Review

Insights on Platelet-Derived Growth Factor Receptor α-Positive Interstitial Cells in the Male Reproductive Tract

Tasuku Hiroshige [1],*, Kei-Ichiro Uemura [1], Kei-Ichiro Nakamura [2] and Tsukasa Igawa [1]

[1] Department of Urology, Kurume University School of Medicine, Kurume 830-0011, Japan
[2] Cognitive and Molecular Research Institute of Brain Diseases, Kurume University School of Medicine, Kurume 830-0011, Japan
* Correspondence: hiroshige_tasuku@kurume-u.ac.jp; Tel.: +(81)-0942-31-7572; Fax: +(81)-0942-34-2605

Abstract: Male infertility is a significant factor in approximately half of all infertility cases and is marked by a decreased sperm count and motility. A decreased sperm count is caused by not only a decreased production of sperm but also decreased numbers successfully passing through the male reproductive tract. Smooth muscle movement may play an important role in sperm transport in the male reproductive tract; thus, understanding the mechanism of this movement is necessary to elucidate the cause of sperm transport disorder. Recent studies have highlighted the presence of platelet-derived growth factor receptor α (PDGFRα)-positive interstitial cells (PICs) in various smooth muscle organs. Although research is ongoing, PICs in the male reproductive tract may be involved in the regulation of smooth muscle movement, as they are in other smooth muscle organs. This review summarizes the findings to date on PICs in male reproductive organs. Further exploration of the structural, functional, and molecular characteristics of PICs could provide valuable insights into the pathogenesis of male infertility and potentially lead to new therapeutic approaches.

Keywords: PDGFRα; interstitial cells; three-dimensional analysis; FIB/SEM; male reproductive tracts

1. Introduction

The issue of infertility in the developed world has reached a level where it is now considered a social concern. Male infertility constitutes a significant portion, with 20–30% of all infertility cases attributed to male factors [1]. These factors encompass hormonal disorders, physical ailments, lifestyle considerations, psychological issues, sexual problems, chromosomal abnormalities, and single-gene defects [1–3]. The primary causes of male infertility are a decreased sperm count and motility. A decline in sperm count may indicate not only reduced sperm production but also impaired transport through the male reproductive tract. Currently, there are no clinical or experimental measurements of sperm transport failure, and only limited objective evidence regarding the mechanisms of sperm transport has been documented. Consequently, many patients with unexplained low sperm count (oligospermia) may experience some degree of transport failure rather than spermatogenesis.

Sperm transport entails various factors, including sperm motility, smooth muscle contractions of the male reproductive tract, and seminal fluid dynamics [4,5]. Spermatozoa within the testes exhibit minimal motility upon ejaculation into the head of the epididymis but acquire motility during transit to the tail of the epididymis [6–8], a phenomenon termed sperm maturation. While mammalian sperm become motile post-ejaculation into the female genital tract, those attaining motility after traversing the epididymis remain inhibited within the male reproductive tract. Consequently, inactive sperm necessitate transportation from the seminiferous tubules to the rete testis via the efferent duct and epididymis to the vas deferens.

The mechanisms governing sperm transport in male reproductive organs have been the subject of various theories, predominantly implicating smooth muscle movement [9–11].

Understanding smooth muscle activity in the male genitalia is crucial for elucidating sperm transport mechanisms, potentially paving the way for male infertility treatment or the development of male contraceptives.

Smooth muscles within the male reproductive tract contract rhythmically to propel sperm along the tract. Their intrinsic activity ensures sperm movement even without neural input, underscoring the redundancy and resilience of the system [12]. While the autonomic nervous system does not directly control rhythmic contractions for sperm propulsion, it does modulate and coordinate these contractions alongside other critical functions throughout the sexual response [13]. Traditionally, discussions on smooth muscle movement have centered on the nerve–smooth muscle relationship. However, in smooth muscle organs such as the gastrointestinal tract and bladder, interstitial cells may interact with nerves to regulate smooth muscle contraction, with implications for various diseases [14–18]. This review focuses on the interplay between stromal cells and smooth muscle movement in the male reproductive tract, exploring the potential of interstitial cell research in this realm.

2. Interstitial Cells Involved in Smooth Muscle Movement

Functional studies of interstitial cells in the male reproductive organs often prioritize Sertoli and Leydig cells, which are associated with spermatogenesis. However, few studies have elucidated the functional relationship between smooth muscle movement and interstitial cells in the male reproductive tract [19].

Interstitial cells of Cajal (ICCs) and platelet-derived growth factor receptor α (PDGFRα)-positive interstitial cells (PICs) are two types of interstitial cells involved in smooth muscle movement, particularly in the gastrointestinal tract and bladder [20,21]. ICCs are renowned for their role in generating pacemaker activity and neurotransmission in the gut, while PICs have been implicated in bladder function and neuromodulation [18,22,23]. Both cell types are electrically coupled to smooth muscle cells (SMCs), forming a functional syncytium that regulates smooth muscle excitability [24].

ICCs, discovered by the Spanish neuroanatomist Santiago Ramón y Cajal, are present around the intermuscular plexus and in the circular muscle layer of the gastrointestinal tract [25]. They have been identified in various parts of the gastrointestinal tract, including the esophagus, stomach, cecum, and small intestine [26,27]. ICCs are thought to act as pacemakers in intestinal peristalsis. ICCs act as electrical pacemakers and generate waves known as slow waves, exhibiting periodic potential fluctuations [28,29]. These slow waves determine the rhythm of spontaneous contractions in the digestive tract. Slow waves originate from ICCs and propagate through gap junctions to surrounding ICCs and smooth muscle cells [30,31]. These slow waves cause an increase in membrane potential, leading to the influx of calcium ions and subsequent muscle cell contraction [32]. The propagation of slow waves depends on IP3 receptors and mitochondrial Ca^{2+} uptake [33]. ICCs, functioning as pacemakers, possess unique ionic conductances that trigger slow wave activity [34,35]. The synaptic-like contacts between nerve terminals and intramuscular ICCs facilitate the rapid diffusion of transmitters, further influencing the propagation of slow waves [31]. Thus, slow waves from ICCs ultimately control the contraction and relaxation cycles of smooth muscles in the gastrointestinal tract.

