Submitted: 30 May, 2025 Accepted: 06 August, 2025 Published: 30 September, 2025 DOI:10.22514/j.androl.2025.028

REV INT ANDROL
Revista Internacional de Andrología

ORIGINAL RESEARCH

Fibroblast distribution and localization in male reproductive organs: a mouse model study via lineage tracing

Tung Shu[1,*,†], Danqing Ren[1,†], Yu-Hsuan Chien[1,2], Baibing Yang[3], Jiajing Li[4], Chunhui Liu[5], Tien C. Ko[4], Yanna Cao[4,*], Run Wang[1,6]

[1]Division of Urology, University of Texas McGovern Medical School, Houston, TX 77030, USA
[2]Division of Urology, Department of Surgery, Linkou Chang Lung Memorial Hospital, 333 Taoyuan, Taiwan
[3]Department of Andrology, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, 210008 Nanjing, Jiangsu, China
[4]Department of Surgery, University of Texas McGovern Medical School, Houston, TX 77030, USA
[5]Department of Urology, Affiliated Zhongda Hospital of Southeast University, 210009 Nanjing, Jiangsu, China
[6]Department of Urology, University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA

***Correspondence**
yanna.cao@uth.tmc.edu
(Yanna Cao);
tung.shu@uth.tmc.edu
(Tung Shu)

† These authors contributed equally.

Abstract

Background: Fibroblasts are vital for tissue structure, repair, and fibrosis in male reproductive organs, but tools for investigating their localization and distribution are limited. This study employed the collagen type I alpha 2 Cre recombinase estrogen receptor tandem dimer Tomato (Col1a2CreER$^{tdTomato\ (Tom)}$) lineage tracing mouse model, enabling tamoxifen-inducible, fibroblast-specific labeling through Cre recombination under the *Col1a2* promoter. This system labels collagen-producing fibroblasts with tdTomato, enabling lineage tracing and visualization of their organization in the penis, prostate, and testis. The study aimed to investigate fibroblast distribution and localization in these organs using this model. **Methods**: Male Col1a2CreER^{Tom} mice (n = 5–6) received tamoxifen (1 mg/day/mouse, intraperitoneal, for 5 consecutive days) to induce Cre recombination and label collagen-producing fibroblasts with tdTomato. Wild type (WT) control mice (n = 3) also received tamoxifen. Two days after the last injection, penis, prostate, and testis tissues were harvested, sectioned, and examined through microscopy on tdTomato epifluorescence. Fibroblasts were quantified as the percentage of tdTomato$^+$ cells relative to total cells. **Results**: Quantification revealed distinct fibroblast distribution patterns among the organs. In the Col1a2CreER^{Tom} mouse, tdTomato$^+$ fibroblasts were most abundant in the penis (75.24 $\pm$ 1.6%), followed by the prostate (26.02 $\pm$ 1.4%) and testis (13.97 $\pm$ 0.9%). Within the penis, the subtunical region had the highest density (93.42 $\pm$ 0.5%). In the prostate, fibroblasts were mainly within the fibromuscular stroma; in the testis, they were in the tunica albuginea and interstitial compartments. No tdTomato$^+$ cells were observed in WT controls. **Conclusions**: The number and location of collagen-producing fibroblasts differ among the penis, prostate, and testis, and vary within penile compartments. These findings reflect the organ- and region-specific distribution of Col1a2-lineage fibroblasts, as revealed by tamoxifen-induced tdTomato lineage tracing. This study provides a useful tool for further investigation of fibroblast function in male reproductive health.

Keywords

Collagen-expressing fibroblasts; Penis; Prostate; Testis; Lineage tracing

Distribución y localización de fibroblastos en los órganos reproductores masculinos: un estudio en modelo murino mediante trazado de linaje

Resumen

Antecedentes: Los fibroblastos son esenciales para la estructura tisular, la reparación y la fibrosis en los órganos reproductivos masculinos, pero existen herramientas limitadas para investigar su localización y distribución. Este estudio empleó el modelo de ratón de trazado de linaje colágeno tipo I alfa 2 Cre recombinasa receptor de estrógeno; tandem dimer Tomato (Col1a2CreER$^{tdTomato\ (Tom)}$), que permite el marcaje específico de fibroblastos inducible por tamoxifeno mediante recombinación Cre bajo el promotor *Col1a2*. Este sistema marca fibroblastos productores de colágeno con tdTomato, permitiendo el trazado de linaje y la visualización de su organización en pene, próstata y testículo. El objetivo fue investigar la distribución y localización de fibroblastos en estos órganos utilizando este modelo. **Métodos**: Ratones machos Col1a2CreER^{Tom} (n = 5–6) recibieron tamoxifeno (1 mg/día/ratón, intraperitoneal, durante 5 días consecutivos) para inducir la recombinación Cre y marcar fibroblastos productores de colágeno con tdTomato. Los ratones control de tipo silvestre (WT, n = 3) también recibieron tamoxifeno. Dos días después de la última inyección, se recolectaron pene, próstata y testículo, seccionaron y examinaron mediante microscopía de epifluorescencia para tdTomato. Los fibroblastos se cuantificaron como el porcentaje de células tdTomato^{+} respecto al total de células. **Resultados**: La cuantificación reveló patrones distintos de distribución fibroblástica entre órganos. En los ratones Col1a2CreER^{Tom}, los fibroblastos tdTomato^{+} fueron más abundantes en el pene (75.24 $\pm$ 1.6%), seguidos por la próstata (26.02 $\pm$ 1.4%) y el testículo (13.97 $\pm$ 0.9%). En el pene, la región subtunical presentó la mayor densidad (93.42 $\pm$ 0.5%). En la próstata, los fibroblastos se localizaron principalmente en el estroma fibromuscular; en el testículo, en la túnica albugínea y los compartimentos intersticiales. No se observaron células tdTomato^{+} en los controles WT. **Conclusiones**: El número y la localización de fibroblastos productores de colágeno difieren entre pene, próstata y testículo, y varían dentro de los compartimentos penianos. Estos hallazgos reflejan la distribución órgano- y región-específica de los fibroblastos de linaje Col1a2, revelada mediante trazado de linaje inducido por tamoxifeno con tdTomato. Este estudio proporciona una herramienta útil para futuras investigaciones sobre la función de los fibroblastos en la salud reproductiva masculina.

Palabras Clave

Fibroblastos que expresan colágeno; Pene; Próstata; Testículo; Trazado de linaje

1. Introduction

Fibroblasts are important for maintaining the structure and function of tissues in many organs [1, 2]. They produce extracellular matrix proteins to support the tissue structure and repair [3]. In the male reproductive system, fibroblasts also play essential roles in maintaining normal tissue homeostasis and function [4–6].

Fibroblasts in the penis have been shown to contribute to the formation of fibrotic plaques in the penile tunica albuginea [7]. Under certain conditions, these fibroblasts produce excessive extracellular matrix proteins, such as collagen, leading to fibrosis and tissue stiffening, which results in penile fibrosis known as Peyronie's disease [8, 9]. Furthermore, recent studies have also shown that fibroblasts are actively involved in regulating erectile function in the mouse penis [4, 10, 11].

