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ORIGINAL ARTICLE

Male Health

# A novel rat model of seminal vesiculitis

Peng Zhang[1], Xiao-Long Wang[2], Zhong-Hua Yang[1], Xin-Jun Su[1], Xing-Huan Wang[1]

**We aimed to establish a novel rat model of seminal vesiculitis that would provide an effective approach to investigate the pathogenesis of this disease in the future. Eight male rats received the same operation, during which the root of one of the two seminal vesicles was partly ligatured with sutures and the other vesicle was left intact. The samples of seminal vesicles were harvested on the 8th day following the operation. Hematoxylin and eosin and Masson's trichrome stains were used to observe the histopathology and the presence of fibrous tissue in seminal vesicles, respectively. Immunoblotting and immunohistochemistry were applied to determine the tumor necrosis factor-alpha and cyclooxygenase-2 levels in seminal vesicle tissues. Real-time fluorescence quantitative polymerase chain reaction was performed to measure the gene expression levels of proinflammatory cytokines. $H_2O_2$ levels in the seminal plasma from the seminal vesicle were also measured. Hematoxylin and eosin staining suggested that there was inflammatory cell infiltration into the seminal vesicles treated by partial root ligation. The tumor necrosis factor-alpha and cyclooxygenase-2 proteins were significantly upregulated in the treated seminal vesicles. The tumor necrosis factor-alpha, cyclooxygenase, interleukin 6, and inducible nitric oxide synthase mRNA expression levels were also upregulated in the treated seminal vesicles. The $H_2O_2$ levels in the seminal plasma from seminal vesicles with partial root ligation were significantly elevated compared with those from vesicle left intact. In conclusion, partially ligating the root of the seminal vesicle via sutures in rats is an effective method to establish a seminal vesiculitis rat model.**

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**Keywords:** cyclooxygenase-2; rat model; seminal vesiculitis; tumor necrosis factor

## INTRODUCTION

Seminal vesiculitis is a common infectious disease in andrology. It includes acute and chronic periods. Urinary tract infection symptoms in the acute period of seminal vesiculitis are similar to those of acute prostatitis. Hematospermia and lower abdominal (or perineal) pain and discomfort are common clinical manifestations.[1,2] Infection and inflammation in the urogenital tract is considered as the major cause of seminal vesiculitis.[3] Furthermore, tuberculosis or other atypical infections can also lead to seminal vesiculitis.[4,5] Although seminal vesiculitis is mostly minimally harmful to the human body, recurrent episodes of hematospermia or pain can lead to anxiety, fear, erectile dysfunction, and even male infertility.[6]

Seminal vesicles are a pair of tubular organs with the twisted and convoluted tubular, and the tubular passage is easily blocked, especially in cases of seminal vesiculitis. Male accessory gland infections (prostatitis, prostate-vesiculitis, and prostate-vesiculo-epididymitis) may impair sperm function and cause male infertility through multiple pathophysiological mechanisms, such as the effects of microorganisms, viruses, oxidative stress, and proinflammatory cytokines.[7] In some clinic cases, we have found that the sperm function in some male patients may be improved after an obstruction of the seminal ductal system is relieved via seminal vesicle endoscopy (data not shown). However, the mechanism of this finding has not yet been elucidated. We designed an animal model that could be used to investigate the seminal vesicle obstructions.

## MATERIALS AND METHODS

### *Study design*

A total of 8 male Sprague–Dawley rats (7 weeks of age) were obtained from the Experimental Animal Centre of Wuhan University (Wuhan, China) and treated in accordance with the Guideline for the Care and Use of Laboratory Animals published by the US National Institutes of Health (Publication No. 85-23, revised 1996). This animal experiment was approved by the Ethics committee of Zhongnan Hospital of Wuhan University (Wuhan, China). The eight male rats received the same operation, during which the root of one seminal vesicle was partially ligatured with sutures and the other vesicle was left intact to be used as a control.

