia, China) weighing 8 weeks old. All mice were randomized the day after giving birth to sham (sham operation), VD2H (vaginal dilation for 2 hours), VD4H (vaginal dilation for 4 hours), OVX (ovariectomy), OVX& VD2H (Vaginal dilation administered at 2 hours following ovariectomy), OVX&&VD4H (Vaginal dilation administered at 4 hours following ovariectomy). The above groupings have 1-month and 2-month time gradients.

This study was reviewed by the Ethical Review Committee of Ningxia Medical University (IACUC-NYLAC-2021-052). All experiments were performed in accordance with relevant Laboratory Animal Welfare and Ethics guidelines and regulations. And we confirmed that complied with the ARRIVE guidelines.

Methods and materials used.

Ovariectomized: Made a 2cm incision above the pubic symphysis to expose the uterine horns, find the ovaries along both sides of the uterus, ligated the ovarian arteries and removed the ovaries. Vaginal dilation: Used 8Fr urinary catheter (Zhanjiang Shida Industrial Co., Ltd., Guangdong Province, China), applied lubricant and inserted it into the vagina, injected 0.6ml warm saline into the water bladder, the urinary catheter is sutured to the vaginal opening with 4 – 0 sutures, and a 30 g weight is suspended from the end of the catheter to hold the catheter under tension (in order to allow the bladder to fully expand the vaginal tissue and not get stuck on the pubic symphysis). Changes before and after vaginal dilation are shown in Supplementary Fig. 1A-C. Vascular occlusion: As indicated by Supplementary Fig. 2, we isolated the blood vessels on both sides of the vagina, and then used high frequency electro tome to divide these vessels into unilateral and bilateral severing.

Sexual behavior and other related sexual responses measured

In this part of the experiment, we refer to the method of Snoeren E and Hernández-Munive A.[22,23]. The process of sexual behavioral analysis can be stated with two conditions to made mice sexually aroused: One with intraperitoneal hormone therapy (24 hours before the test, 3 mg per mouse of estradiol benzoate subcutaneously; followed by progesterone, 1 mg per mouse subcutaneously, 4 hours before the test), and second with nerve stimulation. After then testing **sniff** in the first 5 minutes, **receptive behavior** (called lordosis, which refers to the dorsiflexion of the spinal cord), **preceptive behaviors** (hopping, darting, and ear wiggling) and **aggressive behaviors** (boxing, bites and lateral postures) in 30 minutes. In addition, we also recorded the **return latencies**, defined as the time the female needs to return to the male's chamber after an exit, and the time that the female spends in the male's compartment. All tests were conducted between 1:00 PM and 5:30 PM during the dark cycle in a glass box (80×60×60cm) that had a door and black covering cloth. Attach a remote-controlled camera to the side wall of the cage, and after 5 minutes, place an 8-week-old male C57BL6J mouse with normal sexual function.

Vaginal lubrication test

In this part, we innovatively used our own absorbent paper (0.3*1.0cm) to directly observe the changes in the degree of vaginal lubrication (Supplementary Fig. 1F). Cut the scaled absorbent paper into 0.3cm*1cm lengths, inserted into the same depth at the proximal end of the vagina, and the mice were artificially sexually aroused by stimulating the vaginal branch of the pelvic nerve (Supplementary Fig. 2), each stimulation was 10 seconds, and the interval was 1 minute, observe for 10 minutes.

Vaginal temperature test

The room temperature was always kept at 36.0°C, an electronic thermometer (unit: 0.1°C) was inserted into the vagina, the mice were sexually aroused by stimulating the vaginal branch of the pelvic nerve, and the reading was waited for 1 minute (Supplementary Fig. 1E). After three consecutive measurements, the average value was taken as the temperature of the vagina when the mice were sexually aroused.

Vaginal surface tension

Use the MD3000 biological information acquisition system (Beijing Zhimo Duobao Biotechnology Co., Ltd. China) to detect the surface tension of the vagina, give electrical stimulation with the corresponding pulse width, frequency, and energy (The stimulator voltage was 6V, the pulse interval was 0.8ms, the stimulation duration was 30s, the stimulation frequency was 10 Hz, and the stimulation interval was 5 minutes), and slowly place the vaginal recording electrode in the center of the mouse channel, adjust the electrode position to make the metal probe close to the vaginal wall, to stimulated the vaginal branch of the pelvic nerve and observe changes in vaginal surface tension.

Vaginal morphology

We use different staining, including H&E staining, Masson staining, Glycogen dyeing, Glycine silver staining and transmission electron microscopy to observe the effect of vaginal dilation and ovariectomy on vaginal morphology.

Immunological staining

Immunological chemical analysis: The dehydrated tissue was first subjected to antigen retrieval (boiled with 0.01mol/L sodium citrate buffer for 15min in a microwave oven), and incubated with 0.3% Triton-x 100 for 15min. The serum working solution was blocked at room temperature for 1 hour, and the antibody CD31(1:200, Abcam, USA), VEGF-A (1:200, Abcam, USA), MMP-9(1:500, Cell Signaling, Beverly, MA, USA) was added dropwise, overnight at 4°C; on the second day, rewarmed for 30-60min, added Rabbit anti-mouse IgG/HRP (Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd., China), incubated at 37°C for 30min, and then incubated with DAB developer solution. Immunofluorescence: Except that the secondary antibody uses Goat Anti-Rabbit IgG H&L, other experimental methods and reagents are similar to immunological chemical analysis.

Enzyme-linked immunosorbent assay (ELISA)

Blood was drawn from the heart, centrifuged at 12000 r/min for 10 min, serum was separated, and ELISA detection kit (Wuhan Elite Biotechnology Co., Ltd. China) was used. The serum to be tested in each group was added to the reaction well, 100μl per well, and 4 duplicate wells were set for each group, and incubated at 37°C. 90 min, discard the liquid in the well, add 100 μL of biotinylated antibody working solution, incubate at 37°C for 60 min, discard the liquid in the well, wash 3 times, then add 100 μL of enzyme conjugate working solution, incubate at 37°C for 30 min, then discard remove the liquid in the wells, wash 5 times, add 90 μL of substrate solution to each well, incubate at 37°C for 15 min, add 50 μL of stop solution, detect at 450 nm wavelength, and calculate the content of T, E2 and DA in the serum of mice in each group according to the standard curve.

Western blot

Anterior vagina tissue protein samples were prepared by homogenizing in RIPA lysis buffer. Equal protein (20μg/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: 1000) (Abcam, Cambridge, MA, USA), VEGF (1:1000) (Abcam, Cambridge, MA, USA), CD31(1: 1000) (Abcam, Cambridge, MA, USA) and β-actin(1:1000) (Santa Cruz Biotechnology, CA, USA).

Estrous Cycle Determination

This part of the experiment is only in the experiment of the relationship between blood flow and PGAD. Starting from the first day, testing every 3 days until the day before the end of the experiment, a daily vaginal smear was collected (13:00–14:00 PM) and examined under a light microscope to determine the stage of the estrous cycle. The estrous cycle phase was characterized by vaginal cytologic study based on Cora M [24].

Statistics

The statistical analysis is made using SPSS23.0 software. The results were expressed as the mean ± SD. The normal distribution of the data was checked with the Kolmogorov-Smirnov test. This study was a mixed within/between design, One-Way ANOVA analysis of variance was used for comparison between groups and Tukey Post Hoc test is used to perform multiple comparisons, and use the two-way ANOVA with repeated measures with one within (test-time) and six between (sham, VD2H, VD4H, OVX, OVX&VD2H, OVX&VD4H) factors. Means were considered significantly different at $P < 0.05$.

Results

Effects of vaginal dilation and ovariectomy on sex organs and sex hormone levels

In the study, there is bleeding on the vaginal surface after vaginal dilation, and the vagina is pouch-like dilation (Fig.2-A). The appearance of the vagina after OVX is similar to the sham group, but there is atrophy and hemorrhage when the vagina after OVX & VD, these changes of the mice in each group were the most obvious at 1 month, and in 2 months this damage was less than 1 month, which may be related to self-healing. These phenomena are more pronounced in animal tissues (Supplementary Fig. 3). Further statistics on vaginal and uterine weight and organ index, we also obtained a similar result (Fig.2 B-E): different modeling methods can reduce the weight of vagina ($F=164.2$, $p<0.01$) and uterine ($F=115.4$, $p<0.01$), among which OVX&VD4H mice lost the most, and there were differences between 1 month and 2 months ($F = 390$, $p < 0.01$). In terms of sex hormones, simple vaginal dilation had little effect on estrogen(E2), testosterone(T) and dopamine (DA), but the contents of the above three hormones decreased after ovariectomy. The change of E2 was also affected by vaginal dilation, OVX&VD4H decreased more than OVX&VD2H ($F=546.4$, $p<0.01$), while T and DA were only affected by oophorectomy ($F=112.6$, $p<0.01$; $F=36.5$, $p<0.01$). Expansion had little effect on T and DA, and there was no significant difference between feeding for 1 month and 2 months ($F=0.01337$, $p=0.9135$). (Fig.2 F-H).