Regarding penile erection, nitric oxide (NO) released from the cavernous nerves and penile vascular endothelial cells plays a major role. NO activates guanylate cyclase, raising the levels of cyclic guanosine monophosphate (cGMP) that causes a cascade of protein phosphorylation. As a result, smooth muscle cells relax, allowing blood to fill the cavernous sinuses to induce and maintain an erection [12]. In addition to this well-established mechanism, recent studies have explored the role of fibroblasts in regulating penile erectile function. Fibroblasts make up over half of the cells in the corpus cavernosum, where they regulate the extracellular matrix and interact with smooth muscle cells to maintain the tissue environment [11]. Zhao *et al.* [10] identified six types of fibroblasts in the human corpus cavernosum, showing their role in supporting penile structure and regulating cell communication, which is essential for erectile function. Guimaraes *et al.* [4] demonstrated that fibroblasts also regulate blood flow by controlling norepinephrine levels around blood vessels, promoting vasodilation and supporting erections.

In the prostate, fibroblasts are involved not only in the normal structural framework but also play pivotal roles in pathological conditions, such as benign prostatic hyperplasia and the progression of prostate cancer [13, 14]. In benign prostatic hyperplasia, increased fibroblast activity secretes growth factors like fibroblast growth factor-2 (FGF-2) and insulin growth factor-1 (IGF-1), driving epithelial proliferation and structural changes [15]. In prostate cancer, the loss of fibroblast-dependent androgen receptor (AR) activation contributes to castration-resistant cancer, while cancer-associated fibroblasts, activated by tumor elements, play a central role in supporting tumor progression [16, 17].

Similarly, in the testis, fibroblasts contribute to the regulation of tissue architecture and are implicated in fibrotic events leading to male infertility [18]. Testicular fibrosis can arise from prolonged mast cell inflammation, leading to fibroblast activation [19]. These fibroblasts become myofibroblasts, producing collagen and fibronectin, which causes excessive scarring and tissue overgrowth [20].

Our group has recently [21] developed the collagen type I alpha 2 Cre recombinase estrogen receptor tandem dimer Tomato

(Col1a2CreER$^{tdTomato\ (Tom)}$) mouse model to genetically label collagen-expressing fibroblasts across multiple mouse organs. This model was generated by crossing Col1a2CreER mice, which express tamoxifen-inducible CreER under the control of the fibroblast-specific *Col1a2* promoter, with a tdTomato Cre-reporter line. Upon tamoxifen administration, CreER enters the nucleus and activates tdTomato expression in Col1a2-expressing fibroblasts. This enables permanent and specific red fluorescence labeling of fibroblasts, allowing their distribution and localization to be visualized in mouse tissues. In the current study, we adapted this model to examine the distribution and localization of fibroblasts in the penis, prostate, and testis to further study the role of fibroblasts in male reproductive organs.

2. Materials and methods

2.1 Animals

All animal experiments were performed according to the protocols approved by the Animal Welfare Committee of UTHealth at Houston. All mice were housed in a climate-controlled room with an ambient temperature of 23 °C and a 12:12-h light-dark cycle. Animals were fed standard rodent chow and given water ad libitum. Male mice (6–8 weeks old) were used. All mice were on C57BL/6 background. Col1a2CreER mice [22] (B6.Cg-Tg(Col1a2-cre/ERT,-ALPP)7Cpd/J, Stock #029567, Jackson Laboratory, Bar Harbor, ME, USA) were crossbred with reporter mice tdTomato [21] (B6.Cg-Gt(ROSA)26Sortm14(CAG-tdTomato)Hze/J, Stock #007914, Jackson Laboratory) and produced Col1a2CreER^{Tom}. The resulting animals were genotyped following specific instructions from Jackson Laboratory. The wild-type (WT) sibling littermates were used as control.

2.2 Model description

The Col1a2CreER mouse line harbors a tamoxifen-inducible *CreER* gene under the control of the fibroblast-specific regulatory sequence of the procollagen alpha-2(I) chain (*proα2(I)*) collagen gene (*Col1a2*) [22]. To enable lineage tracing, Col1a2CreER mice were crossbred with a Cre-reporter line carrying a floxed STOP cassette upstream of the tdTomato red fluorescent protein gene, generating the Col1a2CreER^{Tom} model [21]. In this inducible system, Cre recombinase remains cytoplasmic and inactive until tamoxifen is administered. Upon tamoxifen binding, the CreER fusion protein translocates into the nucleus and excises the STOP cassette, enabling permanent tdTomato expression in Col1a2-expressing fibroblasts (Fig. 1). This system allows for temporal control and permanent labeling of fibroblasts under physiological conditions, thereby enabling spatial visualization and quantification of fibroblast distribution in various tissues using epifluorescence microscopy.

2.3 Induction of Cre recombination and tissue harvesting

Two groups of mice were included. The experimental group consisted of 5–6 Col1a2CreER^{Tom} mice, which were used to trace collagen-expressing fibroblasts upon tamoxifen (Serial#: T5648-5G, Lot#: WX22909V, Sigma-Aldrich, St. Louis, MO, USA) induction. The control group consisted of 3 WT littermate mice that received the same tamoxifen treatment but lacked the Cre allele and therefore did not express tdTomato. Only WT mice treated with tamoxifen were used as negative controls in this study, as we have previously validated in other organ systems that Col1a2CreER^{Tom} mice without tamoxifen and tdTomato-only mice do not show background tdTomato expression [21]. Therefore, these groups were not repeated here. Col1a2CreER^{Tom} mice and the control mice were administered tamoxifen (1 mg/day/mouse, for 5 consecutive days, i.p.) to induce Cre recombination. Two days after the final tamoxifen injection, the mice were euthanized using an overdose of isoflurane (J123028, Dechra, Overland Park, KS, USA), followed by cervical dislocation. The penis, testis, and prostate were then harvested for further analysis. This two-day interval allowed time for CreER nuclear translocation, locus of X-over P1 (LoxP) recombination, and the reporter gene expression to reach detectable levels in fibroblasts, as previously demonstrated [22]. The tissue samples were fixed in 10% formalin and paraffin embedded for morphological studies, or fixed in 4% paraformaldehyde and 10% sucrose, followed by optimal cutting temperature (OCT) block preparation for frozen sectioning [23] for epifluorescence.

2.4 Morphological examination

Paraffin-embedded tissue samples of mouse penis, prostate, and testis were sectioned (5-μm thick) in both cross-sectional and longitudinal planes, while prostatic tissues and testicular tissues were sectioned transversely, and stained with hematoxylin and eosin (H&E).