ICCs express c-kit; therefore, immunohistochemistry using an anti-KIT antibody is an effective and widely used method to identify them [27,36]. It was previously thought that ICCs also exist in various smooth muscle organs, other than the gastrointestinal tract. However, many reports of ICCs other than those of the gastrointestinal tract have not been double-stained with tryptase, and it is now possible that most of these reports were mast cells in tissues expressing c-kit [37]. Therefore, PICs are believed to be the main type of interstitial cells involved in the regulation of smooth muscle movement in smooth muscle organs other than the gastrointestinal tract [37,38].

3. Immunohistological Features of PICs

The PDGFR is a type III receptor tyrosine kinase expressed in various organs, including the GI tract [39]. It consists of two subtypes, PDGFRα and PDGFRβ, and has four ligands (PDGF-A, -B, -C, and -D) [40,41]. In the developmental stage of mammals, PDGF–PDGFR signals are crucial for organogenesis, including alveogenesis, hair morphogenesis, spermatogenesis, oligodendrogenesis, palate genesis, angiogenesis, and glomerulogenesis [39,42]. In mature organs, PDGFRα is known to be distributed in several interstitial-type cells such as cardiac fibroblasts, alveolar fibroblasts, dermal fibroblasts, hepatic stellate cells, and renal mesangial cells [39].

As mentioned above, PDGFRα is not specific to PICs and is also expressed on other types of fibroblasts. Therefore, PICs cannot be identified using PDGFRα alone. PICs also express CD34 and vimentin in addition to PDGFRα [43–45]. Additionally, Dennis et al. reported that mouse bladder PICs express multiple canonical fibroblast markers, including Col1a2, CD34, LY6A, and PDGFRα, along with the ubiquitous fibroblast genes *Col15a1* and *Pi16*. These results suggest that PICs are a type of fibroblast [38]. In addition, as will be described later, PICs express small-conductance Ca(2+)-activated K(+) channel 3 (SK3), which is classified as member of a family of calcium-activated potassium channels based on functional characteristics [46,47].

Furthermore, the expression of these markers varies depending on tissue and localization. For example, interstitial cells in the upper lamina propria of the human bladder are positive for vimentin, α-smooth muscle actin, caveolin-1 and 2, PDGFRα, and non-phosphorylated and phosphorylated connexin 43 [45]. In contrast, interstitial cells in the lower lamina propria are positive for vimentin, CD34, and unphosphorylated connexin 43 but negative for α-smooth muscle actin, caveolin-1 and 2, PDGFRα, and phosphorylated connexin 43 [45]. Therefore, heterogeneous immunohistochemical characteristics across animal species and tissues make PIC identification with molecular markers difficult.

4. Morphological Features of PICs

Electron microscopy observations of PICs reveal a distinct morphology characterized by a well-developed and rough endoplasmic reticulum, often featuring extended cisternae, Golgi apparatus, and mitochondria primarily located in the perinuclear region. Notably, these cells lack caveolae and basement membranes [48]. However, the qualitative nature of their morphological features, as observed via transmission electron microscopy (TEM), presents challenges in quantitative morphological characterization due to heterogeneity influenced by the local microenvironment [49].

To address this limitation, recent morphological studies have employed three-dimensional (3D) reconstruction techniques such as serial block-face scanning electron microscopy (SBF/SEM) and focused ion beam scanning electron microscopy (FIB/SEM) [50–52]. Mantani et al. utilized SBF/SEM to demonstrate that type III fibroblast-like cells, analogous to PICs in the lamina propria of the rat ileum, exhibit thin, mesh-like cell bodies and thinly branched cell processes immediately beneath the epithelium [49]. Similarly, Takeya et al., employing FIB/SEM, illustrated that PICs in guinea pig seminal vesicles possess sheet-like cell bodies with thicknesses ranging from 0.1 to 0.5 μm, along with sheet-like broad cell processes that overlap selectively [53]. Furthermore, Neuhans et al., utilizing both SBF/SEM and FIB/SEM, reported that PICs in the lamina propria of the human bladder exhibit sheet-like cell bodies and elongated cell processes, as revealed through 3D reconstruction [54]. A consistent morphological feature across these studies is the presence of very thin, mesh- or sheet-like cell bodies, contrasting with the spindle-like structures observed in conventional TEM-based 2D morphological analyses.

5. Functional Features of PICs

Neural regulation of smooth muscle movement in the gastrointestinal tract, an organ where functional studies of PICs have been conducted, involves both excitatory and inhibitory mechanisms. Excitatory mechanisms are primarily initiated by excitatory motor

neurons releasing neurotransmitters like acetylcholine and substance P to enhance SMC excitability [55–57]. These neurotransmitters bind to muscarinic type 3 receptors and neurokinin 1 receptors expressed by ICCs [58,59]. Gq-coupled plasma membrane receptors activate phospholipase C (PLC), leading to the production of IP3 and diacylglycerol, which in turn trigger Ca^{2+} release and activate protein kinase C [60]. Diacylglycerol further amplifies this process by enhancing IP3 production through positive feedback on PLC activity [61]. The IP3-induced Ca^{2+} release is mediated by IP3 receptors (IP3Rs) in the endoplasmic reticulum (ER) [62]. The intense release of Ca^{2+} activates anoctamin 1 channels in the plasma membrane, coupled with IP3Rs [63], resulting in a depolarizing response that conducts to SMCs via gap junctions, ultimately leading to smooth muscle contraction.