### *Operating procedure*

The rats were anesthetized by peritoneal injection with 30 mg $kg^{-1}$ pentobarbital sodium (Mercker Co., Darmstadt, Germany). A midline incision was made in the lower abdomen of the rats, and the pair of seminal vesicles in the rear of the prostate was exposed. Next, the root section that was proximal to one seminal vesicle was tied with sutures (diameter: 0.200–0.249 mm), and the syringe needle (outer diameter: 0.71 mm) (Shandong Boda Medical Products Co., Ltd., Heze, China) was inserted between the root and the sutures to control the ligature tightness (**Figure 1a** and **1b**). Then, the abdomen incision was sutured, rendering the operation complete. Seminal vesicle tissues and the seminal plasma were harvested from the rats on the 8th postoperative day.

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***Hematoxylin and eosin (H&E) and Masson's trichrome staining***
Harvested seminal vesicle tissues were fixed with 4% paraformaldehyde (Sinopharm, Wuhan, China). Then, the fixed tissue samples were embedded in paraffin (Paraplast, Sigma-Aldrich, St. Louis, MO, USA). Paraffin sections (5 μm) were made on slides and dewaxed with xylene (Sinopharm). Dewaxed tissue sections were rehydrated in an alcohol gradient and rinsed with deionized water. An H&E staining assay (Wuhan Guge Biological Technology Co., Ltd., Wuhan, China) was used on seminal vesicle tissues. Similarly, seminal vesicle tissues were also subjected to Masson's trichrome staining (Wuhan Guge Biological Technology Co., Ltd.). Slides were dehydrated using graded alcohol and finally made transparent with xylene. The stained tissues were visualized under a CX-21 microscope (Olympus, Tokyo, Japan). The smooth muscle-to-collagen ratio in the tissues was determined on Masson's trichrome staining photographs using a high-power lens.

***Western blot***
Harvested parts of rat seminal vesicles were lysed in triple-detergent radioimmunoprecipitation assay (RIPA) buffer with a protease inhibitor cocktail (Sigma-Aldrich) for 20 min. Total protein was obtained from the supernatant of the lysate after centrifugation at 14 489 *g* for 10 min at 4°C. The protein quantity was determined by the bicinchoninic acid (BCA) method (Beyotime, Shanghai, China). Quantified protein samples were mixed with sodium dodecyl sulfate (SDS)-polyacrylamide gel-loading buffer (Biyuntian, Shanghai, China) and denatured at 95°C for 5 min. A protein sample (30 μg) was loaded in a lane of a 10% discontinuous SDS-polyacrylamide gel for electrophoresis and then transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, MO, USA). The membranes were blocked with 5% skim milk (BD Biosciences, San Jose, CA, USA) in Tris-buffered saline Tween-20 (TBST) buffer (Biyuntian) for 30 min at ambient temperature and then incubated with anti-tumor necrosis factor-alpha (TNF-α) antibody (1:1000; Abcam, San Francisco, CA, USA), anti-cyclooxygenase-2 (COX-2) antibody (1:1000; Abcam), and anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (1:5000; BioWorld, Nanjing, China) overnight. Following incubation with a secondary antibody at ambient temperature for 1 h, signals were visualized by enhanced chemiluminescence (Bio-Rad, Hercules, CA, USA).

***Immunohistochemistry***
Paraffin-embedded seminal vesicle sections were dewaxed in xylene, then rehydrated in an alcohol gradient and rinsed in deionized water. After the endogenous peroxidase was blocked with 3% hydrogen peroxide ($H_2O_2$), the sections were stained with antibodies to TNF-α (1:500) and COX-2 (1:200), followed by immunoperoxidase staining with peroxidase-conjugated secondary antibodies (Aspen, Wuhan, China) and diaminobenzidine (Sigma-Aldrich) as a chromogen. Horseradish peroxidase (HRP)-conjugated secondary antibodies (Santa Cruz Biotechnology, Dallas, TX, USA) were used to identify primary antibody targets. Cell nuclei in the sections were stained using hematoxylin (Wuhan Guge Biological Technology Co., Ltd.). Immunostained tissues were visualized under a CX-21 microscope. Five microscopic fields were randomly acquired by the camera at high magnification for each tissue sample. The optical density of photographs was analyzed using the ImageJ software (LOCI, Madison, WI, USA).