2.5 Quantification of fibroblasts

Frozen tissue blocks were sectioned at 5 μm using a cryostat (Leica CM1850, Leica Biosystems, Buffalo Grove, IL, USA) for epifluorescence of tdTomato. Penile tissues were sectioned in both cross-sectional and longitudinal planes, while prostatic tissues and testicular tissues were sectioned transversely. Nuclei were counterstained with VECTASHIELD Antifade Mounting Medium with DAPI (DAPI: 4′,6-diamidino-2-phenylindole; Lot#: H-1200, Vector Laboratories, Newark, CA, USA). Epifluorescence images were captured using a Nikon Ti-E inverted fluorescence microscope (Nikon Instruments, Melville, NY, USA) equipped with appropriate filter sets for tdTomato and DAPI. Non-overlapping high-power field images (4–6 images/section) were acquired using a Nikon Tie microscope, and tdTomato$^+$ fibroblasts were quantified using Nikon Elements BR 5.30.05 software (Nikon Instruments, Melville, NY, USA) as previously described [24]. The number of tdTomato$^+$ cells was quantified by identifying cells with colocalization of tdTomato fluorescence and DAPI nuclear staining. The result was expressed as a percentage

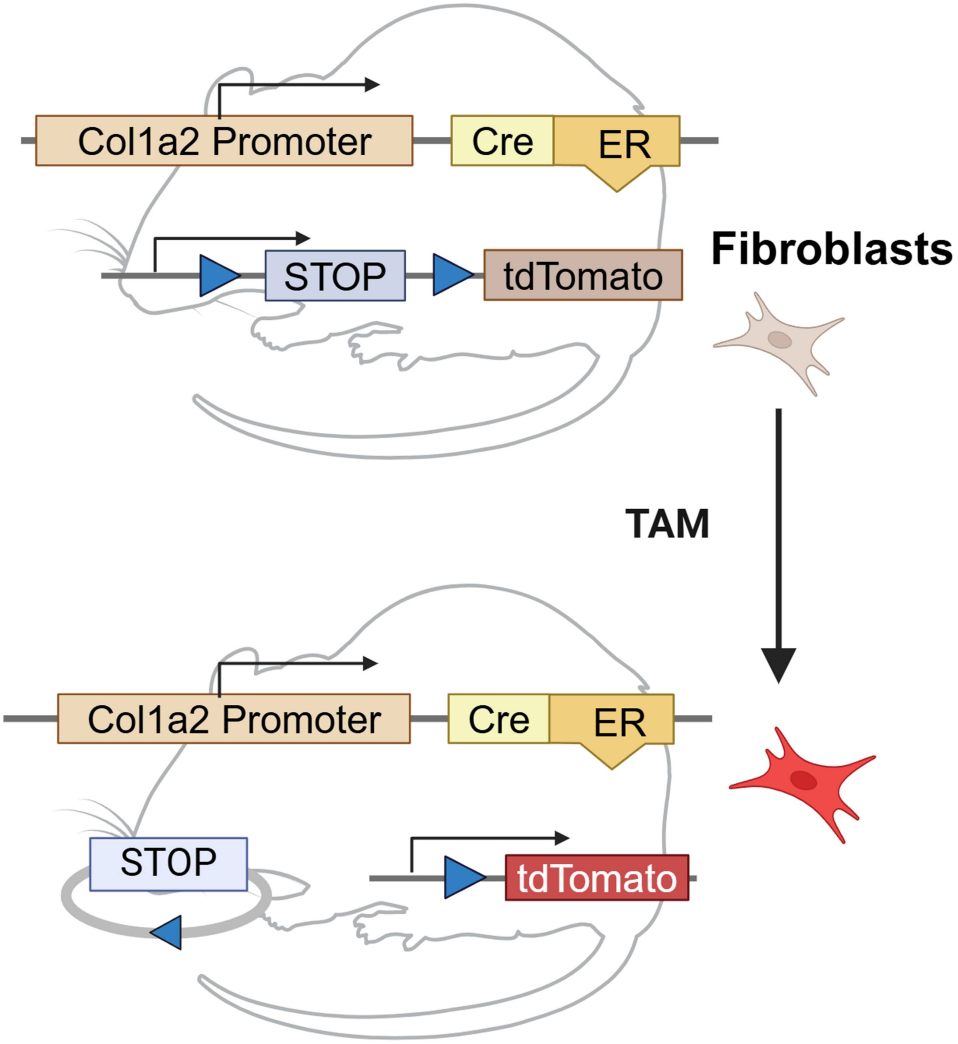


FIGURE 1. The Col1a2CreER^{Tom} mouse model. The Col1a2CreER mice carry a *CreER* gene under the control of a fibroblast-specific promoter segment from the *proα2(I)* collagen gene (*Col1a2*). These mice were bred with tdTomato reporter mice, generating the Col1a2CreER^{Tom} mice. TAM administration induces Cre recombination and the resulting Cre recombinase cleaves the stop codon, leading to tdTomato expression in collagen-expressing fibroblasts. Cre: cre recombinase; ER: estrogen receptor; tdTomato: tandem dimer Tomato; TAM: tamoxifen.

of tdTomato$^+$ cells relative to the total number of DAPI$^+$ nuclei within each tissue region. tdTomato fluorescence represents fibroblasts that have undergone tamoxifen-induced Cre-mediated recombination in the Col1a2CreER^{Tom} mouse model. Thus, all quantified tdTomato$^+$ cells reflect lineage-traced collagen-producing fibroblasts [21].

2.6 Statistical analysis

Data are expressed as mean ± standard error (SEM). Statistical significance was determined using one way Analysis of Variance (ANOVA). p values < 0.05 were considered statistically significant. All statistics were performed using GraphPad Prism version 9 (GraphPad Software, Inc., San Diego, CA, USA).

3. Results

3.1 Collagen-producing tdTomato$^+$ fibroblasts in penis, prostate, and testis were efficiently labeled and tracked

As shown in Fig. 2A, tdTomato$^+$ fibroblasts in the studied organ tissues of the Col1a2CreER^{Tom} mouse appeared red. After tamoxifen injection, tdTomato$^+$ fibroblasts constituted 75.24 ± 1.6% of total cells in the penis, 26.02 ± 1.4% in the prostate, and 13.97 ± 0.9% in the testis of the Col1a2CreER^{Tom} mouse (Fig. 2B). The distribution of tdTomato$^+$ fibroblasts among these three reproductive organs demonstrated a statistically significant difference ($p < 0.05$). Specifically, the percentage of tdTomato$^+$ fibroblasts was 49.22% higher in the penis than in the prostate and 61.27% higher in the penis than in the testis. In contrast, as shown in tdTomato epifluorescence images, no tdTomato$^+$ cells were observed in these organs of tamoxifen-injected WT control mice (Fig. 2A). Based on the previous findings in the pancreas in our group [21], tdTomato$^+$ cells were positive for the fibroblast marker desmin [25], but negative for peanut