Effects of vaginal dilation and ovariectomy on the sexual behavior

In the experiment, we artificially induced sexual arousal in mice by intraperitoneal injection of estrogen and progesterone, and then observed the changes in the sexual response of different groups of mice under normal and aroused states. In table 1, at 1 month, the number of sniff (in the first 5 minutes) and proceptivity (hops, ear wiggling, and darting) decreased except VD4H, OVX and VD2H&OVX groups. Aggressive (the number of boxing, bites and lateral postures) increased in VD4H, OVX, VD2H+OVX and VD4H+OVX ($F=98.3$, $p<0.01$). While the return latencies only increased statistically in the VD4H+OVX group ($F=177.9$, $p<0.01$). Compared the 1 month and 2 months, we found that except for VD4H&OVX, the sniff and proceptivity of mice in other groups were lower than sham group, and there was no significant difference between the different modeling groups and the sham group.

Effects of vaginal dilation and ovariectomy on the sexual response

We stimulated the vaginal branch of the pelvic nerve to achieve sexual arousal in mice after anesthesia, and then measured changes in vaginal temperature, vaginal lubrication and vaginal surface tension. Vaginal lubrication showed great differences between different groups. Compared with the sham group, the vaginal lubrication was reduced in all groups except for VD2H, and the decrease was more obvious in OVX&VD4H ($F=94.3$, $p<0.01$), but there is no significant difference between 1 month and 2 months ($F=1.301$, $p=0.3177$) (Fig.3A). There was no statistical difference in the changes of vaginal temperature ($F=0.485$, $P=0.7834$) (Fig.3B). In terms of lordosis (Fig.3C-D), the effect of VD2H is not very distinctively, but the VD4H, OVX, OVX&VD2H and OVX&VD4H are obviously descending, besides, the lordosis score and lordosis quotient of each group have different degrees of recovery at 2 months. In vaginal surface tension, we found that the sham mice exhibited regular contractions when stimulated, and regular contraction peaks could be detected (Fig. 3-E), but in OVX or VD4H mice, this contraction became irregular, and the effective contraction peak was also reduced, which was more obvious in ovariectomy combined with vaginal dilation mice, and particular in OVX&VD4H mice, there was irregular vaginal tremor, and no effective contraction peak appeared.

Morphological analysis after vaginal dilation and ovariectomy

We observed the difference in the gross morphology, collagen fibers, nerve fibers and glycogen in vaginal tissue by different staining methods (Fig.4). Through H&E staining, we found that the vagina of OVX OVX&VD2H and OVX&VD4H mice showed obvious signs of atrophy, the epithelial layer was thinned. VD2H did not show obvious changes, but the epithelial layer appeared loose and broken, which was most obvious in VD4H. Except for the OVX&VD4H group, the vaginal morphology of the other combination groups improved at 2 months. In Masson staining, VD2H or OVX had no significant effect on vaginal fibrosis, but when VD4H OVX&VD2H,OVXVD4H was present, the vaginal epithelium appeared keratinized, muscle fibers (red) were significantly reduced, and collagen fibers (orange) increased, indicating that the degree of fibrosis was aggravated. And the degree of fibrosis was further aggravated when vaginal dilation combined with ovariectomy**,** particularly, OVX&VD4H was more obvious than OVX& VD2H. We used glycogen staining to observe the glycogen content in vaginal tissue: we found that ovariectomy had a greater effect on glycogen content than vaginal dilation, neither VD2H nor VD4H had a significant effect on glycogen content, but after ovariectomy, the glycogen was significantly reduced, and the reduction was more obvious after vaginal dilation and ovariectomy. Except for OVX&VD4H, the glycogen content of the other groups of mice recovered at 2 months. Finally, we stained the nerve fibers in the vaginal tissue

and found that there was no obvious change after oophorectomy, but the nerve fibers were significantly broken and reduced after vaginal dilation, especially after VD4H. At 2 months, the number of nerve fibers in VD2H, OVX and OVX&VD2H all recovered, while VD4H and OVX&VD4H were similar to 1 month without significant change.

Effects of vaginal dilation and ovariectomy on vaginal ultrastructure

We further observed the ultrastructural changes of vaginal tissue after vaginal dilation and ovariectomy by transmission electron microscopy. We found that, compared with the sham group, the vaginal epithelium became looser after vaginal dilation, the cell-to-cell space became larger, and there was extensive fusion between adjacent cells (Fig.5). However, after ovariectomy, the space between the epithelial cells did not change significantly, but the glycogen content in the cytoplasm was significantly reduced compared with the vaginal dilation group. When vaginal dilation was combined with oophorectomy, the normal structure of the vaginal epithelium was destroyed, and the content of glycogen in the cytoplasm was also reduced. At 2 months, the injured vaginal tissue recovered to varying degrees, but the recovery of VD4H and OVX&VD4H was not obvious.

Microvascular and MMP-9 changes after vaginal dilation and ovariectomy

As shown in the Figure 6, VD2H has little effect on vaginal microvascular, VD4H and OVX are significantly reduced, and the combination of the 2 methods will make the reduction more obvious. At 2 months, the number of microvascular in the VD2H, OVX, OVX&VD2H groups all increased significantly ($F=91.8$, $p<0.01$, Fig.6B), but the VD4H and OVX&VD4H did not recover significantly ($F=311.5$, $p<0.01$). In addition to CD31, we also found that different modeling methods ($F=36.91$, $p<0.01$) had effects on VEGF, although VD2H and OVX were reduced at 1 month, they recovered significantly at 2 months. VD4H and OVX&VD4H both were significantly lower than the sham group, and the recovery was not obvious at 2 months (Fig.6C). Similar changes were also found in MMP-9: both vaginal dilation and ovariectomy increased MMP-9 levels to varying degrees, and this change was statistically significant ($F=167.9$, $p<0.01$), at 1 month, except for the VD2H group, MMP-9 levels in other groups were significantly increased, but at 2 months, MMP-9 in all groups were significantly recovered except OVX and OVX&VD4H ($F=311.5$, $p<0.01$) (Fig.6D).

Changing of sexual organs and sexual behavior by vaginal vascular injury

In order to verify the effect of vaginal blood on PGAD, we observed the changes in sexual organs and behavior by unilaterally or bilaterally blocking blood vessels around the vagina. We found that the vagina showed signs of atrophy through blood flow occlusion after 1 month, and bilateral occlusion was more pronounced than unilateral occlusion (Fig.7A-C) ($p < 0.05$). However, the effect of blood flow occlusion on the uterus should not be obvious. The weight of the uterus was not significantly different between unilateral and bilateral occlusion ($p < 0.05$) (Fig.7D-E), only the uterine organ index after bilateral occlusion was lower than sham group ($p >0.05$). Regarding the sexual behavior, blood flow changes did not have an effect on lordosis quotient, either unilaterally or bilaterally ($p>0.05$), only bilaterally blocked had lower lordosis scores than sham group ($p<0.01$) (Fig.7F-G). We also found that when the blood flow was blocked, the estrous cycle of the mice gradually to be disordered. The estrous cycle of the mice with unilateral blockade appeared to be disordered to varying degrees, while the estrous cycle with bilateral blood flow blockage gradually stayed in estrus (Fig.7H-I).

Effects of blood flow occlusion on vaginal histomorphology and VEGF-ERK signaling pathway

Through H&E staining, we found the vagina had obvious atrophy after blocking the vaginal vessels. This atrophy is showed uniform atrophy of the whole thickness of the vagina (Fig.8A). About Masson staining, we found that after vaginal blood occlusion, vaginal tissue fibrosis appeared, and this fibrosis was more obvious in bilateral occlusion than unilateral occlusion. Similarly, immunohistochemical staining showed that after blood occlusion, the number of microvascular in the vaginal muscle layer was significantly less, and bilateral occlusion was more obvious than unilateral occlusion (Fig.8B). Correspondingly, the content of MMMP-9 serotonin also decreased with blood flow occlusion, and bilateral occlusion decreased more than unilateral occlusion (Fig.8C). We further analyzed the protein expression levels of VEGF, ERK and p-ERK in vaginal tissue of mice in each group. (Fig.8D-E). We found that after blood flow occlusion, the levels of proteins associated with angiogenesis such as VEGF, ERK and p-ERK were all reduced, similarly, bilateral occlusion decreased more than unilateral occlusion.

Discussion

Sexual health is one of the important aspects in women's physical and mental health today, Due to the increasing incidence of PGAD[25], it is urgent to clarify the pathogenesis. In the early stage, many researchers have continuously explored the PGAD model: Scientists initially used genital smooth muscle cell culture, genital strip organ bath from rabbits and rats to study the physiology and pharmacology of peripheral female genital arousal responses[26], but we know that sexual response is an integral activity of the whole body[27], individual organs or cells lack integrity which is difficult to reflect the essential characteristics of sexual behavior; Subsequently, some researchers according to the characteristics of different types of sexual dysfunction to established sexual motive dysfunction[28], sexual Arousal dysfunction[29] and orgasm

dysfunction[15]. However, women's sexual response process is not staged and unidirectional, there is no sequence that sexual desire, sexual arousal and orgasm are often mixed together, so this model cannot reflect the real process of sexual response. At present, most researchers are accustomed to using ovariectomized female rats as the objection of PGAD[22], this only represents the sexual dysfunction caused by menopause, and cannot represent the characteristics of premenopausal[30,31]. Because of these, we need a comprehensive animal model which can fully reflects the sexual characteristics of PGAD patients.