***Quantitative real-time polymerase chain reaction (qRT-PCR)***
Portions of harvested seminal vesicle tissues, stored at −80°C, were ground to powder in liquid nitrogen and dissolved in the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) to extract total RNA from the seminal vesicles according to the manufacturer's instructions. A ReverTra Ace qPCR RT kit (Toyobo, Tokyo, Japan) was used to synthesize cDNA. The reaction contained 4 μl of 5 × buffer, 1 μl of reverse transcriptase, 1 μg of total RNA, and RNase-free $H_2O$ up to 20 μl. The relative expression levels of TNF-α, COX-2, interleukin 6 (IL-6), and inducible nitric oxide synthase (iNOS) were determined by quantitative real-time polymerase chain reaction (qRT-PCR) using a standard SYBR Green RT-PCR kit (Bio-Rad). The reaction contained 10 μl of RT-PCR mix (from the above RT-PCR kit), 1 μl of cDNA, 1 μl of each primer (50 nmol $l^{-1}$), and 7 μl of RNase-free $H_2O$. The specific primer pairs are shown in **Supplementary Table 1**. The relative expression of TNF-α, COX-2, IL-6, and iNOS was quantified using GraphPad Prism 4.0 software (GraphPad Software, San Diego, CA, USA) and the $2^{-\Delta\Delta Ct}$ method.

***Measurement of $H_2O_2$ levels***
A hydrogen peroxide assay kit (Beyotime) was used to determine $H_2O_2$ levels in the seminal plasma from seminal vesicles according to the manufacturer's protocol. Then, 10 μl seminal plasma sample received the measurement process in the assay. A microplate reader (Bio-Rad), set at 560 nm, was used to measure the absorbance of the reaction mixture in 96-well plates.

***Statistical analyses***
Statistical analyses were performed using the GraphPad Prism 5 data analysis software (GraphPad Software, La Jolla, CA, USA). Student's *t*-test was used to determine the differences in continuous data with normal distribution between different experimental interventions. Differences were considered statistically significant at $P < 0.05$. Bar charts were generated using mean and standard deviation (s.d.) values.

## RESULTS

***Histologic inflammation***
The shape of the epithelium in the seminal vesicles became distorted, and there was proliferation of fibrous tissue. Inflammatory cell infiltration was also found in the operated seminal vesicles, but not in normal seminal vesicles from the same rat (**Figure 1c** and **1d**).

***Fibrous connective tissue measurement***
A notably increased fibrous connective tissue ratio was obtained for the operated seminal vesicles compared with that for the normal seminal vesicles ($P < 0.0001$; **Figure 2**).

***TNF-α and COX-2 expression in seminal vesicle tissues***
Immunohistochemistry assay showed that TNF-α and COX-2 were mainly expressed in the cytoplasm of endothelial and stromal cells in seminal vesicles. The TNF-α and COX-2 expression levels were significantly higher in the operated seminal vesicles than in the normal seminal vesicles from the same rat ($P < 0.0001$; **Figure 3** and **4a–4c**). Western blot analysis showed that TNF-α and COX-2 were elevated in the operated seminal vesicles, compared with the normal seminal vesicles. The operated seminal vesicles and the normal seminal vesicles are a pair of seminal vesicle organs (**Figure 3** and **4d**).

***TNF-α, COX-2, IL-6, and iNOS mRNA expression in seminal vesicle tissues***
The mRNA expression of TNF-α, COX-2, IL-6, and iNOS was observably higher in the operated seminal vesicles than in the normal seminal vesicles from the same rat (**Figure 5**).