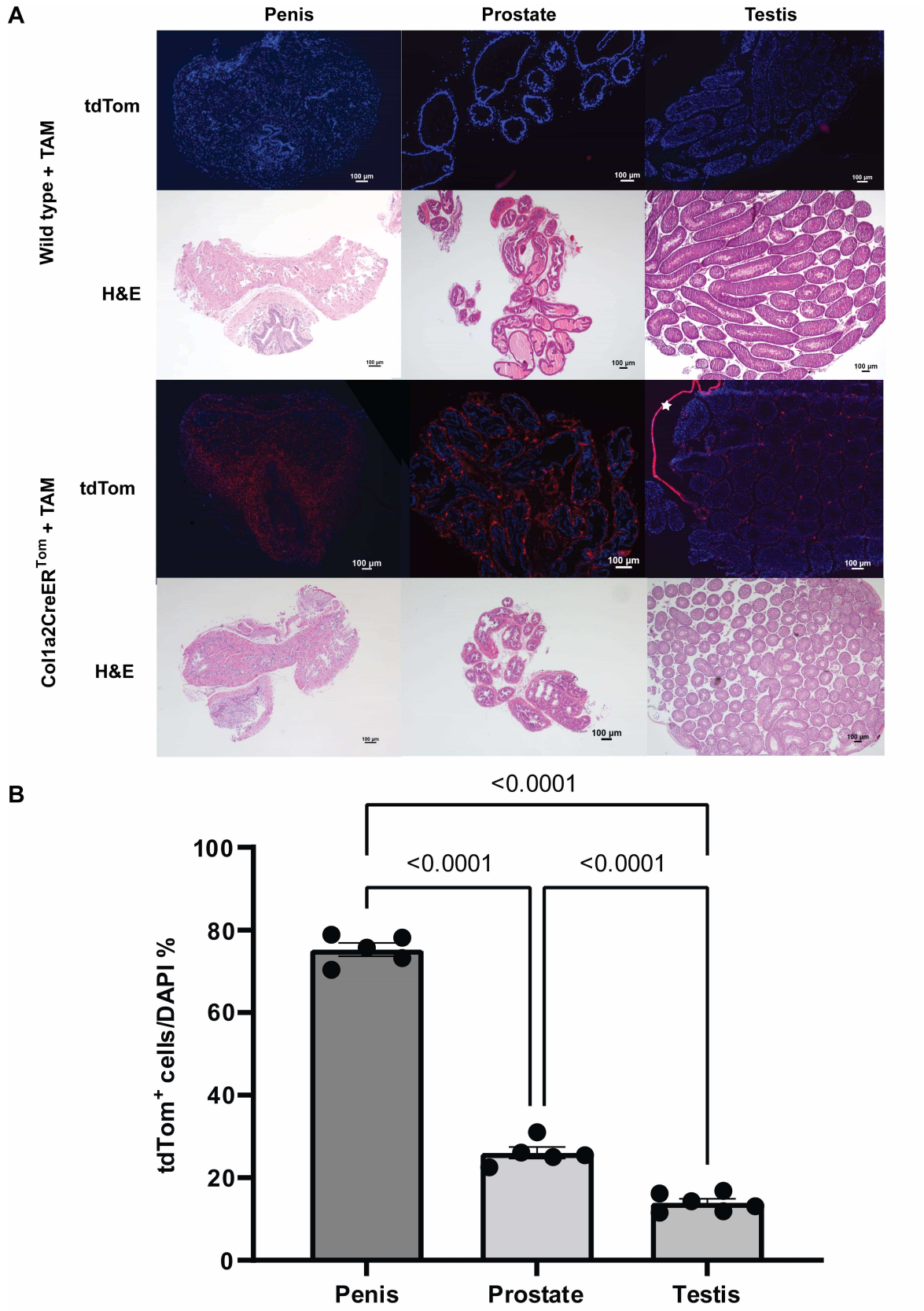


FIGURE 2. The tdTomato$^+$ fibroblasts in the penis, prostate, and testis of Col1a2CreER^{Tom} mouse were efficiently labeled and tracked. (A) Representative images of tdTomato epifluorescence and H&E. White asterisk = tunica albuginea around the testis with tdTomato$^+$ fibroblasts. Scale bar = 100 μm. (B) The amount of tdTomato$^+$ fibroblasts in Col1a2CreER^{Tom} mouse penis, prostate, and testis. Data are presented as mean ± SEM. n = 5–6 mice/group. DAPI: 4′,6-diamidino-2-phenylindole; TAM: tamoxifen; tdTom: tandem dimer Tomato; tdTomato$^+$: tdTomato positive; H&E: hematoxylin and eosin; Col1a2CreER^{Tom}: collagen type I alpha 2 Cre recombinase estrogen receptor tandem dimer Tomato. $p < 0.05$.

agglutinin (PNA, an acinar epithelial cell marker) and cluster of differentiation 31 (CD31) (an endothelial cell marker), confirming that the labeled cells were fibroblasts. This specificity ensures that the model can be reliably used to trace and study fibroblast populations in the other organs.

3.2 Distribution and localization of tdTomato$^+$ fibroblasts in penis

In both cross sections (Fig. 3A) and longitudinal sections (Fig. 3B) of the Col1a2CreER^{Tom} mouse penis, tdTomato$^+$ fibroblasts were seen spread throughout the penis, but predominantly distributed around the vascular structures, with the majority primarily surrounding the sinusoids within the corpus cavernosum (Fig. 3A, white asterisk).

The tdTomato$^+$ fibroblasts in the Col1a2CreER^{Tom} mouse penis were quantified both overall and at three divided regions as previously studied by Guimaraes *et al.* [4]: the tunica albuginea, the subtunical, and the trabecular region in the corpora cavernosa. The overall percentage of tdTomato$^+$ fibroblasts was around 75.24 $\pm$ 1.6% of total cells (Fig. 3C). A significant difference was observed among the three regions ($p < 0.05$), with a 39.44% higher tdTomato$^+$ fibroblast percentage in the subtunical (93.42 $\pm$ 0.5%) compared to the trabecular region (53.98 $\pm$ 2.6%), and a 15.09% difference between the subtunical and tunica albuginea (78.33 $\pm$ 1.8%) (Fig. 3C). This shows that fibroblasts are not evenly distributed, and their presence may relate to the function of each area.

3.3 Distribution and localization of tdTomato$^+$ fibroblasts in prostate and testis

In the prostate lobes, tdTomato$^+$ fibroblasts were mostly found in the fibromuscular stroma (Fig. 4A, white asterisk), surrounding the secretory epithelial cells. These fibroblasts were spread evenly across the stromal areas, with a percentage of 26.02 $\pm$ 1.4% (Fig. 2).

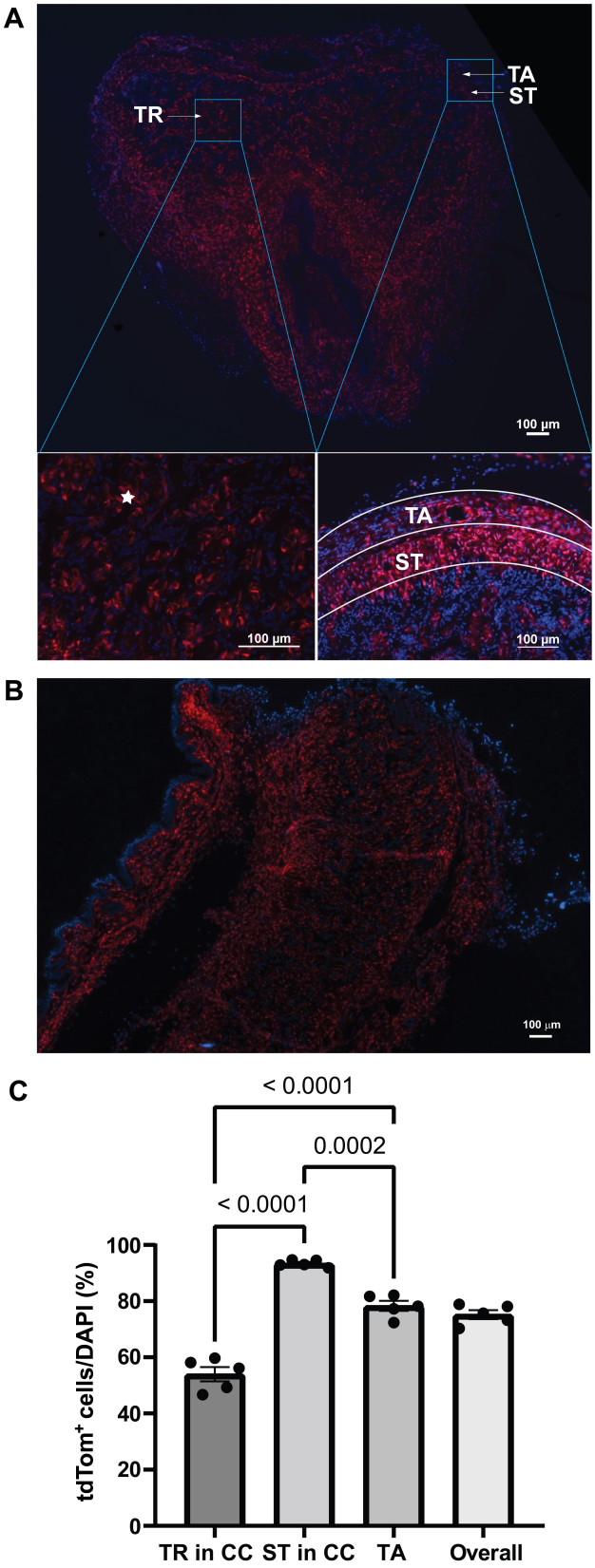
The testis is covered by a thin, tough fibrous layer called the tunica albuginea. The seminiferous tubules are surrounded by a basal lamina that separates them from the interstitial space, provides structural support, and controls the function of nearby cells [26]. From our observations, the tdTomato$^+$ fibroblasts in testis were mostly located in the tunica albuginea around the testis (Fig. 2A, white asterisk), in the basal lamina surrounding the seminiferous tubules (Fig. 4B, yellow asterisk), and around individual stromal cells located between the seminiferous tubules in the interstitial compartment (Fig. 4B, green asterisk). The tunica albuginea of the testis is thin, compact, and easy to separate from testis, making it hard to quantify tdTomato$^+$ fibroblasts under epifluorescence imaging. Therefore, we could only count them in the seminiferous tubules and stromal areas, which constitute 13.97 $\pm$ 0.9% of total cells (Fig. 2).