In contrast, inhibitory mechanisms are mediated by inhibitory motor neurons through the release of neurotransmitters such as NO, ATP, β-nicotinamide adenine dinucleotide (β-NAD), and other purines [64–66]. ATP or β-NAD released from purinergic nerve terminals initially binds to P2Y1 receptors on PICs [47], which couple to G-protein Gq/11, activating PLC to increase intracellular IP3 [67]. IP3 stimulates Ca^{2+} release from intracellular calcium pools, activating SK3 channels and PICs, thereby causing cell hyperpolarization [47,68]. This results in the hyperpolarization of SMCs via gap junctions, leading to smooth muscle relaxation. Deep sequencing of gene transcripts in the small bowel and colonic PICs, purified by fluorescence-activated cell sorting (FACS), has revealed that these cells also express various receptors for additional neurotransmitters, hormones, and inflammatory mediators [69]. Growing evidence suggests that PICs act as a brake on gastrointestinal motility, integrating inhibitory inputs from intrinsic and extrinsic nerves, hormones, and inflammatory mediators [70]. Not only in the gastrointestinal smooth muscle but also in the detrusor smooth muscle, purines activate SK currents primarily via P2Y1 receptors in PICs [46,68].

6. Smooth Muscle Movement in Seminiferous Tubules

Spermatozoa lack active motility within the seminiferous tubules and are thus passively transported via testicular fluid from these tubules to the caput of the epididymis through the rete testis and efferent ductules. Although previous studies have noted bulk movement of luminal content [71,72], quantitative data on sperm transport within the seminiferous tubules are lacking. Earlier findings have suggested that contractile tubule movements are mediated by smooth-muscle-like testicular peritubular myoid cells (TPCs) [73,74]. Fleck et al. demonstrated that TPC contractions induce directional sperm movement within mouse seminiferous tubules both in vitro and in vivo [9].

TPCs encompass the seminiferous tubules in the mammalian testis. They exhibit contractile function and express cytoskeletal markers typical of smooth muscle, such as alpha-isoactin and smooth muscle myosin [75,76]. ATP [9], vasopressin [77], oxytocin [78], prostaglandins [79], and endothelin [80] have been identified as signaling factors that act on TPCs. ATP secretion from both Sertoli cells and germ cells occurs in a stimulus-dependent manner [81]. This secreted ATP activates TPCs through P2X and P2Y receptors, leading to seminiferous tubule contraction. However, the detailed mechanism underlying tubule contraction remains largely unknown.

7. PICs in Seminiferous Tubules

Morphological evidence supporting the presence of PICs surrounding the seminiferous tubules has been documented in humans [82], mice [83], rats [84], and Chinese soft-shelled turtles [85]. Immunohistochemistry and double immunofluorescence analyses revealed the co-expression of CD34, while testing negative for vimentin, α-SMA, c-kit, and CD31 [82,84,85]. PICs were observed outside the layer of TPCs surrounding the seminiferous tubules, characterized by a cytoplasm and a small cell body containing mitochondria around the nucleus. Adjacent PICs exhibited physical contact and formed a network connecting the TPCs, Leydig cells, and blood vessels [82,84,85]. Additionally,

secretory vesicles were noted between PICs [82,84,85], suggesting the potential for direct cell conjugation, vesicle release, and paracrine and/or autocrine signaling.

As previously mentioned, while morphological studies on testicular PICs have been recently reported, functional studies remain absent. It remains unclear whether these cells express P2X and P2Y receptors; however, given the possibility of seminiferous tubule contraction via purinergic signaling [9,81], it is highly probable that PICs do indeed express P2X and P2Y receptors and play a role in seminiferous tubule contraction. Moreover, they may interact with Leydig and myoid cells, as well as blood vessels, potentially facilitating the transport of substances such as testosterone, crucial for spermatogenesis, to and from the seminiferous tubules.

8. Smooth Muscle Movement in Epididymal Ducts

Reabsorption of luminal fluid resulting from epithelial secretions and movements of ciliated cells may contribute to the transport of spermatozoa, but transportation mainly relies on the contractile activity of the SMC layer surrounding the epididymal epithelium [10,86,87]. However, actual sperm transport as a result of SMC contractility has only occasionally been reported.

The epididymal duct is characterized by spontaneous rhythmic contractions which decrease in frequency but increase in amplitude from head to tail, as shown in vivo [88–90] and in vitro [91–93]. There is also a report that the distal portion of the tail, which stores sperm until ejaculation, only sporadically contracts but does not spontaneously contract [94,95]. Additionally, adrenergic nerves are present in the caudal region, and it is believed that smooth muscle movement is mainly controlled by nerve input [10,96].

Spontaneous contractions in the epididymis are myogenic, suggesting the presence of pacemaking cells such as the ICC. Mewe et al. reported that cells isolated from bovine epididymal tracts exhibit three morphologically distinct contractile cell types: typical smooth muscle cells in the tail, myofibroblast-like cells along the ducts, and atypical myocytes with filaments. These cell types also demonstrated distinct biophysical properties. Atypical myocytes with filaments are speculated to provide electrical coupling between myofibroblasts, which is essential for the generation of regular myogenic activity [97].

Regarding the control of smooth muscle movement, it has been reported that not only neural input but also hormones, such as testosterone [98,99] and estrogen [100], as well as the influence of epithelial [101], sperm, and luminal factors [97], play a role. Cyclic guanosine monophosphate (cGMP) signaling plays a crucial role in the relaxation of SMCs in the epididymis [101]. This relaxation is mediated by the activation of cGMP-dependent protein kinase I and the subsequent inhibition of myosin light chain phosphorylation [102,103].

The involvement of purine signaling in the epididymis is also significant, with P2X1 and P2X2 receptors identified in the SMC layer, suggesting a role in regulating epididymal contractility and sperm ejection [104]. Additionally, the activation of P1 and P2 purinergic receptors by ATP and adenosine is proposed to play a role in luminal acidification, a crucial process for sperm maturation and storage [105].