***$H_2O_2$ levels in the seminal plasma***
The $H_2O_2$ levels in the seminal plasma from the seminal vesicles with root ligation were markedly elevated compared to those from the vesicles left intact ($P < 0.05$; **Figure 6**).

## DISCUSSION

The present study aimed to provide an effective rat model of seminal

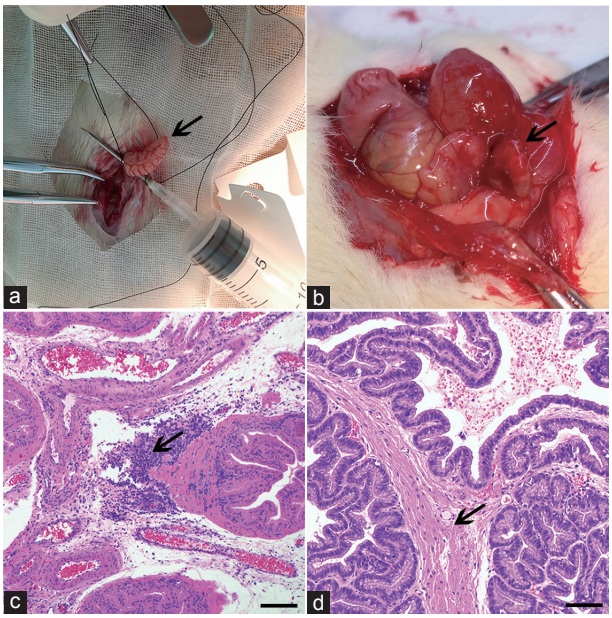


**Figure 1:** (**a**) The root section of one of two seminal vesicles was tied by sutures (diameter: 0.200–0.249 mm), and the syringe needle (outer diameter: 0.71 mm) was placed between the root and the sutures to control tightness. (**b**) Seminal vesicle appearance at the 8th postoperative day; black arrows show the partially ligatured root proximal to seminal vesicle. From H&E staining for seminal vesicles, (**c**) obviously increased proliferation of fibrous tissue was found in the treated seminal vesicles, and inflammatory cell infiltration was also found in the operated seminal vesicles (black arrows show inflammatory cell infiltration in the stroma of the tissue) compared to (**d**) normal seminal vesicles in the same rat (black arrows show the lack of inflammatory cells in the stroma of the tissue). Scale bars = 50 μm. H&E: hematoxylin and eosin.

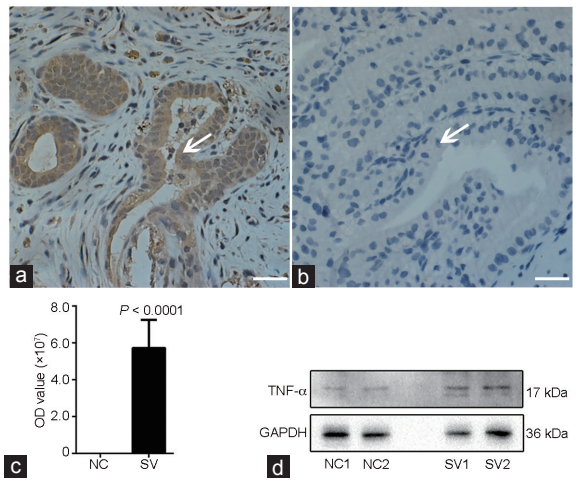


**Figure 3:** Seminal vesicle TNF-α staining and western blot. TNF-α expression levels were significantly increased in (**a**) the operated seminal vesicles compared to (**b**) the normal control seminal vesicles in the same rat on immunostaining; white arrows show the section with TNF-α staining. Scale bars = 20 μm. (**c**) The histogram shows the immunohistochemical analysis results. (**d**) Western blot results show TNF-α expression levels were elevated in the operated seminal vesicles. NC1, NC2, SV1 and SV2 were operated (SV1, SV2) and normal (N1 and N2) seminal vesicle samples in two independent rats marked as 1 and 2, respectively. $P < 0.0001$, SV group compared to NC group. OD: optical density; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; COX-2: cyclooxygenase-2; TNF-α: tumor necrosis factor-alpha; SV: seminal vesiculitis; NC: normal control.