4. Discussion

Studying fibroblasts is challenging due to their heterogeneity and the lack of specific markers. Genetic tools in mice, such as colorimetric reporters and Cre recombinase, have been used to study fibroblasts in organs prone to fibrosis, like the heart, kidney, liver, lung, and skeletal muscle [27]. The *Col1a2* gene has been used as a model to study how transcription is regulated, especially in connection to the deposition of the extracellular matrix in normal and fibrotic conditions [28]. Col1a2CreER is a transgenic mouse model where the *CreER* gene is controlled by the Col1a2 promoter, which targets cells that produce type I collagen. Recently, our group [21] used the Col1a2CreER^{Tom} model for lineage tracing of collagen-expressing fibroblasts in both normal pancreas and during acute pancreatitis, demonstrating specificity in labeling pancreatic fibroblasts. We also investigated [21] tdTomato$^+$ fibroblasts across multiple organs in this mouse model and observed a wide variation in their distribution pattern, with significantly higher numbers in the lung, skin, and colon, and much lower levels in the kidney, heart, and liver. A recent study by Guimaraes *et al.* [4] used the solute carrier family 1 member 3-Cre recombinase estrogen receptor T2; Rosa26-loxP-tdTomato (Slc1a3-CreER^{T2}; Rosa26-tdTomato) mice to mark and trace fibroblasts expressing the Slc1a3 protein, which allowed for tracking fibroblast behavior in the penile tissue.

In the current study, we used the Col1a2CreER^{Tom} mouse model to identify and quantify collagen 1a2-producing fibroblasts in mouse reproductive organs—the penis, prostate, and testis. We found that tdTomato$^+$ collagen-expressing fibroblasts are present in these three organs, but their amount and distribution are variable.

Research on penile fibroblasts primarily focuses on their role within the tunica albuginea, the fibrous layer surrounding the corpora cavernosa, as they are involved in Peyronie's disease [29, 30]. In our study, we observed that tdTomato$^+$ fibroblasts are distributed throughout the penis, with the highest density in the subtunical region of the corpora cavernosa, followed by the tunica albuginea, and the lowest density in the trabecular region. Similarly, the study by Guimaraes *et al.* [4] utilized the Slc1a3-CreER^{T2}; R26R-tdTomato mouse model to label Slc1a3 + tdTomato$^+$ cells, primarily fibroblasts, located mostly in the corpora cavernosa. They found that fibroblasts are the most abundant cell type in the corpora cavernosa, significantly exceeding the numbers of vascular smooth muscle cells and endothelial cells. These fibroblasts are broadly distributed in the subtunical and trabecular areas near the vasculature, as well as in the tunica albuginea [4]. Taken together, our findings and those from Guimaraes *et al.* [4] demonstrated that, the spatial arrangement of fibroblasts is predominantly in the subtunical region and tunica albuginea, suggesting a potentially distinct role in supporting vasodilation and maintaining structural integrity within the corpora cavernosa. tdTomato$^+$ fibroblasts are concentrated around vascular structures, especially sinusoids in the corpus cavernosum, further implicating a role in modulating blood flow during erection. Recent research on the pathophysiology of erectile dysfunction (ED) has explored the important role of fibroblasts in the corpus cavernosum and how they contribute to the disease through the combination of various techniques, such as, single-cell RNA sequencing (scRNA-seq), cell-cell communication networks, *etc.* [10, 11, 31, 32]. Previously seen as static cells, fibroblasts are now understood to regulate blood flow, fibrosis, and extracellular matrix changes, reflecting the complex interactions between fibroblasts, smooth muscle



F I G U R E 3. Distribution of tdTomato$^+$ fibroblasts in both cross (A) and longitudinal (B) sections of the Col1a2CreER^{Tom} mouse penis. (A) Cross sections showing representative images of tdTomato epifluorescence and quantification in three regions of penis: the tunica albuginea (TA), the subtunical (ST) and the trabecular region (TR) in the corpora cavernosa (CC). White asterisk = sinusoids with tdTomato$^+$ fibroblasts. Scale bar = 100 μm. (B) Longitudinal sections of the penis. Scale bar = 100 μm. (C) tdTomato$^+$ fibroblasts quantification in the three regions mentioned in (A). Data are presented as mean ± SEM. n = 6 mice/group. tdTomato$^+$: tdTomato positive; DAPI: 4′,6-diamidino-2-phenylindole. $p < 0.05$.