9. PICs in Epididymis

We have identified PICs in the epididymis of mice (Figure 1) [106]. They are classified into two subtypes, each possessing distinct immunohistochemical properties. The first subtype, located just beneath the epithelium, was CD34(−) and was observed from the corpus to the cauda but not in the initial segment (IS). The second subtype, CD34(+), was observed in the interstitial space, including the muscle layers of all segments. Furthermore, the density of CD34(+) PICs increased from the IS to the tail to match the thickness of the smooth muscle layer. Nerve fibers in the epididymis closely associated with CD34(+) PICs, which were observed both within and outside the smooth muscle layer. Therefore, CD34(+) PICs are presumed to interact with nerves, while CD34(−) PICs beneath the epididymal epithelium may be involved in transmitting information from the epithelium to the smooth muscle.
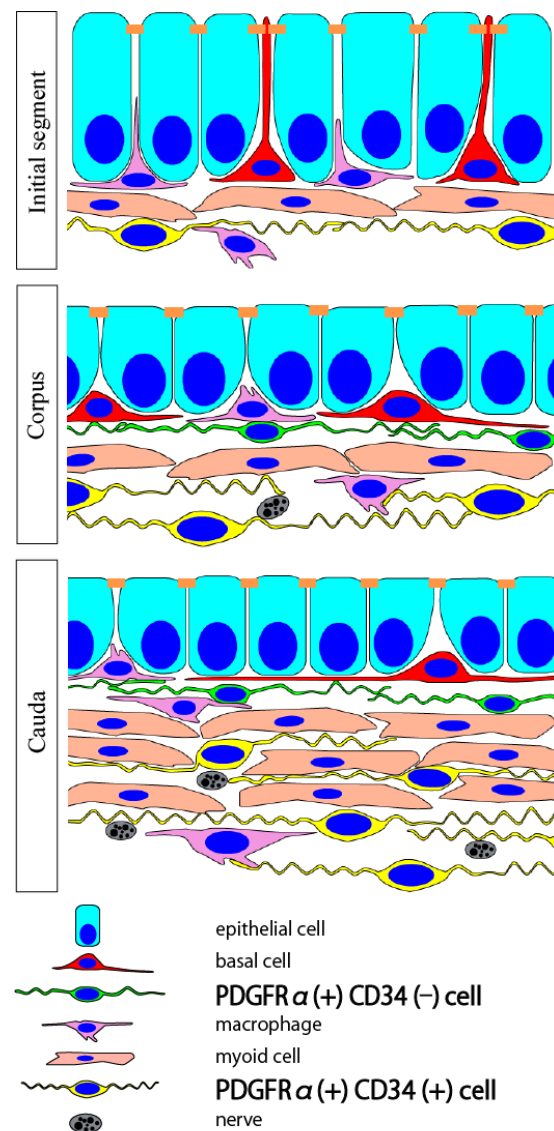
Figure 1. Schematic model of PIC subtypes present in the interstitial space of the murine epididymis. In the initial segment, 1–2 layers of CD34(+) PICs are present. In the other segments, the number of CD34(+) PIC layers increases toward the cauda. In the cauda, CD34(+) PICs are present between smooth muscle bundles. CD34(−) PICs are present beneath the epithelium in the other segments but not in the initial segment. PIC; PDGFRα-positive interstitial cell. Reprinted from [106] with permission of Elsevier, GmbH (Amsterdam, The Netherlands).

TEM analysis revealed that the PICs in the murine epididymis lacked a basement membrane, exhibited a small amount of perinuclear cytoplasm, and displayed elongated cell processes (Figure 2) [106]. These ultrastructural characteristics closely resembled those of PICs previously described in other organs. However, no apparent differences were observed in the two-dimensional ultrastructure of the two PIC subtypes distinguishable by immunohistochemical features. Future research employing three-dimensional observations may uncover morphological disparities between these PIC subtypes.
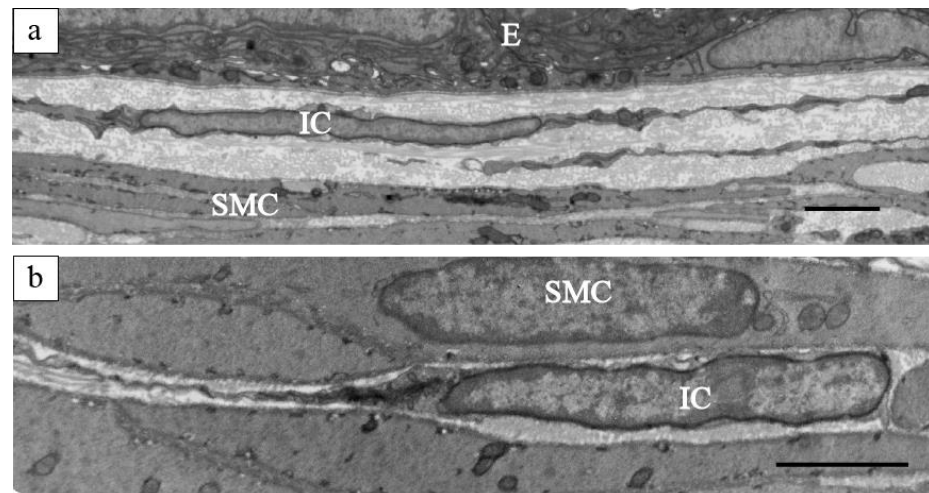
Figure 2. Ultrastructural images of PICs in the epididymal duct. (**a**) A PIC beneath the epithelium; (**b**) A PIC in the smooth muscular layer. IC, PDGFRα-positive interstitial cells; E, epithelium; SMC, smooth muscle cells. Reprinted from [106] with permission of Elsevier, GmbH. Scale bars: 10 μm (**a**), 500 nm (**b**).