vesiculitis. After the root proximal to the seminal vesicle was ligatured partially by sutures for 8 days, protein and mRNA expression levels of

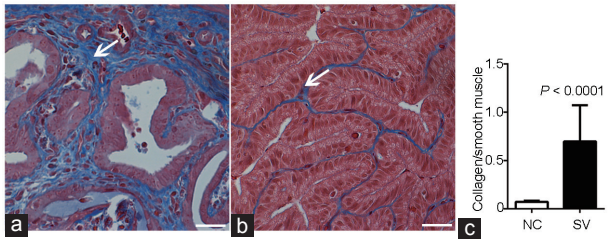


**Figure 2:** Massontrichrome stain for seminal vesicles. It was shown that there was a significantly increased fibrous connective tissue ratio found in (**a**) the operated seminal vesicles (white arrow indicates fibrous connective tissue) compared to (**b**) normal seminal vesicles (white arrow indicates fibrous connective tissue). Scale bars = 20 μm. (**c**) The fibrous connective tissue ratio in SV group compared to NC group. SV: seminal vesiculitis; NC: normal control.

proinflammatory mediators (TNF-α and COX-2) became significantly elevated in tissues from the treated seminal vesicle, based on the western blot assay, immunohistochemistry analysis, and RT-PCR. In addition, IL-6 and iNOS were found to significantly increase in tissues from the treated seminal vesicles. The increased fibrous connective tissue ratio and inflammatory cell infiltration further strengthened the findings, suggesting an inflammatory process. These results showed that our method successfully created a rat model of seminal vesiculitis.

Seminal vesiculitis is a common disease frequently encountered by urologists and andrologists in clinical settings. The definite cause and pathological mechanisms of this disease remain unclear, and the disease is highly related to male infertility. Liu *et al*.[8] collected seminal vesicle tissues from a living body and found that abnormal expression of semenogelin I (Sg I) was closely related to seminal vesiculitis. A review by La Vignera *et al*.[9] indicated that male accessory gland infection may impair the sperm function and cause male infertility. Castiglione *et al*.[7] found that increased oxidative stress in infertile men and increased proinflammatory interleukins in patients with male accessory gland infection (especially when infection is extended to seminal vesicles) can lead to seminal hyperviscosity. To investigate the pathogenesis of seminal vesiculitis, it is very important to explore the mechanisms of related male infertility with low seminal parameters. Nevertheless, there are few studies on this issue, and a relevant animal model is lacking.

There are two types of animal models of inflammation, bacterial and nonbacterial. In our study, the root proximal to the seminal vesicles of rats was ligated partially, and the degree of ligature tightness was controlled. The root distal to the seminal vesicles connects to the ejaculatory duct which is unblocked. The sperm from the epididymis can join into the seminal fluid from the treated seminal vesicles. In this rat model, we can conclude that the suture was a kind of foreign body to the seminal vesicle, which can cause an inflammatory response, but it was unable to rule out bacterial infection due to the lacking of bacterial identification. The seminal ductal system was blocked incompletely when the root proximal to the seminal vesicle was ligatured partially. The obstruction may lead to the accumulation of seminal fluid in the seminal vesicle. The seminal fluid may be affected by inflammatory factors from the inflammatory reaction. The abnormal seminal fluid may impair the sperm from epididymides via vas deferens. More work is needed to determine whether this animal model can be used to test if seminal vesiculitis can cause the poor semen quality. The certain inflammation change in the treated seminal vesicle could provide a seminal vesiculitis rat model for scientists to study its pathogenesis and promote the development of anti-inflammatory agents to the seminal vesiculitis.