Previously, by analyzing a large number of clinical cases, we found that menopause and birth trauma are the main causes of PGAD, which are the direct factors leading to genital sexual dysfunction except to psychological factors and social influences[32]. Most patients are prone to PGAD in postpartum, and this is closely related to the mode of delivery (natural delivery or cesarean section) and the number of births[33]. Relevant studies have pointed out that the incidence of PGAD before pregnancy is 1–38%, and it increases to 49–83% after childbirth[34], with the advent of menopause, the structure and function of the pelvic floor are further relaxed due to lack of hormone nutrition[35]. And under the joint cooperation of the above reasons, it leads to the abnormality of female sexual function organs, sex hormones, and nerves, and finally induces the occurrence of PGAD[36]. Therefore, we plan to simulate birth trauma and menopause to establish a representative PGAD model.

The etiology had been largely conceptualized as a psychogenic model leaving little room for physiological evaluation in treating sexual dysfunction of women with PGAD. Under sexual stimulation, women will show clitoral erection, labia congestion and swelling, vaginal lubrication, regular vaginal muscle contraction and perineal heat sensation due to the action of neurovascular[27,37], so we also need a set of indicators that can comprehensively assess and quantify sexual behavior and response in this model. Referring to the performance of normal women during sexual response and the clinical characteristics of PGAD patients, we measured the changes in vaginal temperature, vaginal lubrication, and vaginal smooth muscle tension through behavioral, functional, morphological and electrophysiological aspects. Based on the above detection, OVX&VD2H and rearing for 1 month are the best modeling method of PGAD for mice.

In the experiment, we found that the temperature of the vagina of mice did not change significantly before and after sexual arousal (Fig. 3). After changing the method of sexual arousal (estrogen and progesterone treatment or stimulation of the pudendal nerve), and adjusting the laboratory temperature, no significant changes were found. Giuliano F's[38] research found that through electrical stimulation of the pelvic nerve, the vaginal temperature of rat continued to increase until the end of the electrical stimulation, which may be due to insufficient intensity of our stimulation, or this is because of the differences between rats and mice. But what excited us is that there are obvious differences in vaginal lubrication between different groups, and inn this part, we also pioneered the use of self-invented absorbent paper with scale to detect the vaginal lubrication, this method is more intuitive and easier to operate than the weight of cotton balls reported by Pei L [39], and avoid errors caused by repeated weighing. Giuliano F's [38] studies have shown that the vagina of rats will contract regularly in sexual arousal, which is consistent with the sexual response of women[27]. In this study, we also observed the changes of vaginal tension by stimulating the internal pudendal nerve, such as Fig. 2E, vaginal dilation and ovariectomy will have varying degrees of influence vaginal contraction ability, among which VD4H is too serious due to the damage to the vagina, and caused the contraction appears disordered and irregular.

Except to these manifestations of sexual behavior, we also assessed specific changes in sex organs in the model. After observing the general shape and ultrastructure of the vagina, we found that vaginal dilation mainly causes mechanical damage to the vagina, breaks the connection between cells and the muscle fibers, enlarges the cell space, which is similar to the damage caused by the prolongation of the second stage of labor in women during childbirth[40]. In addition, the effect of oophorectomy on the vagina is mainly manifested as atrophy and glycogen reduce., this mainly because the nutritional effect of estrogen is lost after oophorectomy[41] (47). Usually, glycogen is regulated by the level of estrogen and is mainly used to maintain the normal acidic environment, the content of glycogen can indirectly reflect the function of the vagina. Similarly, the nerve fibers distributed in the vagina play an important role in the regulation of sexual response[42]. Matrix metalloproteinase 9(MMP-9) is a protein that can represent the content of extracellular matrix, which can well reflect the degree of tissue saturation or atrophy (40, 41). We also found that vaginal dilation and oophorectomy increased the content of MMP-9, among which OVX&VD4H increased the most obvious and irreversible.

Most studies understanding the presentation of symptoms have undertaken a case wise analysis rather than an empirical model. This study employs an animal model to replicate the occurrence of PGAD to investigate the role of vascular damage exclusively the signal pathway. Preliminary studies have shown decreased vaginal perfusion both in PGAD patients and animal models[43–45], and vaginal or clitoral blood flow can be increased by administering vascular-promoting drugs such as PDE-4 or PDE-5[46]. During the experiment, we found that microvascular in vaginal tissue were reduced after VD or OVX (Fig. 8), and OVX combined with VD had a synergistic effect on vascular damage. However, what is the relationship between blood flow and PGAD, whether there is a sequence and causal relationship between the two, and clarifying this may provide an important reference for elucidating the pathogenic mechanism and clinical treatment of PGAD. Therefore, we examined the specific relationship between PGAD and blood flow by blocking microvascular entering the vagina. We dissected multiple mice and found the vascular distribution around the mouse vagina as shown in Suplemment1. In order to fully block the blood flow into the vagina, we divided the

microvascular into the one side and two sides disconnection to create a blood flow block model. We found that the sexual response of mice after blood flow occlusion was significantly weakened, the vaginal lubrication and vaginal tension were significantly lower than sham group, and bilateral occlusion was more obvious than unilateral occlusion. From the morphological, after the blood flow is blocked, the vaginal tissue shrinks significantly, which is caused by the loss of the nutrition from microvascular in the vaginal tissue. Furthermore, through protein analysis, we found that changes in VEGF-ERK signaling pathway were involved in the reduction of blood vessels, and there was a close relationship between VEGF signaling pathway and ERK signaling pathway, which could influence each other and participate in angiogenesis together[47,48].

Conclusion

In this study, we used a microscope to simulate vaginal birth and menopause to establish a scientifically reasonable, simple and reproducible PGAD mouse model, and screened out a comprehensive set of methods for evaluating mouse sexual behavior. In addition, we further verified the causal relationship between PGAD and blood flow, that is, microvascular damage and blood flow changes are the main causes of PGAD, and further clarified that the VEGF-ERK signaling pathway is a possible mechanism involved in microvascular reduction.

Strengths and Limitations:

In this work, we used a microscope to simulate vaginal birth and menopause to establish a scientifically reasonable, simple and reproducible PGAD mouse model. In our experiments, we innovatively used a variety of assays to assess the sexual function, and screened out a comprehensive set of methods for evaluating mouse sexual behavior

However, there are also some unavoidable limitations in this study. First, PGAD can be caused by pathophysiological, pharmacological and psychological triggers. In this study, we only focused on PGAD caused by pathophysiological and did not take into account psychoactive and social factors. Second, due to lack of advanced technology, we don't have investigated the influence of vaginal blood flow in different groups, this leads us to lose one of the most intuitive data to detect changes in vaginal blood. What's more, we barely verified the causal relationship between microvascular injury and PGAD, and failed to further clarify the specific mechanism between them, which will be further explored in our follow-up study.

Declarations

Acknowledgments: The authors would like to acknowledge the National Natural Science Foundation of China for their support in this project. The authors would also like to acknowledge the Laboratory Animal Center of Ningxia Medical University for their helpful assistance.

Author contributions statement:

Guangyong Li and Rui He conceived the original idea and funding securedby; Puguang Yu conducted research design, data analysis, and writing the first draft of the paper; Xiaojiang Chen, Shuai Ren and Qiangqiang Wang are mainly responsible for the feeding of experimental animals and some morphological experiments; Yashan Su, Keming Chen and Jiajin Feng in charge of experiments in molecular biology, Hetao Liu and Huaiqin Han mainly made repeated revisions to the manuscript. All authors reviewed the manuscript.

Funding

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

Competing interests

The authors have no conflicts of interest to declare.

Data availability statement

The data used to support the findings of this study are included within the article.

References

1. Goldstein, I. *et al.* International Society for the Study of Women's Sexual Health (ISSWSH) Review of Epidemiology and Pathophysiology, and a Consensus Nomenclature and Process of Care for the Management of Persistent Genital Arousal Disorder/Genito-Pelvic Dysesthesia (PGAD/GPD). **18**, 665–697, doi:10.1016/j.jsxm.2021.01.172 (2021).