Connexin 43 was expressed in PICs spanning from the corpus to the cauda. Electron microscopy unveiled an electron-dense region proximal to the PICs, indicative of a gap junction (Figure 3) [106]. This observation suggests the potential existence of electrical interconnections among PICs within the epididymis through these gap junctions. Furthermore, their adjacency to nerves and smooth muscle implies a possible role in mediating smooth muscle contraction through physical interaction within the epididymis.
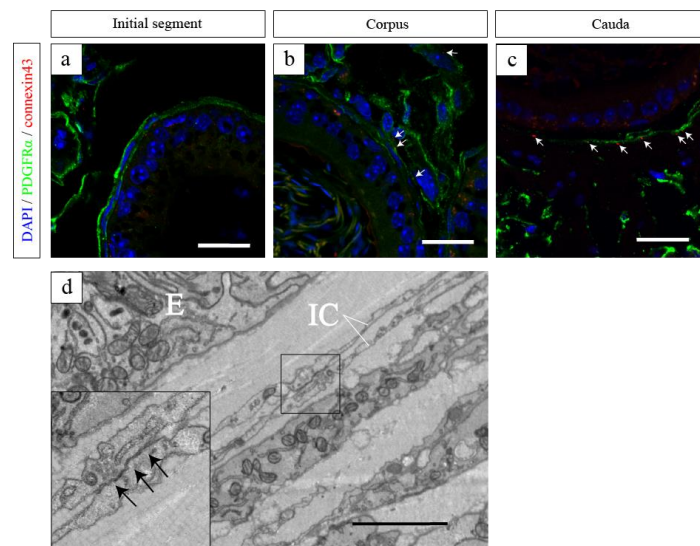
Figure 3. Representative images of the initial segment (**a**), corpus (**b**), and cauda (**c**) with double immunostaining for PDGFRα (green) and connexin 43 (red) [106]. (**a**–**c**) Nuclei were counterstained in blue with 4′,6-diamidino-2-phenylindole (DAPI). Co-localization PDGFRα and connexin 43 (white arrows of (**b**,**c**)). (**d**) Ultrastructural findings of PICs in the cauda. A close proximity area between cellular processes of PICs. High magnification of the black line square area of (**d**) (inset of (**d**)). An electron-dense region proximal to the PICs, indicative of a gap junction (black arrows, inset of (**d**)). IC, PDGFRα-positive interstitial cell; E, epithelium. Reprinted from [106] with permission of Elsevier, GmbH. Scale bars: 20 μm (**a**–**c**), 2 μm (**c**).

CD34(−) PICs situated directly beneath the epithelium were closely associated with basal cells and macrophages (Figure 4) [106]. Macrophages localized near the epididymal duct are hypothesized to play dual roles: maintaining the integrity of the epithelial barrier and facilitating peripheral tolerance to auto-antigenic spermatozoa during maturation [107,108]. Simultaneously, they may also engage in immune surveillance, combating and monitoring pathogens that pose constant threats to the reproductive tract. Additionally, basal cells within the epididymis have been identified as luminal sensors regulating the activities of principal and clear cells, while also exhibiting scavenger functions [109,110]. Consequently, CD34(−) PICs may participate in various epididymal functions beyond sperm transport, including immunoregulation or epithelial activities.
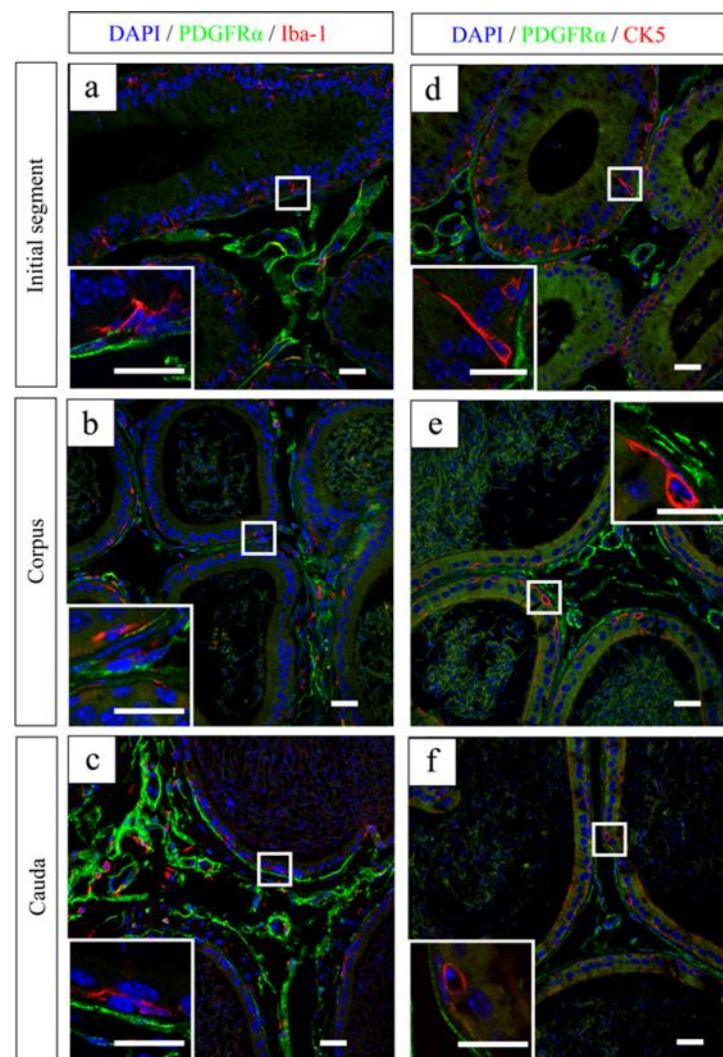
Figure 4. Representative images of the initial segment (**a**), corpus (**b**), and cauda (**c**) with double immunostaining for PDGFRα (green) and ionized calcium-binding adaptor molecule 1 (Iba-1; red). Nuclei were counterstained in blue with 4′,6-diamidino-2-phenylindole (DAPI). Representative images of the initial segment (**d**), corpus (**e**), and cauda (**f**) with double immunostaining for PDGFRα (green) and cytokeratin 5 (CK5; red) [106]. (**a**–**f**) Insets in (**a**–**f**): Higher magnification of the white square areas in (**a**–**f**). Reprinted from [106] with permission of Elsevier, GmbH. Scale bars: 20 μm.