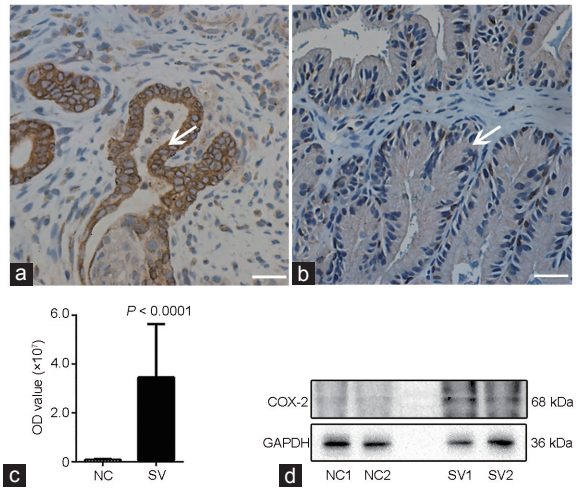


**Figure 4:** Seminal vesicle COX-2 staining. COX-2 expression levels were significantly increased in (**a**) the operated seminal vesicles compared to (**b**) the normal control seminal vesicles in the same rat on immunostaining; white arrows show the section with COX-2 staining. Scale bars = 20 μm. (**c**) The histogram shows the immunohistochemical analysis results. (**d**) Western blot results show TNF-α expression levels were elevated in the operated seminal vesicles; NC1, NC2, SV1 and SV2 were operated (SV1, SV2) and normal (N1 and N2) seminal vesicle samples in two independent rats marked as 1 and 2, respectively. $P < 0.0001$, SV group compared to NC group. OD: optical density; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; COX-2: cyclooxygenase-2; SV: seminal vesiculitis; NC: normal control.

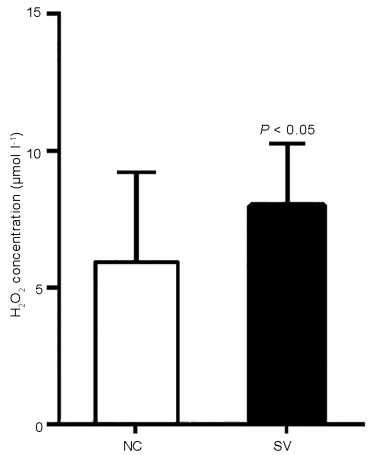


**Figure 6:** Levels of $H_2O_2$ in the seminal plasma from the seminal vesicles in rats. $P < 0.05$, SV group compared to NC group. $H_2O_2$: hydrogen peroxide; SV: seminal vesiculitis; NC: normal control.

In our study, we used immunoblotting and immunohistochemistry assays to determine the TNF-α and COX-2 levels in the seminal vesicle tissues. The results suggested that TNF-α and COX-2 proteins were significantly upregulated in one of the seminal vesicles in rats after the root proximal to the seminal vesicle was ligatured partially with suture for 8 days. The mRNA expression of other proinflammatory cytokines (IL-6 and iNOS) was also found to significantly increase in the seminal vesicles ligated with sutures. Interestingly, we also found that $H_2O_2$, an important reactive oxygen species, increased in the seminal plasma from the seminal vesicle with inflammatory. A research has suggested an inverse relationship between sperm quality (motility, viability, and intact acrosome percentages), and the concentration of $H_2O_2$ and the duration of incubation.[10] In a previous study, we conducted a pilot