2. Jackowich, R. *et al.* Symptom Characteristics and Medical History of an Online Sample of Women Who Experience Symptoms of Persistent Genital Arousal. **44**, 111–126, doi:10.1080/0092623x.2017.1321598 (2018).
3. Segraves, R. J. C. j. o. p. R. c. d. p. Female sexual disorders: psychiatric aspects. **47**, 419–425, doi:10.1177/070674370204700502 (2002).
4. Calmasini, F., Klee, N., Webb, R. & Priviero, F. J. S. m. r. Impact of Immune System Activation and Vascular Impairment on Male and Female Sexual Dysfunction. **7**, 604–613, doi:10.1016/j.sxmr.2019.05.005 (2019).
5. Bitzer, J. & Alder, J. J. T. U. R. t. [Female sexual dysfunction]. **67**, 105–116, doi:10.1024/0040-5930/a000021 (2010).
6. Levin, R. *et al.* The Physiology of Female Sexual Function and the Pathophysiology of Female Sexual Dysfunction (Committee 13A). **13**, 733–759, doi:10.1016/j.jsxm.2016.02.172 (2016).
7. Dick, B. *et al.* Application of Botulinum Neurotoxin in Female Sexual and Genitourinary Dysfunction: A Review of Current Practices. **9**, 57–63, doi:10.1016/j.sxmr.2020.01.003 (2021).
8. Fiala, L., Lenz, J. & Bob, P. J. M. Effect of psychosocial trauma and stress on sexual dysfunction in women with endometriosis. **100**, e26836, doi:10.1097/md.0000000000026836 (2021).
9. Traish, A., Botchevar, E. & Kim, N. J. T. j. o. s. m. Biochemical factors modulating female genital sexual arousal physiology. **7**, 2925–2946, doi:10.1111/j.1743-6109.2010.01903.x (2010).
10. Gabrielson, A., Sartor, R. & Hellstrom, W. J. S. m. r. The Impact of Thyroid Disease on Sexual Dysfunction in Men and Women. **7**, 57–70, doi:10.1016/j.sxmr.2018.05.002 (2019).
11. Sansone, A. *et al.* Sexual Dysfunction in Men and Women with Diabetes: A Reflection of their Complications? **18**, e030821192147, doi:10.2174/1573399817666210309104740 (2022).
12. Motta-Mena, N., Puts, D. J. H. & behavior. Endocrinology of human female sexuality, mating, and reproductive behavior. **91**, 19–35, doi:10.1016/j.yhbeh.2016.11.012 (2017).
13. Calabrò, R. *et al.* Neuroanatomy and function of human sexual behavior: A neglected or unknown issue? **9**, e01389, doi:10.1002/brb3.1389 (2019).
14. Marson, L., Giamberardino, M., Costantini, R., Czakanski, P. & Wesselmann, U. J. S. m. r. Animal Models for the Study of Female Sexual Dysfunction. **1**, 108–122, doi:10.1002/smrj.14 (2013).
15. Giraldi, A. *et al.* Physiology of female sexual function: animal models. **1**, 237–253, doi:10.1111/j.1743-6109.04037.x (2004).
16. Snoeren, E. *et al.* A new female rat animal model for hypoactive sexual desire disorder; behavioral and pharmacological evidence. **8**, 44–56, doi:10.1111/j.1743-6109.2010.01998.x (2011).
17. Yildirim, E., Hacioglu, M., Essizoglu, A., Kucukparlak, I. J. J. o. s. & therapy, m. Persistent genital arousal disorder misdiagnosed because of Islamic religious bathing rituals: a report of three cases. **38**, 436–444, doi:10.1080/0092623x.2011.606888 (2012).
18. Markos, A., Dinsmore, W. J. I. j. o. S. & AIDS. Persistent genital arousal and restless genitalia: sexual dysfunction or subtype of vulvodynia? **24**, 852–858, doi:10.1177/0956462413489276 (2013).
19. Bell, C. *et al.* Persistent sexual arousal in a woman with associated cardiac defects and raised atrial natriuretic peptide. **18**, 130–131, doi:10.1258/095646207779949592 (2007).
20. Thubert, T. *et al.* [Persistent genital arousal disorder: a systematic review]. **22**, 1043–1050, doi:10.1016/j.purol.2012.07.016 (2012).
21. Facelle, T., Sadeghi-Nejad, H. & Goldmeier, D. J. T. j. o. s. m. Persistent genital arousal disorder: characterization, etiology, and management. **10**, 439–450, doi:10.1111/j.1743-6109.2012.02990.x (2013).
22. Snoeren, E. *et al.* Combination of testosterone and vardenafil increases female sexual functioning in sub-primed rats. **8**, 989–1001, doi:10.1111/j.1743-6109.2010.02177.x (2011).
23. Hernández-Munive, A., Rebolledo-Solleiro, D. & Fernández-Guasti, A. J. T. j. o. s. m. Does Chronic Hyperglycemia Affect Female Rat Sexual Behavior? Differences in Paced and Non-Paced Mating. **16**, 1130–1142, doi:10.1016/j.jsxm.2019.05.017 (2019).
24. Cora, M., Kooistra, L. & Travlos, G. J. T. p. Vaginal Cytology of the Laboratory Rat and Mouse: Review and Criteria for the Staging of the Estrous Cycle Using Stained Vaginal Smears. **43**, 776–793, doi:10.1177/0192623315570339 (2015).
25. Klifto, K. & Dellon, A. J. S. m. r. Persistent Genital Arousal Disorder: Review of Pertinent Peripheral Nerves. **8**, 265–273, doi:10.1016/j.sxmr.2019.10.001 (2020).
26. Munarriz, R., Kim, S., Kim, N., Traish, A. & Goldstein, I. J. T. J. o. u. A review of the physiology and pharmacology of peripheral (vaginal and clitoral) female genital arousal in the animal model. **170**, S40-44; discussion S44-45, doi:10.1097/01.ju.0000075352.03144.15 (2003).
27. Basson, R. J. H. o. c. n. Human sexual response. **130**, 11–18, doi:10.1016/b978-0-444-63247-0.00002-x (2015).
28. Le Moëne, O. & Ågmo, A. J. F. i. b. n. Modeling Human Sexual Motivation in Rodents: Some Caveats. **13**, 187, doi:10.3389/fnbeh.2019.00187 (2019).

29. Agmo, A. J. H. & behavior. On the intricate relationship between sexual motivation and arousal. **59**, 681–688, doi:10.1016/j.yhbeh.2010.08.013 (2011).
30. McCool-Myers, M., Theurich, M., Zuelke, A., Knuettel, H. & Apfelbacher, C. J. B. w. s. h. Predictors of female sexual dysfunction: a systematic review and qualitative analysis through gender inequality paradigms. **18**, 108, doi:10.1186/s12905-018-0602-4 (2018).
31. Santos, A. *et al.* Sexual function in women of fertile age with epilepsy. **125**, 108399, doi:10.1016/j.yebeh.2021.108399 (2021).
32. Jahan, M., Billah, S., Furuya, H., Watanabe, T. J. T. j. o. o. & research, g. Female sexual dysfunction: facts and factors among gynecology outpatients. **38**, 329–335, doi:10.1111/j.1447-0756.2011.01648.x (2012).
33. Gutzeit, O., Levy, G. & Lowenstein, L. J. S. m. Postpartum Female Sexual Function: Risk Factors for Postpartum Sexual Dysfunction. **8**, 8–13, doi:10.1016/j.esxm.2019.10.005 (2020).
34. Casscells, W. *et al.* Thermal detection of cellular infiltrates in living atherosclerotic plaques: possible implications for plaque rupture and thrombosis. **347**, 1447–1451 (1996).
35. Scavello, I., Maseroli, E., Di Stasi, V. & Vignozzi, L. J. M. Sexual Health in Menopause. **55**, doi:10.3390/medicina55090559 (2019).
36. Giuliano, F., Rampin, O., Allard, J. J. J. o. s. & therapy, m. Neurophysiology and pharmacology of female genital sexual response. 101–121, doi:10.1080/00926230252851230 (2002).
37. Rieger, G., Savin-Williams, R., Chivers, M., Bailey, J. J. J. o. p. & psychology, s. Sexual arousal and masculinity-femininity of women. **111**, 265–283, doi:10.1037/pspp0000077 (2016).
38. Giuliano, F. *et al.* Vaginal physiological changes in a model of sexual arousal in anesthetized rats. **281**, R140-149, doi:10.1152/ajpregu.2001.281.1.R140 (2001).
39. Pei, L. *et al.* Expression of aquaporin proteins in vagina of diabetes mellitus rats. **10**, 342–349, doi:10.1111/j.1743-6109.2012.02989.x (2013).
40. Kopas, M. J. J. o. m. & health, w. s. A review of evidence-based practices for management of the second stage of labor. **59**, 264–276, doi:10.1111/jmwh.12199 (2014).
41. Chung, H., Lee, H., Park, K. J. I. & urology, c. Estrogen modulates epithelial progenitor cells in rat vagina. **62**, 349–353, doi:10.4111/icu.20200513 (2021).
42. Castiglione, F. *et al.* Pelvic nerve injury negatively impacts female genital blood flow and induces vaginal fibrosis-implications for human nerve-sparing radical hysterectomy. **122**, 1457–1465, doi:10.1111/1471-0528.13506 (2015).
43. Salonia, A. *et al.* Physiology of women's sexual function: basic knowledge and new findings. **7**, 2637–2660, doi:10.1111/j.1743-6109.2010.01810.x (2010).
44. Kim, N. *et al.* Effects of tamoxifen on vaginal blood flow and epithelial morphology in the rat. **6**, 14, doi:10.1186/1472-6874-6-14 (2006).
45. Angulo, J., Cuevas, P., Cuevas, B., Bischoff, E. & Sáenz de Tejada, I. J. I. j. o. i. r. Vardenafil enhances clitoral and vaginal blood flow responses to pelvic nerve stimulation in female dogs. **15**, 137–141, doi:10.1038/sj.ijir.3900985 (2003).
46. Leddy, L. *et al.* Influence of sildenafil on genital engorgement in women with female sexual arousal disorder. **9**, 2693–2697, doi:10.1111/j.1743-6109.2012.02796.x (2012).
47. Fish, J. *et al.* Dynamic regulation of VEGF-inducible genes by an ERK/ERG/p300 transcriptional network. **144**, 2428–2444, doi:10.1242/dev.146050 (2017).
48. Shah, A., Birdsey, G. & Randi, A. J. V. p. Regulation of endothelial homeostasis, vascular development and angiogenesis by the transcription factor ERG. **86**, 3–13, doi:10.1016/j.vph.2016.05.003 (2016).