10. Smooth Muscle Movement in the Vas Deferens

Sperm within the vas deferens are primarily transported through smooth muscle contraction. While numerous studies have investigated smooth muscle movement in the vas deferens compared to the seminiferous tubules and epididymis, relatively fewer

studies have been conducted on the latter. The smooth muscle of the vas deferens is primarily regulated by autonomic nerves, with adrenergic nerves being the predominant group of nerve fibers supplying the vas deferens in mammals [111–114]. Research across various species has identified two distinct components of neurogenic contraction in the vas deferens. The first component involves rapid phasic contractions triggered by ATP acting on P_{2X} receptors, while the second component entails slower tonic contractions induced by noradrenaline and is independent of membrane depolarization [115–118]. These findings are corroborated by studies demonstrating the role of ATP and noradrenaline as co-transmitters in the vas deferens [116,118]. The initiation of these contractions is correlated with the rate and degree of depolarization, with phasic contraction being more sensitive to a reduction in extracellular calcium [119]. The selective blockade of ATP, noradrenaline, and electrically evoked contractions by nifedipine further underscores the involvement of ATP in contractile responses [120]. In the human vas deferens, Amobi et al. demonstrated that the stimulation of P2X1 purinoceptors elicited an excitatory effect leading to longitudinal muscle contraction [121], subsequently activating 4-aminopyridine-sensitive (KV) and iberiotoxin-sensitive (BKCa) K^+ channels [122].

ATP acting on P2X1 receptors stimulates an influx of Ca^{2+} to produce excitatory junctional potentials (EJPs) that summate to depolarize the membrane sufficiently to activate VOCs [123]. The entry of Ca^{2+} then activates ryanodine receptors (RYRs) to release Ca^{2+} from the internal store. The initial release occurs proximal to the plasma membrane and subsequently propagates intracellularly through the regenerative release of Ca^{2+} by the RYRs and/or via inositol 1,4,5-trisphosphate (InsP3) receptors, manifesting as an intracellular Ca^{2+} wave [124]. The Ca^{2+} near the membrane activates large-conductance Ca^{2+}-sensitive K^+ (BK) channels and the resulting spontaneous transient outward currents hyperpolarize the membrane and help to terminate the activation process [125,126]. Noradrenaline (NA) acts by stimulating α1-adrenoreceptors to produce InsP3, which then releases Ca^{2+} that may induce an intracellular Ca^{2+} wave similar to that triggered by the ATP-dependent entry of external Ca^{2+}. In addition, the α1-adrenoreceptors also activate the smooth muscle Rho/Rho kinase signaling pathway that serves to increase the Ca^{2+} sensitivity of the contractile machinery [127].

In vivo, there is also evidence suggesting that P2X1 receptors mediate postzygotic excitatory responses to ATP in the vas deferens and play a role in male reproductive function. Vas deferens tissue from P2X1 receptor-deficient (P2X1 receptor $-/-$) mice failed to respond to exogenously applied ATP or α,β-meATP, and these tissues lacked spontaneous and evoked EJPs [128]. This genetic deletion resulted in a 90% reduction in male fertility, attributed to a decreased spermatozoa count in ejaculated semen.

11. PICs in Vas Deferens

In the murine vas deferens, PICs are widely distributed across the lamina propria, smooth muscle, and serosal layers [129]. They have been categorized into two types, similar to those found in the epididymis: PDGFRα(+) CD34($-$) PICs located just below the epithelium and PDGFRα(+) CD34(+) PICs observed within the interstitial space containing the muscle layer. PDGFRα(+) CD34($-$) PICs exhibit a gradual increase in number from the testis side of the vas deferens, characterized by its thin smooth muscle layer, toward the urethra side, which features a thicker smooth muscle layer. The escalated distribution of PICs in both the epididymis and vas deferens, relative to the thickness of the smooth muscle layer, suggests their involvement in smooth muscle movement.

The cell bodies of PICs appear relatively small, with sparse cytoplasm containing some mitochondria observed around the nuclei (Figure 5a–f). Notably, a basement membrane is absent, while several elongated cell processes are evident, facilitating proximity among PICs (Figure 5a–c). Additionally, a few caveolae are discernible in the cytoplasm (inset of Figure 5d). Connexin 43 expression is notable in PICs, with gap-junction-like structures observed via electron microscopy (Figure 6f,g). Morphologically, PICs within the vas deferens exhibit electrical connections with each other and are closely situated near nerves

and smooth muscles (Figure 6c,e). Furthermore, PICs within the lamina propria are in close proximity not only to nerves and smooth muscle but also to the epithelium (Figure 6a,d), blood vessels (Figure 6d), and macrophages (Figure 6b).
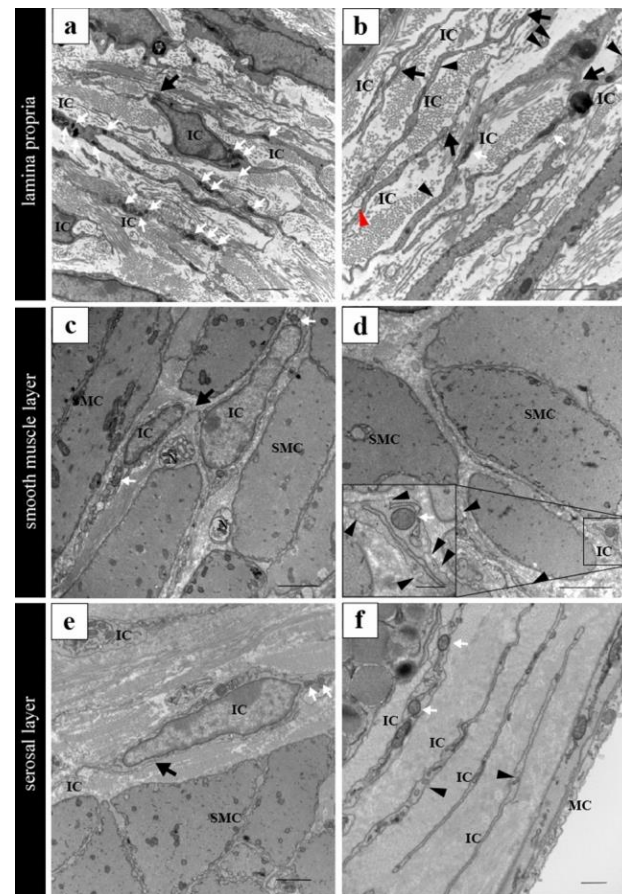