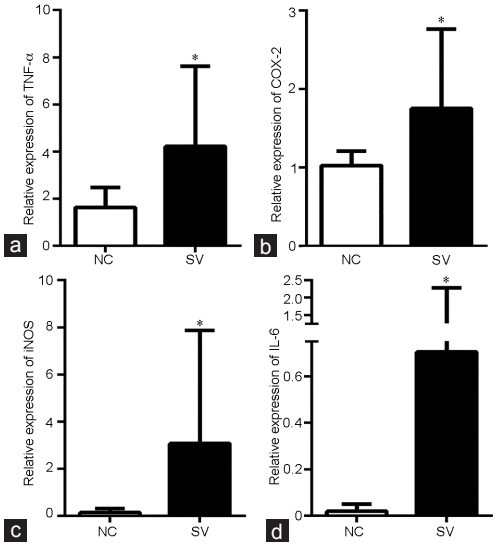


**Figure 5:** Proinflammatory cytokine mRNA expression in the seminal vesicles. (**a**) TNF-α, (**b**) COX-2, (**c**) IL-6, and (**d**) iNOS mRNA expression levels were significantly upregulated in the treated seminal vesicles. $^*P < 0.05$, SV group compared to NC group. TNF-α: tumor necrosis factor-alpha; COX-2: cyclooxygenase-2; IL-6: interleukin 6; iNOS: inducible nitric oxide synthase; SV: seminal vesiculitis; NC: normal control.

experiment to confirm that the ideal time for tissue harvesting was the 8th day after the operation, based on the appearance of the seminal vesicle. TNF-α, COX-2, IL-6, and iNOS are cell signaling proteins (cytokines) involved in systemic inflammation and in the makeup of the acute-phase reaction.[11] Significantly elevated proinflammatory mediators' (TNF-α, COX-2, IL-6, and iNOS) levels in the treated seminal vesicle showed that there was an inflammatory reaction in the tissue. We evaluated that the endothelium of the seminal vesicle was the main region of the inflammatory reaction. It was possible that the inflammatory reaction affected the seminal fluid present in the seminal vesicle because the endothelium of the seminal vesicle is in contact with the seminal fluid. The specific mechanisms need to be explored in future studies. Our animal study was designed as a self-control study, which could eliminate numerous biases from confounding factors.

There were several limitations in our study. First, no detection of sperm motility in the seminal plasma from seminal vesicles was performed. Second, the degree of tightness of the ligation at the root of the seminal vesicle was not completely consistent in each group. Finally, it was unable to distinguish whether the type of seminal vesiculitis in the rat was bacteria or nonbacterial because we did not perform any bacterial cultures for the seminal vesicle tissue after the partial ligation of the root.

## CONCLUSION

Partially ligating the root of the seminal vesicle via suture in rats is an effective method to build a novel animal mode of seminal vesiculitis.

## AUTHOR CONTRIBUTIONS

PZ, XLW, and ZHY conceived and designed the experiment; PZ and XJS performed the experiments; PZ and XLW analyzed the data; PZ wrote the paper; PZ, XLW, and XJS participated in supervision; XHW participated in supervision and funding raising. All authors read and approved the final manuscript.

## COMPETING INTERESTS

All authors declared no competing interests.

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Supplementary Information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

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**Supplementary Table 1: Primer sequence used**

| *Gene* | *Forward primer* | *Reverse primer* |
|---|---|---|
| *COX-2* | 5'-TTTgCCCAgCACTTCACTC-3' | 5'-ACTCTgTTgTgCTCCCgAA-3' |
| *TNF-α* | 5'-CCACCACgCTCTTCTgTCTA-3' | 5'-CTCCTCTgCTTggTggTTTg-3' |
| *IL-6* | 5'-CCACTgCCTTCCCTACTTCA-3' | 5'-TTCTgACAgTgCATCATCgC-3' |
| *iNOS* | 5'-gAgATTTTTCACgACACCCTT-3' | 5'-TTCTATTTCCTTTgTTACggC-3' |
| *GAPDH* | 5'-AACgACCCCTTCATTgACCT-3' | 5'-CCCCATTTgATgTTAgCggg-3' |

*COX-2*: cyclooxygenase-2; *TNF-α*: tumor necrosis factor-alpha; *IL-6*: interleukin 6; *INOS*: inducible nitric oxide synthase; *GAPDH*: glyceraldehyde phosphate dehydrogenase