Tables

Tab 1. The number of Sniff in the first 5 minutes, Proceptivity, aggressive and the return latencies during 30 min test in different group.

		Sniff in the first 5 minutes		Proceptivity (hops, ear wiggling, and darting)		Aggressive (boxing, bites and lateral postures)		Return latencies((sec))	
		Normal	Arousing	Normal	Arousing	Normal	Arousing	Normal	Arousing
Sham	1 month	77.3±13.3	81.7±9.6	134.3±31.2**	166.2±26.0	6.8±0.5	5.4±0.6	12.8±0.1	16.0±0.0
	2 months	84.5±26.1	69.3±11.5	112.4±22.3	183.6±31.9	7.4±0.9	6.7±0.1	14.3±0.6	18.7±0.4
VD2H	1 month	91.7±7.9*	76.8±14.2*	137.2±11.1	156.4±14.1*	5.1±0.7	7.8±0.4	8.2±0.4*	11.2±0.1
	2 months	132.8±34.3	92.3±21.4	124.1±26.9	115.3±18.7	7.2±0.5	9.1±0.7**	9.1±0.3	6.6±0.9
VD4H	1 month	66.2±18.7	89.6±6.9	134.3±31.4*	126.3±19.2	9.9±0.1*	8.0±0.8	9.6±0.8	9.2±0.8
	2 months	81.4±8.6**	73.4±17.1	114.5±10.4	156.2±23.8**	7.9±0.2	7.0±0.7	13.5±0.3	11.3±0.7
OVX	1 month	61.5±5.6	83.3±13.5	127.3±14.9*	115.4±18.6	5.2±0.1	3.1±0.1	10.6±0.6	14.6±0.1
	2 months	78.9±7.2	61.4±4.8	133.3±24.0	156.3±26.5	6.1±0.1	5.3±0.4	9.4±0.6	11.2±0.3**
OVX&VD2H	1 month	64.1±3.5*	72.6±13.2	109.4±13.9	136.4±22.7*	6.2±0.6	2..8±0.5	8.8±0.2	12.4±0.8
	2 months	92.9±15.2**	87.6±19.1	124.3±13.6	133.7±15.5	4.2±0.4	2..3±0.2	4.3±0.1**	5.6±0.9
OVX&VD4H	1 month	59.4±4.3*	63.4±7.7	96.4±10.2	86.4±7.9	6.9±0.1	4.0±0.8*	9.6±0.8	9.2±0.8
	2 months	75.6±7.2**	82 ± 9	104.4±11.2	116.3±25.1	5.6±0.2**	7.0±0.7	11.6±0.2	7.2±0.5

These analyses indicated that there was a significant effect of different states (normal or rousing) and time (1 month or 2 month), as well as the other between factors

at different modeling methods. *Indicates significantly different effect of different modeling methods in 1 month, compared with sham. ** Indicates significantly different effect of different modeling methods in 2 months, compared with sham.

Figures

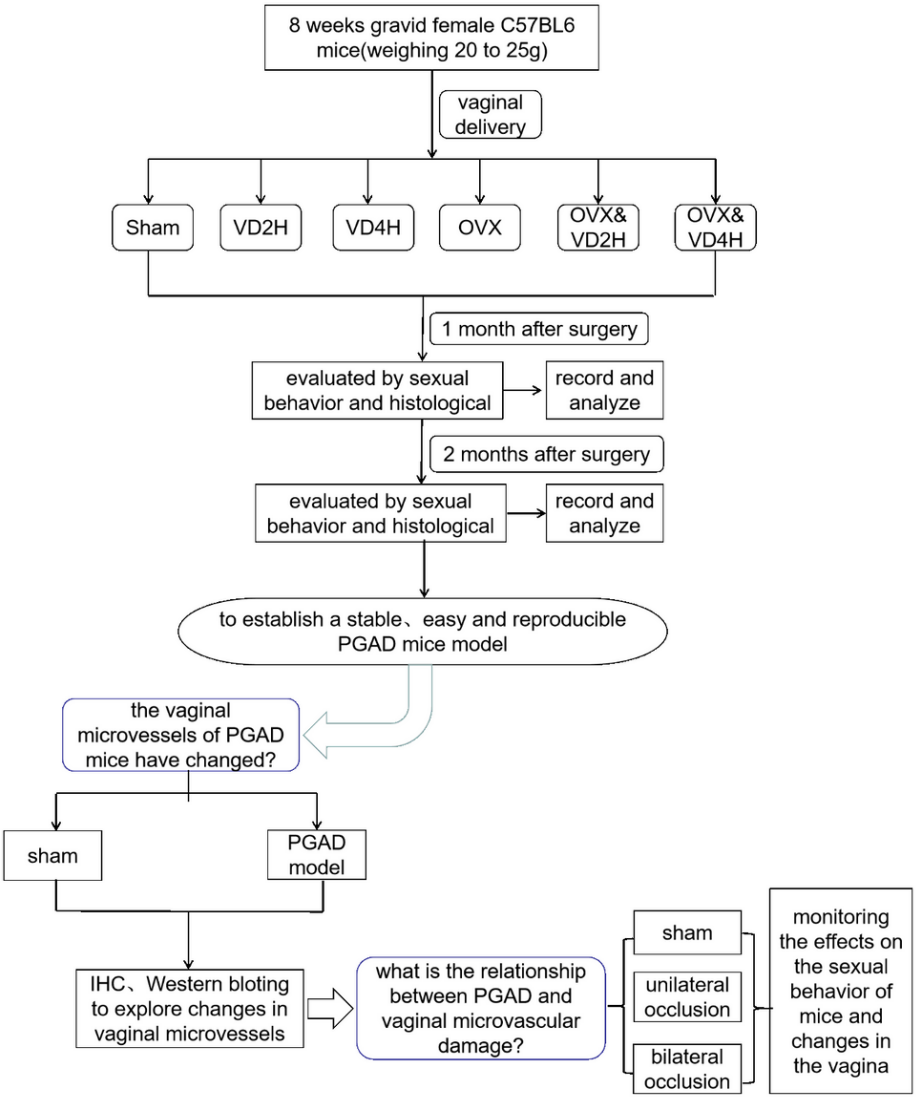

Figure 1

Flowchart from the creation of the process and overview of the research. VD: vaginal dilation; OVX: ovariectomized; PGAD: persistent genital arousal disorder