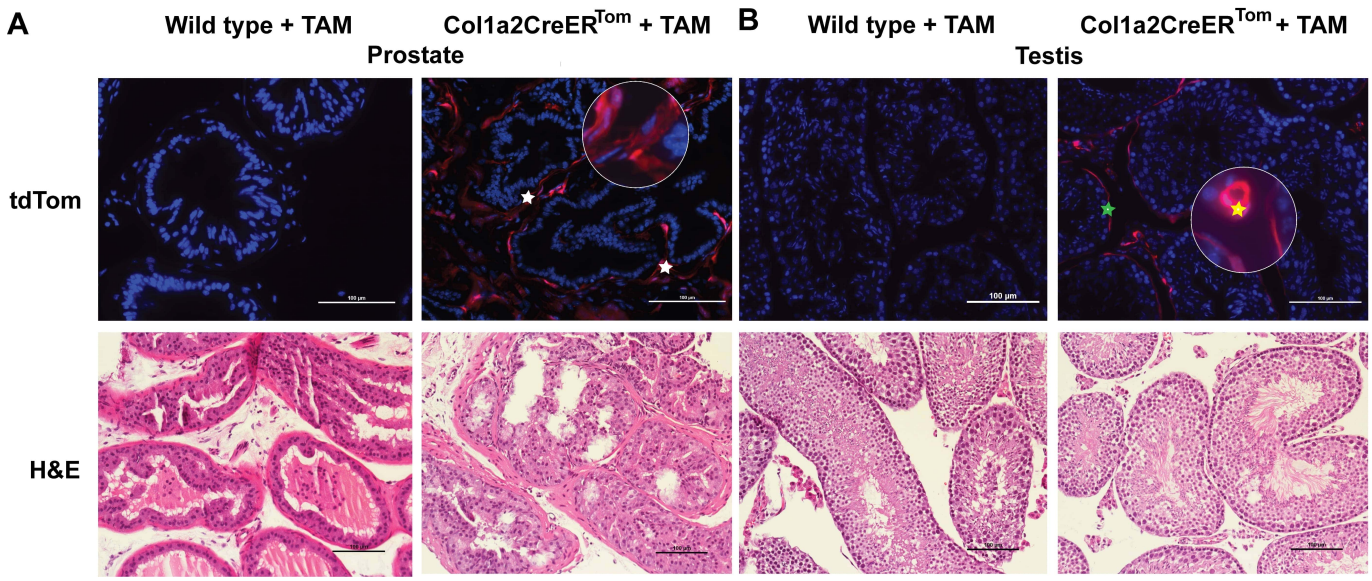


FIGURE 4. The tdTomato$^+$ fibroblasts distribution and localization in Col1a2CreER^{Tom} mouse prostate and testis. Representative images of tdTomato epifluorescence and H&E. (A) Prostate. White asterisk = fibromuscular stroma with tdTomato$^+$ fibroblasts. (B) Testis. Yellow asterisk = basal lamina with tdTomato$^+$ fibroblasts. Green asterisk = individual stromal cells with tdTomato$^+$ fibroblasts. Scale bar = 100 μm. TAM: tamoxifen; tdTom: tdTomato; H&E: hematoxylin and eosin; Col1a2CreER^{Tom}: collagen type I alpha 2 Cre recombinase estrogen receptor tandem dimer Tomato.

cells, and endothelial cells. These findings could provide a starting point for new therapeutic options for ED [4, 10, 11, 31, 32]. The quantification ability of the Col1a2CreER^{Tom} mouse model will help in studying the complex functions of fibroblasts and support further research into the mechanisms of ED management.

The prostate, a fibromuscular gland that produces prostatic fluid, relies on fibroblasts for development and cancer progression [33]. Studies have aimed to identify fibroblast populations in normal mouse and human prostates, but the diversity of fibroblasts and the lack of specific markers are challenging [5, 16, 33–35]. Lineage tracing studies could help clarify fibroblast behavior and their role in prostate health. In this study, we found that tdTomato$^+$ fibroblasts were distributed throughout the fibromuscular stroma of the mouse prostate, comprising 26.02% of the total cell population. To our knowledge, no prior studies have reported the absolute proportion of fibroblasts in the wild-type mouse prostate. Previous histological work [33] estimated fibroblast-to-smooth muscle density ratios in defined regions, but organ-wide quantification of fibroblasts has not been described. As our labeling method does not perturb fibroblast homeostasis, we believe this measurement accurately reflects the endogenous abundance of fibroblasts. This quantitative reference provides a valuable baseline for future investigations of stromal remodeling under pathological conditions, including prostate cancer. Our findings support previous reports indicating that fibroblasts are integral components of the prostate stroma [36], potentially contributing to tissue structure and function in both normal and pathological contexts, such as benign prostatic hyperplasia [15, 37]. Furthermore, given the critical role of fibroblasts in modulating the tumor microenvironment, our findings may have implications for understanding stromal remodeling in prostate cancer. Establishing this fibroblast distribution profile in the normal prostate lays the groundwork for future research exploring how fibroblast abundance and activation states may change during disease progression.

In the mouse testis, we observed that most tdTomato$^+$ fibroblasts are mainly distributed in the tunica albuginea surrounding the testis, within the basal lamina surrounding the tubules, and around individual stromal cells between the seminiferous tubules in the interstitial compartment. We measured the proportion of tdTomato$^+$ fibroblasts only in the areas around the seminiferous tubules and in the interstitial compartment and found that they account for about 13.97% of the total cells in these regions. Their location supports the idea that fibroblasts may help maintain the basal lamina and assist the peritubular myoid cells, which play a key role in spermatogenesis [38]. Recent studies using single-cell technologies reveal how fibroblasts interact with other testicular cells, helping to maintain tissue structure [39]. The Col1a2CreER^{Tom} mouse model, with its ability to quantify and locate fibroblasts, could become a useful tool for tracking fibroblast activity in the testis, helping to understand their specific role in the testicular microenvironment. When used together with advanced single-cell methods like scRNA-seq, this model may help mapping of fibroblast functions and provide insights into their broader role in maintaining testicular health.

Furthermore, given that the reproductive organs investigated in this study (penis, prostate, and testis) are androgen-dependent [40, 41], our characterization of fibroblast distribution and localization provides an essential foundation for future hormonal investigations. Specifically, fibroblasts expressing collagen may interact closely with AR signaling pathways, potentially modulating tissue responses to hormonal changes [17, 42, 43]. Our findings thus highlight the importance of exploring how hormonal status and androgen signaling influence fibroblast function and remodeling within these organs, which

could be pivotal for understanding reproductive physiology and pathology.

This study presents several novelties. Firstly, expanding upon the work of Guimaraes *et al*. [4], we further confirmed the distribution and localization of fibroblasts within the mouse penis. We used a distinct lineage tracing mouse model and quantified the percentage of fibroblasts across different functional regions of the penis. This presents a more detailed understanding of spatial distribution for further study of the potential roles of fibroblasts within these regions. Additionally, we extended our investigation to the testis and prostate, examining the distribution of fibroblasts in these organs as well. To our knowledge, this is the first study to specifically investigate fibroblast populations in the testis and prostate of male mice using the lineage tracing. This is an important first step toward understanding how fibroblasts may contribute to the function of these reproductive organs.

Despite these novelties, our study also has limitations. Firstly, we did not employ organ-specific markers in addition to the tdTomato labeled fibroblasts. Secondly, this study is descriptive and lacks functional experiments to directly assess the clinical relevance of our findings.

Our current work specifically addresses fibroblast distribution and homeostasis under physiological conditions, providing essential baseline data for normal tissue maintenance. Importantly, this study did not investigate fibrosis or pathological remodeling, and our findings are not intended to imply any fibrotic process. However, future studies incorporating histological methods such as Masson's trichrome or Sirius Red staining will be valuable to assess whether fibroblast populations contribute to fibrotic changes under pathological conditions.

In addition, evaluating organ-specific biomarkers and performing correlation analyses with fibroblast distribution will be an important next step. Future investigations involving immunofluorescent co-labeling with functional markers such as AR and endothelial nitric oxide synthase (eNOS), as well as hormonal manipulation models, will be crucial to further elucidate the roles of fibroblasts in androgen-regulated reproductive tissues. These approaches will help clarify the functional roles of fibroblasts in different reproductive organs and provide insights into how fibroblast heterogeneity contributes to tissue-specific physiology and disease.