Figure 5. Ultrastructural findings in PICs of murine vas deferens. (**a**) PICs in the lamina propria of the vas deferens. (**b**) Cell processes of PICs in the lamina propria. Exosome-like structure between cell processes of PICs (red arrowhead). (**c**) PICs in the smooth muscle layer of the vas deferens. (**d**) Cell processes of PICs in the smooth muscle layer. High magnification of the solid black line square area (inset at the lower left corner of (**d**)). (**e**) PICs in the serosal layer of the vas deferens. (**f**) Cell processes of ICs in the serosal layer. PICs' cell bodies are relatively small, with sparse cytoplasm containing several mitochondria surrounding the nucleus (white arrows). PICs were in close proximity to each other (black arrows). Caveolae in the cytoplasm of PICs in all the layers (black arrowheads). IC, PDGFRα-positive interstitial cells; SMC, smooth muscle cells; MC, mesothelial cells. Images shown are taken from a paper [129]. Scale bars: 2 μm (**a**–**e**); 500 nm ((**f**), inset at the lower left corner of (**d**)).

A 3D reconstruction of PICs in the vas deferens, performed using FIB/SEM, revealed that PICs possess sheet-like structures rather than spindle-like structures, consistent with previous three-dimensional analyses of PICs in other organs (Figure 7a,b) [130,131]. In other words, the findings observed by TEM suggest a cross-section of a sheet-like cell body of PICs. Furthermore, the 3D reconstruction of PICs using FIB/SEM highlighted morphological differences between PICs in the lamina propria and those in the smooth muscle layer.
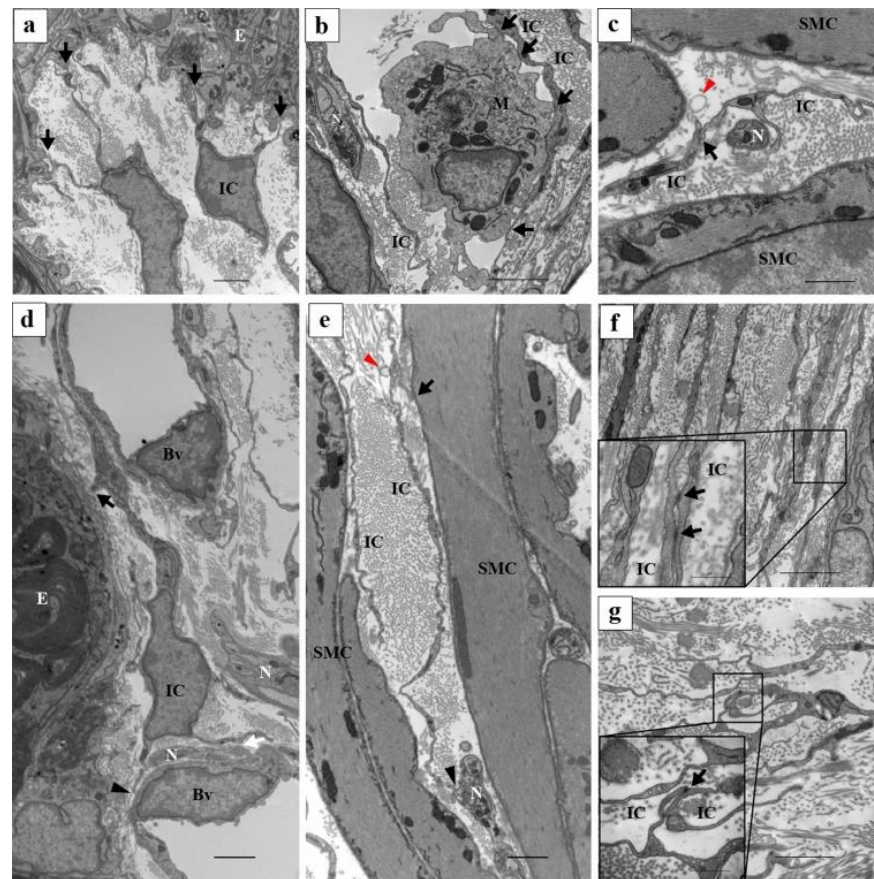
Figure 6. Ultrastructural findings showing the relationship between PICs and surrounding cells in murine vas deferens. (**a**) The cell processes of the ICs were in close proximity to the epithelium (black arrows). (**b**) A cell process of the IC was in close proximity to a macrophage (black arrows). A cell process of the IC wraps nerves and is in close proximity to the cell process of the other IC (black arrows). (**c**) Exosome-like structure near the cell process of the IC (red arrowhead). (**d**) The ICs in the lamina propria were in close proximity to surrounding tissues. The IC in the lamina propria was in close proximity to the epithelial cell (black arrow), a vascular endothelial cell (black arrowhead), and a nerve (white arrow). (**e**) The IC in the smooth muscle layer was in close proximity to the surrounding tissues. The IC in the smooth muscle layer was in close proximity to a smooth muscle cell (black arrow) and a nerve (black arrowhead). Exosome-like structure near the cell processes of the IC (red arrowhead). (**f**,**g**) TEM images of contact areas between ICs. Higher magnification of the solid black line square area (inset at the lower left corner of (**f**,**g**)). Electron-dense lines exist in the contact area between ICs (black arrows). ICs, interstitial cells; E, epithelium; N, nerves; Bv, blood vessels; M, macrophages. Images shown are taken from a paper [129]. Scale bars: 2 μm (**a**–**g**); 500 μm (inset at the lower left corner of (**f**,**g**)).