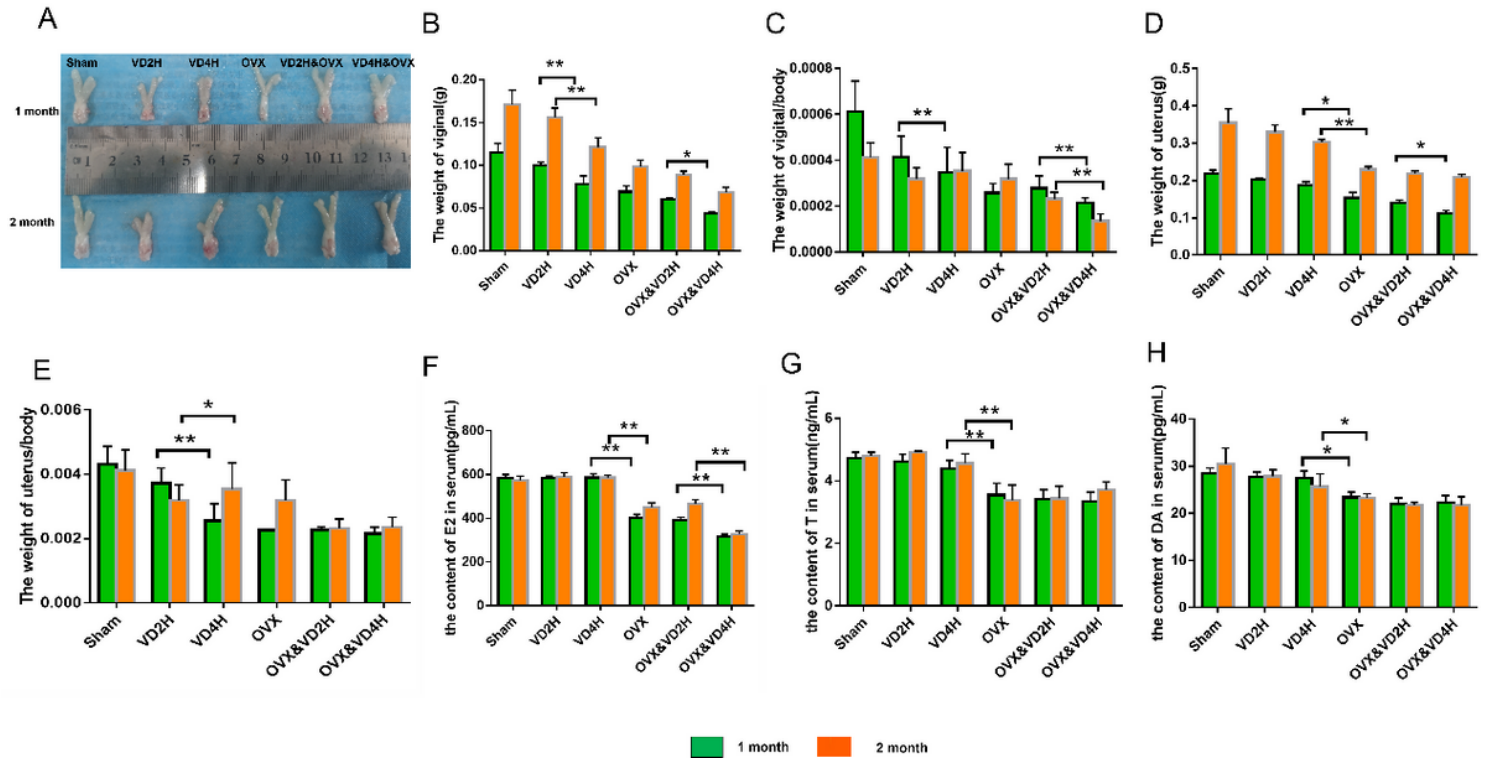

Figure 2

Effects of vaginal dilation and ovariectomy on sex organs and sex hormone levels. A. Effects of different time and methods on the vaginal morphology. B. Vaginal weights of different groups(g) (Row factor: F=164.2, p<0.01; column factor: F=390, p<0.01). C. Vaginal organ index (vaginal weight/body weight) in different groups. (Row factor: F=23.62, p<0.01; column factor: F=9.697, p=0.0357). D. Uterus weights of different groups(g). (Row factor: F=115.4, p<0.01; column factor: F=736.2, p<0.01). E. Uterus organ index (Uterus weight/body weight) in different groups. (Row factor: F=21.19, p<0.01; column factor: F=8.414, p<0.01). F. Serum estrogen levels in different groups(pg/ml). (Row factor: F=546.4, p<0.01; column factor: F=71.7, p=0.0011). G. Serum testosterone levels in different groups(pg/ml). (Row factor: F=112.6, p<0.01; column factor: F=57.2, p<0.01) H. Serum dopamine levels in different groups(pg/ml). (Row factor: F=36.51, p<0.01; column factor: F=0.01337, p=0.9135). Note: sham: control group with sham operation; VD2H: vaginal dilation with 2 hours; VD4H: vaginal dilation with 4 hours; OVX: ovariectomized; OVX+VD2H: ovariectomy combined with vaginal dilation 2 hours; OVX+VD4H: ovariectomy combined with vaginal dilation 4 hours; E2: estrogen; T: testosterone; DA: dopamine. Each bar depicts the mean (±SEM) from n=6-8 mice per group. *P <0 .05, **P <0 .001.

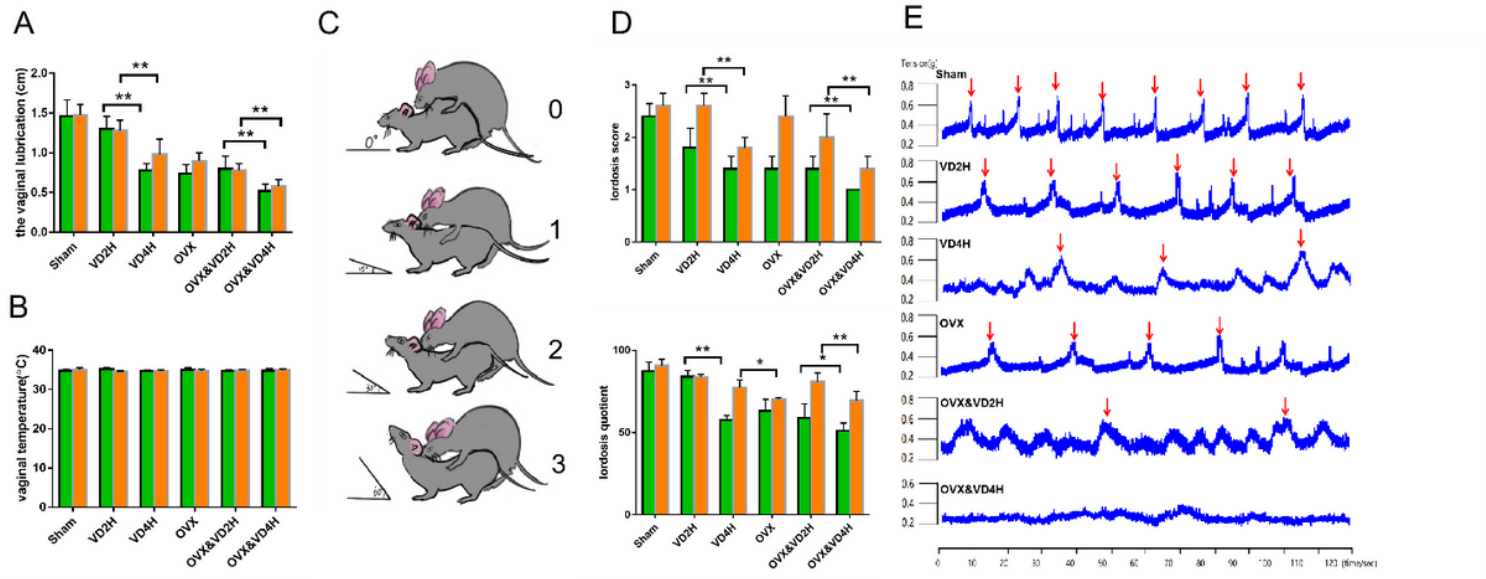

Figure 3

Effects of vaginal dilation and ovariectomy on the sexual response. A. Vaginal lubrication of different groups(cm). (Row factor: F=94.3, p<0.01; column factor: F=1.301, p=0.3177). B. The vaginal temperature in different groups (℃). (Row factor: F=0.485, p=0.7834; column factor: F=0.6286, p=0.4722). C. Schematic diagram of lordosis. D. Lordosis score and lordosis quotient in different groups. (Row factor: F=38.63, p<0.01; column factor: F=159.6, p<0.01). E. vaginal surface tension in different groups. Note: sham: control group with sham operation; VD2H: vaginal dilation with 2 hours; VD4H: vaginal dilation with 4 hours; OVX: ovariectomized; OVX+VD2H: vaginal dilation with 2 hours combined with ovariectomized; OVX+VD4H: vaginal dilation with 4 hours combined with ovariectomy; Each bar depicts the mean (±SEM) from n=6-8 mice per group. *P < 0.05, ** P <0 .01.