5. Conclusions

This study employed the Col1a2CreER^{Tom} mouse model to identify and quantify collagen-expressing fibroblasts in the penis, testis, and prostate. The distinct distribution patterns of the fibroblasts in these male reproductive organs suggested their possible specific roles in tissue structure and function. For example, the high fibroblast density in the subtunical of the penis may contribute to structural integrity and vascular regulation essential for erectile function, while fibroblasts in the prostate stroma may influence epithelial–stromal interactions relevant to benign prostatic hyperplasia or cancer progression. In the testis, fibroblasts localized to the tunica albuginea and interstitial space may play supportive roles in spermatogenesis and testicular immune regulation.

Looking forward, the Col1a2CreER^{Tom} model may serve as a platform to explore how fibroblasts contribute to specific disease processes in male reproductive organs. In this study, tdTomato^{+} fibroblasts were mainly located around vascular structures in the subtunical and trabecular areas of the penis. These regions are important for blood filling during erection. The presence of fibroblasts in these areas suggests they may regulate vascular tone and extracellular matrix stiffness, thereby influencing erectile function. Previous studies also showed that fibroblasts interact with smooth muscle cells and modulate neurotransmitter levels, such as norepinephrine, around blood vessels. These interactions may be related to blood flow and penile tissue relaxation. Future studies can combine this Col1a2CreER^{Tom} model with animal models of ED to investigate fibroblast behavior under disease conditions. This may have relevance to aging-related ED or fibrotic disorders such as Peyronie's disease, where abnormal fibroblast activity leads to localized penile fibrosis and curvature. Functional experiments are needed to test how fibroblast activation or depletion affects penile structure and erection. Single-cell RNA sequencing or spatial transcriptomics may help identify fibroblast subtypes that are more active in fibrotic or vasoregulatory pathways. These approaches can improve understanding of fibroblast roles in ED and may help find new therapeutic targets.

In the prostate, this model can be applied to test how different fibroblast subtypes contribute to stromal growth in benign prostatic hyperplasia or support tumor progression in prostate cancer, where fibroblast–epithelial interactions are key components of disease pathology. In the testis, further investigations are warranted to elucidate fibroblast roles in supporting the spermatogenic environment or promoting fibrosis related to infertility.

Overall, this study provides essential baseline data on fibroblast distribution and homeostasis under physiological conditions. These findings lay the groundwork for future functional studies aimed at understanding fibroblast dynamics in male reproductive organ health and disease. The Col1a2CreER^{Tom} model may also facilitate the discovery of novel fibroblast markers or therapeutic targets for diagnostic and interventional strategies in male reproductive diseases.

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on reasonable request from the corresponding author.

AUTHOR CONTRIBUTIONS

TS, DR, BY, JL, CL and YC—conducted relevant experiments. TS, DR, YHC, YC and RW—contributed to the data acquisition; processing; analysis; and interpretation. TS and DR—drafted the manuscript. DR, YHC, YC, TCK and RW—revised and refined the manuscript. The study was conceptualized and designed through the collaborative efforts of all authors. All authors have read and approved the final version of the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All animal experiments were performed according to the protocols (AWC-23-2024) approved by the Animal Welfare Committee of UTHealth at Houston.

ACKNOWLEDGMENT

The authors thank the HistoCore Lab at UTHealth Houston for the tissue process and H&E staining, and Amanda C. Bean from the University of Texas McGovern Medical School for her support with English language editing and stylistic revisions.

FUNDING

This study is partially supported by the NIH1R21 AA027014-01A1 and Jack H Mayfield M.D. Distinguished Professorship in Surgery (TCK). Additional support was provided by the Weatherhead Fund for Translational Research (TS) and the Cecil M. Crigler, M.D. Chair in Urology Endowment Fund (RW). The above sponsors were not involved in the study design, collection, analysis, or interpretation of data.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Forte E, Ramialison M, Nim HT, Mara M, Li JY, Cohn R, *et al*. Adult mouse fibroblasts retain organ-specific transcriptomic identity. eLife. 2022; 11: e71008.

[2] Gauthier V, Kyriazi M, Nefla M, Pucino V, Raza K, Buckley CD, *et al*. Fibroblast heterogeneity: keystone of tissue homeostasis and pathology in inflammation and ageing. Frontiers in Immunology. 2023; 14: 1137659.

[3] Roman J. Fibroblasts—warriors at the intersection of wound healing and disrepair. Biomolecules. 2023; 13: 945.

[4] Guimaraes EL, Dias DO, Hau WF, Julien A, Holl D, Garcia-Collado M, *et al*. Corpora cavernosa fibroblasts mediate penile erection. Science. 2024; 383: eade8064.

[5] Henry GH, Malewska A, Joseph DB, Malladi VS, Lee J, Torrealba J, *et al*. A cellular anatomy of the normal adult human prostate and prostatic urethra. Cell Reports. 2018; 25: 3530–3542.e5.

[6] Miki H, Manresa MC. Novel fibroblast phenotypes in homeostasis and chronic inflammation: from functions to potential regulators. The Journal of Physiology. 2023; 601: 2273–2291.

[7] Ilg MM, Bustin SA, Ralph DJ, Cellek S. TGF-β1 induces formation of TSG-6-enriched extracellular vesicles in fibroblasts which can prevent myofibroblast transformation by modulating Erk1/2 phosphorylation. Scientific Reports. 2024; 14: 12389.

[8] Ilg MM, Harding S, Lapthorn AR, Kirvell S, Ralph DJ, Bustin SA, *et al*. Temporal gene signature of myofibroblast transformation in Peyronie's disease: first insights into the molecular mechanisms of irreversibility. Journal of Sexual Medicine. 2024; 21: 278–287.

[9] Şahin A, Babayev H, Cirigliano L, Preto M, Falcone M, Altıntas E, *et al*. Unveiling the molecular hallmarks of peyronie's disease: a comprehensive narrative review. International Journal of Impotence Research. 2024; 36: 801–808.

[10] Zhao L, Han S, Su H, Li J, Zhi E, Li P, *et al*. Single-cell transcriptome atlas of the human corpus cavernosum. Nature Communications. 2022; 13: 4302.

[11] Fang D, Tan XH, Song WP, Gu YY, Pan JC, Yang XQ, *et al*. Single-cell RNA sequencing of human corpus cavernosum reveals cellular heterogeneity landscapes in erectile dysfunction. Frontiers in Endocrinology. 2022; 13: 874915.

[12] Toda N, Ayajiki K, Okamura T. Nitric oxide and penile erectile function. Pharmacology & Therapeutics. 2005; 106: 233–266.

[13] Jin S, Liu Z, Xiang P, Fu M, Zhang G, Li J, *et al*. Activation of the cGMP/PKG/ERK signaling pathway associated with PDE5Is inhibits fibroblast activation by downregulating autophagy in early progressive benign prostatic hyperplasia. World Journal of Urololgy. 2024; 42: 333.

[14] Pacheco-Torres J, Sharma RK, Mironchik Y, Wildes F, Brennen WN, Artemov D, *et al*. Prostate fibroblasts and prostate cancer associated fibroblasts exhibit different metabolic, matrix degradation and PD-L1 expression responses to hypoxia. Frontiers in Molecular Biosciences. 2024; 11: 1354076.

[15] Giri D, Ittmann M. Interleukin-8 is a paracrine inducer of fibroblast growth factor 2, a stromal and epithelial growth factor in benign prostatic hyperplasia. The American Journal of Pathology. 2001; 159: 139–147.

[16] Owen JS, Clayton A, Pearson HB. Cancer-associated fibroblast heterogeneity, activation and function: implications for prostate cancer. Biomolecules. 2022; 13: 67.