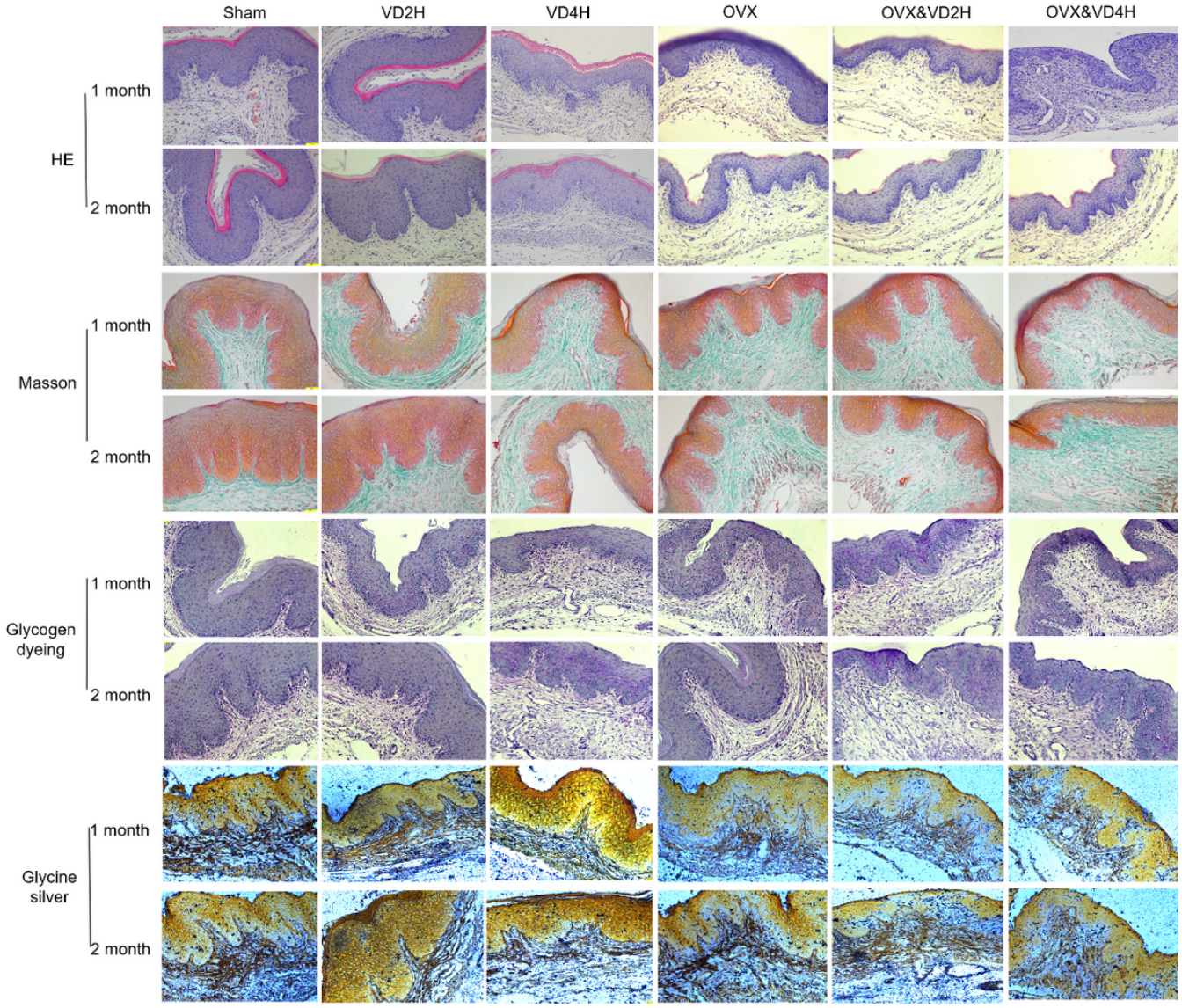

Figure 4

Effects of vaginal dilation and ovariectomy on vaginal morphology. In Masson staining, collagen fibers are blue and muscle fibers are red. In Glycogen dyeing, glycogen present in the cytoplasm combines with leucoma magenta in Schiff's reagent to form a purplish red dye that is deposited at the location of intracellular polysaccharides. In Glycine sliver staining, the axons of nerve fibers are argentophilic, so after being treated with acidic formaldehyde solution, they react with glycine silver solution to deposit silver ions on the axons that are argentophilic, making the axons black or brownish black. Scale bar=50μm.

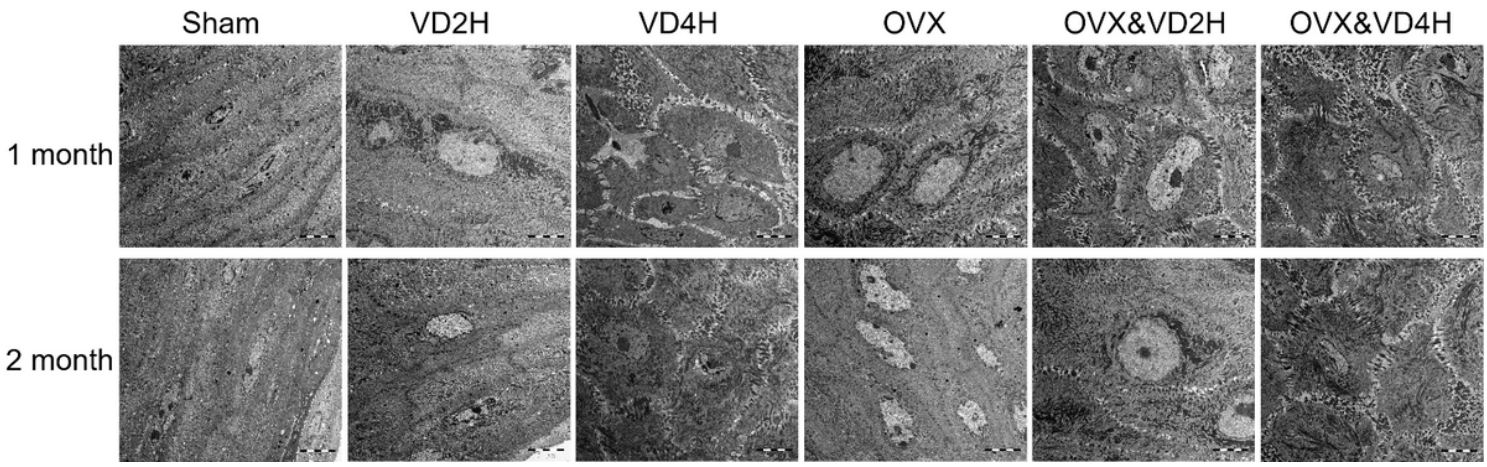

Figure 5

Effects of vaginal dilation and ovariectomy on vaginal ultrastructure. Scale bar=5μm

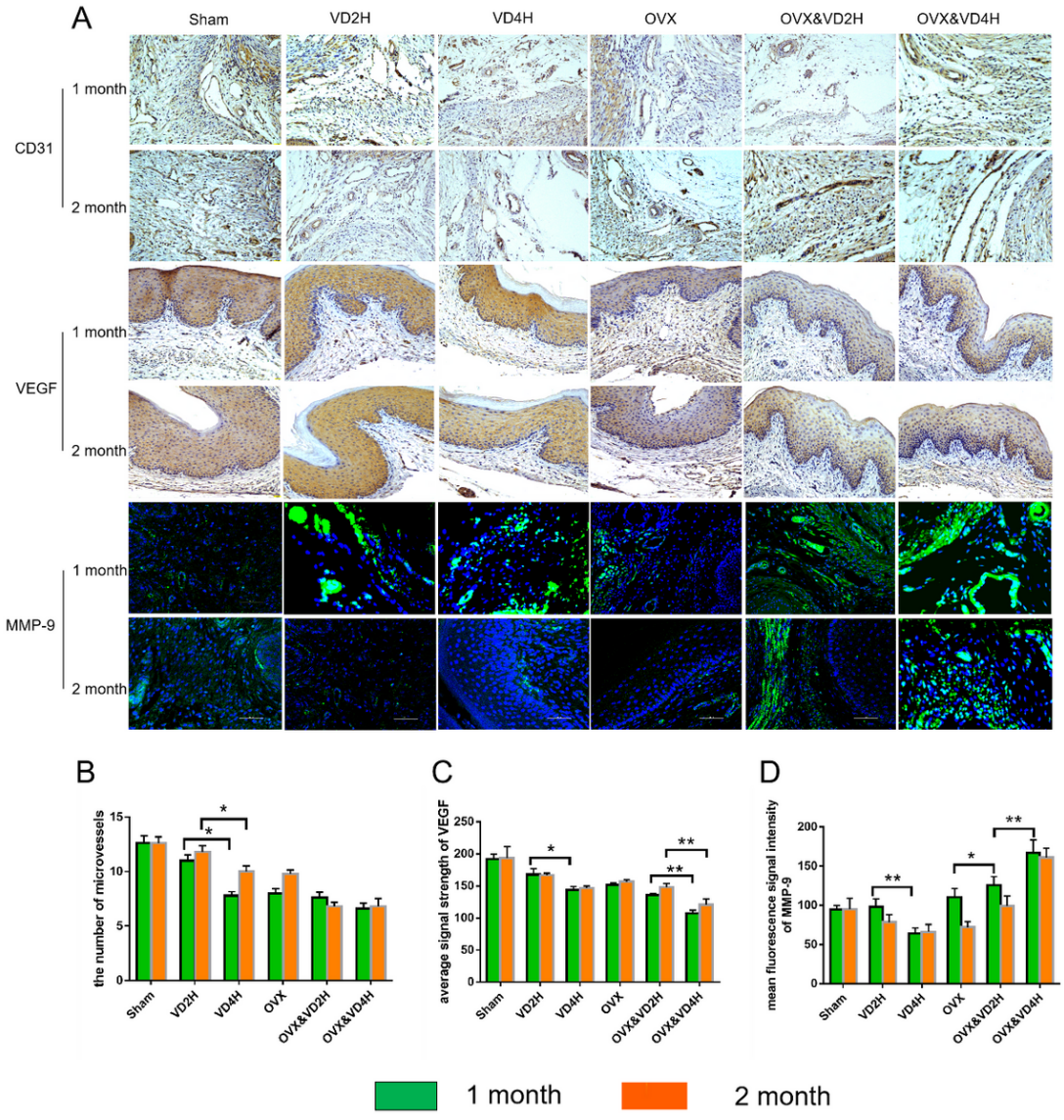

Figure 6

Effects of vaginal dilation and ovariectomy on the microvascular of mouse vaginal.A.CD31, VEGF and MMP-9 immunohistochemical/immunofluorescence in different groups. In immunohistochemical, microvascular are clearly shown in the muscle fiber

layer. In immunofluorescence, Nuclei were labeled with 4,6-diamidino-2-phenylindole (DAPI, blue), MMP-9 were labeled with green staining. B. Mean ROI of VEGF in different groups. (Row factor: F=36.91, p<0.01; column factor: F=1.529, p=0.2840). C. The number of CD31-labeled microvascular in different groups. (Row factor: F=91.8, p<0.01; column factor: F=12.65, p=0.0237) D. Mean fluorescence intensity of MMP-9 in vaginal tissues of mice in different groups. (Row factor: F=167.9, p<0.01; column factor: F=45.93, p=0.0025). VEGF: vascular endothelial growth factor, MMP-9: matrix metalloproteinase-9. Each bar depicts the mean (±SEM) from n=6-8 mice per group. Scale bar, 50μm. *P <0 .05, ** P < 0.001.

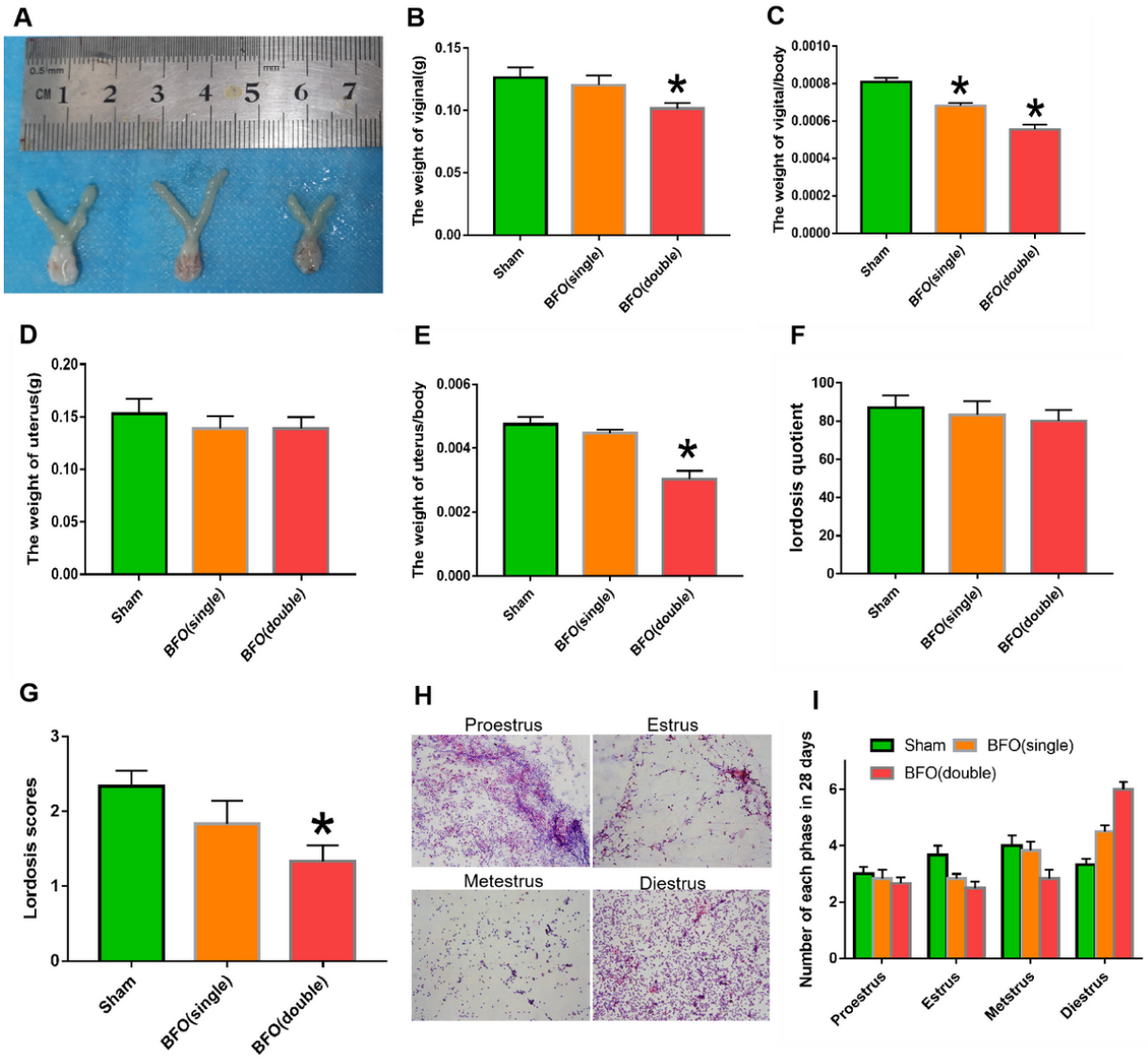

Figure 7

Changing of sexual organs and sexual behavior in vaginal vascular injury. A. Effects of unilateral and bilateral blood flow occlusion on vaginal morphology in mice. B. Vaginal weights of unilateral and bilateral blood flow occlusion(g). C. Vaginal organ index (vaginal weight/body weight) of unilateral and bilateral blood flow occlusion. D. Uterus weights of unilateral and bilateral blood flow occlusion(g). E. Uterus organ index (Uterus weight/body weight) of unilateral and bilateral blood flow occlusion. F. Lordosis quotient of unilateral and bilateral blood flow occlusion. G. Lordosis score of unilateral and bilateral blood flow occlusion. H. H&E staining of four different stages of the estrous cycle. I. The proportion of each estrous cycle in different groups. Each bar depicts the mean (±SEM) from n=6-8 mice per group. *P <0 .05, ** P <0 .001.

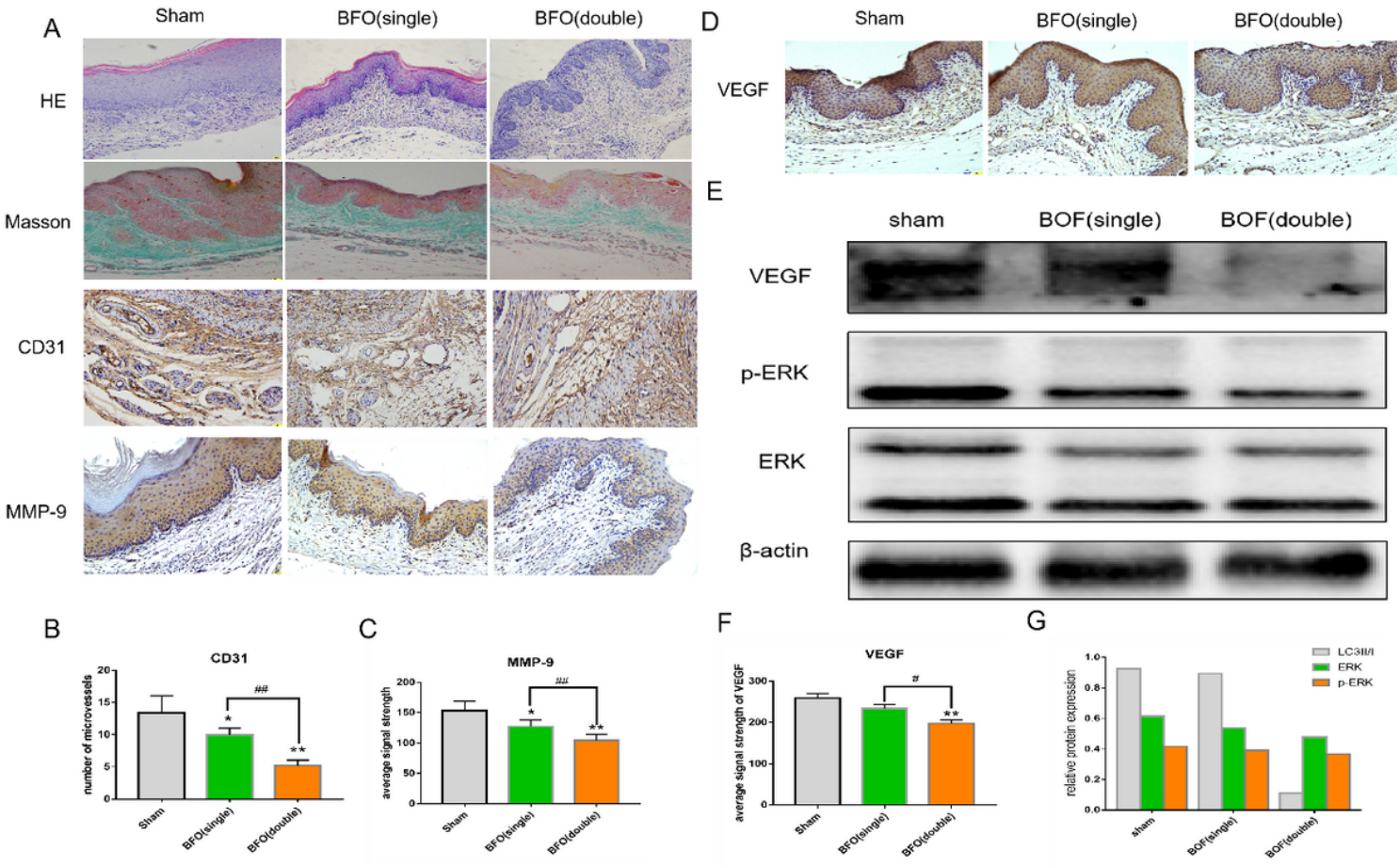

Figure 8

Effects of blood flow occlusion on vaginal morphology, microvascular and MMP-9. A. H&E staining, Masson staining, CD31 and MMP-9 immunohistochemical in different groups. In Masson staining, collagen fibers are blue and muscle fibers are red CD31 immunohistochemical labeled microvascular in different groups, MMP-9 immunohistochemical Used to display the content of MMP-9. B. The number of CD31-labeled microvascular in different groups. C. Mean signal intensity of MMP-9 in different groups. D. VEGF immunohistochemical in different groups. E. Expression of VEGF-ERK related protein in vaginal tissue of mice in each group detected by Western bloting. F. Mean signal intensity of MMP-9 in different groups. G. Relative expression levels of VEGF, ERK and p-ERK proteins in vaginal tissue of mice in each group. Note: sham: control group with sham operation; BFO(Single): Unilateral blood flow occlusion; BFO(Double): bilateral blood flow occlusion; HE: hematoxylin-eosin staining. Scale bar,50μm. Each bar depicts the mean (±SEM) from n=6-8 mice per group. *P < 0.05, ** P < 0.001.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigures.docx
- blotsSupplementaryInformationfile..pdf