[17] Ishii K, Matsuoka I, Sasaki T, Ishii K, Matsuoka I, Sasaki T, *et al*. Loss of fibroblast-dependent androgen receptor activation in prostate cancer cells is involved in the mechanism of acquired resistance to castration. Journal of Clinical Medicine. 2019; 8: 1379.

[18] Albrecht M, Rämsch R, Köhn FM, Schwarzer JU, Mayerhofer A. Isolation and cultivation of human testicular peritubular cells: a new model for the investigation of fibrotic processes in the human testis and male infertility. The Journal of Clinical Endocrinology and Metabolism. 2006; 91: 1956–1960.

[19] Atiakshin D, Kulchenko N, Kostin A, Ignatyuk M, Protasov A, Klabukov I, *et al*. Cyto- and histopographic assessment of CPA3-positive testicular mast cells in obstructive and non-obstructive azoospermia. Cells. 2024; 13: 833.

[20] Eming SA, Wynn TA, Martin P. Inflammation and metabolism in tissue repair and regeneration. Science. 2017; 356: 1026–1030.

[21] Qin A, Shi K, Tindall RR, Li J, Cheng B, Li J, *et al*. Characterization of pancreatic collagen-expressing fibroblasts in mouse acute pancreatitis. Gastro Hep Advances. 2025; 4: 100557.

[22] Zheng B, Zhang Z, Black CM, de Crombrugghe B, Denton CP. Ligand-dependent genetic recombination in fibroblasts: a potentially powerful technique for investigating gene function in fibrosis. The American Journal of Pathology. 2002; 160: 1609–1617.

[23] Kusser KL, Randall TD. Simultaneous detection of EGFP and cell surface markers by fluorescence microscopy in lymphoid tissues. The Journal of Histochemistry and Cytochemistry. 2003; 51: 5–14.

[24] Gao X, Cao Y, Yang W, Duan C, Aronson JF, Rastellini C, *et al*. BMP2 inhibits TGF-β-induced pancreatic stellate cell activation and extracellular matrix formation. American Journal of Physiology-Gastrointestinal and Liver Physiology. 2013; 304: G804–G813.

[25] Garcia PE, Scales MK, Allen BL, di Magliano MP. Pancreatic fibroblast heterogeneity: from development to cancer. Cells. 2020; 9: 2464.

[26] Middendorff R, Müller D, Mewe M, Mukhopadhyay AK, Holstein AF, Davidoff MS. The tunica albuginea of the human testis is characterized by complex contraction and relaxation activities regulated by cyclic GMP. The Journal of Clinical Endocrinology and Metabolism. 2002; 87: 3486–3499.

[27] Aguado-Alvaro LP, Garitano N, Abizanda G, Larequi E, Prosper F, Pelacho B. Comparative evaluation of inducible Cre mouse models for fibroblast targeting in the healthy and infarcted myocardium. Biomedicines. 2022; 10: 2350.

[28] Ramirez F, Tanaka S, Bou-Gharios G. Transcriptional regulation of the human α2(I) collagen gene (COL1A2), an informative model system to study fibrotic diseases. Matrix Biology. 2006; 25: 365–372.

[29] Gundogdu G, Nguyen T, Namasivayam A, Starek S, Gelman J, Mauney JR. Characterization of a novel rabbit model of Peyronie's disease. International Journal of Impotence Research. 2024; 36: 269–274.

[30] Mitsui Y, Yamabe F, Hori S, Uetani M, Kobayashi H, Nagao K, *et al*. Molecular mechanisms and risk factors related to the pathogenesis of Peyronie's disease. International Journal of Molecular Sciences. 2023; 24: 10133.

[31] Liu Q, Song Y, Cui Y, Hu C, Luo Y, Hu D, *et al*. Heterogeneity of fibroblasts is a hallmark of age-associated erectile dysfunction. The International Journal of Biochemistry & Cell Biology. 2023; 156: 106343.

[32] Luo C, Peng Y, Gu J, Li T, Wang Q, Qi X, *et al*. Single-cell RNA sequencing reveals critical modulators of extracellular matrix of penile cavernous cells in erectile dysfunction. Scientific Reports. 2024; 14: 5886.

[33] Kwon OJ, Zhang Y, Li Y, Wei X, Zhang L, Chen R, *et al*. Functional heterogeneity of mouse prostate stromal cells revealed by single-cell RNA-seq. iScience. 2019; 13: 328–338.

[34] Joseph DB, Henry GH, Malewska A, Reese JC, Mauck RJ, Gahan JC, *et al*. Single-cell analysis of mouse and human prostate reveals novel fibroblasts with specialized distribution and microenvironment interactions. The Journal of Pathology. 2021; 255: 141–154.

[35] Peng YC, Levine CM, Zahid S, Wilson EL, Joyner AL. Sonic hedgehog signals to multiple prostate stromal stem cells that replenish distinct stromal subtypes during regeneration. Proceedings of the National Academy of Sciences of the United States of America. 2013; 110: 20611–20616.

[36] Josson S, Matsuoka Y, Chung LWK, Zhau HE, Wang R. Tumor stroma co-evolution in prostate cancer progression and metastasis. Seminars in Cell & Developmental Biology. 2010; 21: 26–32.

[37] Park H, Park S, Kim KH, Cho MS, Sung SH, Ro JY. Stromal nodules in benign prostatic hyperplasia: morphologic and immunohistochemical characteristics: stromal nodules of prostate. The Prostate. 2014; 74: 1433–1443.

[38] Zhou R, Wu J, Liu B, Jiang Y, Chen W, Li J, *et al*. The roles and mechanisms of Leydig cells and myoid cells in regulating spermatogenesis. Cellular and Molecular Life Sciences. 2019; 76: 2681–2695.

[39] Zhang G, Sun Y, Guan M, Liu M, Sun S. Single-cell and spatial transcriptomic investigation reveals the spatiotemporal specificity of the beta-defensin gene family during mouse sperm maturation. Cell Communication and Signaling. 2024; 22: 267.

[40] Rhee H, Gunter JH, Heathcote P, Ho K, Stricker P, Corcoran NM, *et al*. Adverse effects of androgen-deprivation therapy in prostate cancer and their management. BJU International. 2015; 115: 3–13.

[41] Dohle GR, Smit M, Weber RFA. Androgens and male fertility. World Journal of Urology. 2003; 21: 341–345.

[42] Cioni B, Nevedomskaya E, Melis MHM, van Burgsteden J, Stelloo S, Hodel E, *et al*. Loss of androgen receptor signaling in prostate cancer-associated fibroblasts (CAFs) promotes CCL2- and CXCL8-mediated cancer cell migration. Molecular Oncology. 2018; 12: 1308–1323.

[43] Holterhus PM, Hiort O, Demeter J, Brown PO, Brooks JD. Differential gene-expression patterns in genital fibroblasts of normal males and 46,XY females with androgen insensitivity syndrome: evidence for early programming involving the androgen receptor. Genome Biology. 2003; 4: R37.

How to cite this article: Tung Shu, Danqing Ren, Yu-Hsuan Chien, Baibing Yang, Jiajing Li, Chunhui Liu, *et al*. Fibroblast distribution and localization in male reproductive organs: a mouse model study via lineage tracing. Revista Internacional de Andrología. 2025; 23(3): 34-44. doi: 10.22514/j.androl.2025.028.