PICs in the smooth muscle layer had two types of 3D sheet-like structures: flat and curled (Figure 8a). Various spatial relationships have been observed between PICs, neurons (Figure 8b–d), and smooth muscle cells (Figure 8e–g), which form a complex 3D. In addition, many extracellular vesicle-like structures were observed around PICs. Since the sheet-like structure increases cell surface area and is a very effective structure for the delivery of extracellular vesicles and for physical interactions and exchange of humoral factors by such vesicles, PICs may be involved in neuromuscular signal transduction (Figure 8h).
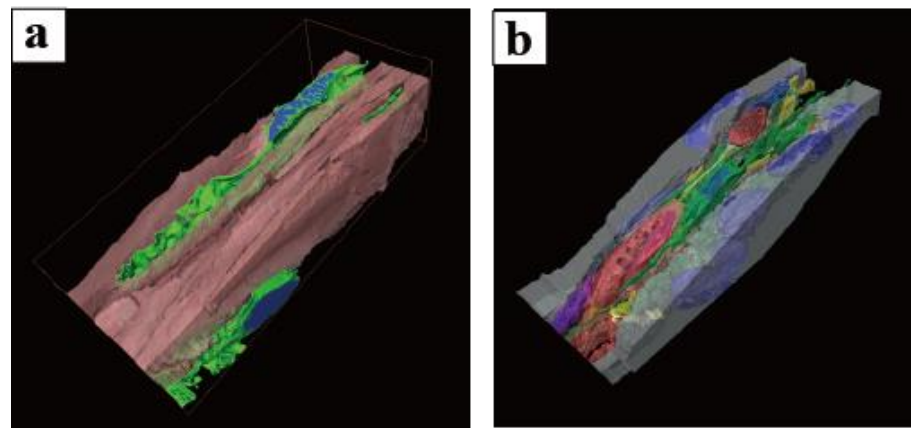
Figure 7. High-magnification images within the murine vas deferens for which a series of sequential images were acquired using FIB/SEM: (**a**) smooth muscle layer; (**b**) lamina propria. The object surfaces are color-coded: PDGFRα-positive interstitial cells, light green; nuclei, blue; smooth muscle cell, coral; epithelium, grey; vessels, red; nerves, yellow. The same images following deconvolution and 3D reconstruction using the Avizo software (version 9.1.1) are available at https://www.fei.com/software/avizo3d/ (last accessed date: 2 March 2024). Reprinted from [130,131] with permission of Elsevier, GmbH.
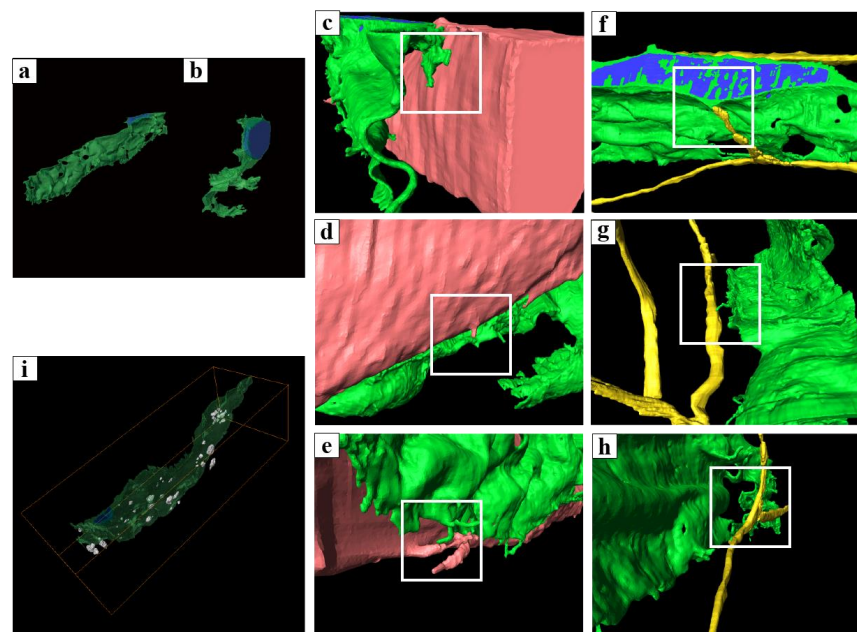
Figure 8. 3D reconstructed image of PICs in the smooth muscle layer and 3D reconstruction of the interfaces between surrounding tissue and PICs. (**a**) Stereoscopic 3D reconstructed image of the flattened, sheet-like PICs. (**b**) Stereoscopic 3D reconstructed image of the curled, sheet-like PICs. (**c**–**e**) 3D reconstruction of the interfaces between smooth muscle cells and PICs (white squares). (**f**–**h**) 3D reconstruction of the interfaces between nerves and PICs (white squares). (**h**) 3D reconstruction of a PIC and the exosome-like structures around it. Object surfaces are color-coded: PICs, light green (**a**–**h**), transparent green (**i**); nuclei, blue; smooth muscle cell, coral; nerves, yellow; exosome-like structures, grey. The same images following deconvolution and 3D reconstruction using the Avizo software (version 9.1.1) are available at https://www.fei.com/software/avizo3d/ (last accessed date: 2 March 2024). Reprinted from [130] with permission of Elsevier, GmbH.

The PICs in the lamina propria exhibited a flat, sheet-like structure with cytoplasm and multiple cellular processes (Figure 9a). Additionally, two types of 3D structures comprising

cell processes were observed: a rod-shaped structure (Figure 9b) and an accordion-fold-like one (Figure 9c). PICs were positioned parallel to the epithelium (Figure 9d) and were interconnected through gap junctions or adherens junctions (Figure 9e–g). Notably, PICs in the lamina propria were folded akin to a screen between the epithelial basement membrane and the smooth muscle, suggesting their potential involvement in regulating smooth muscle movement by the epithelium.
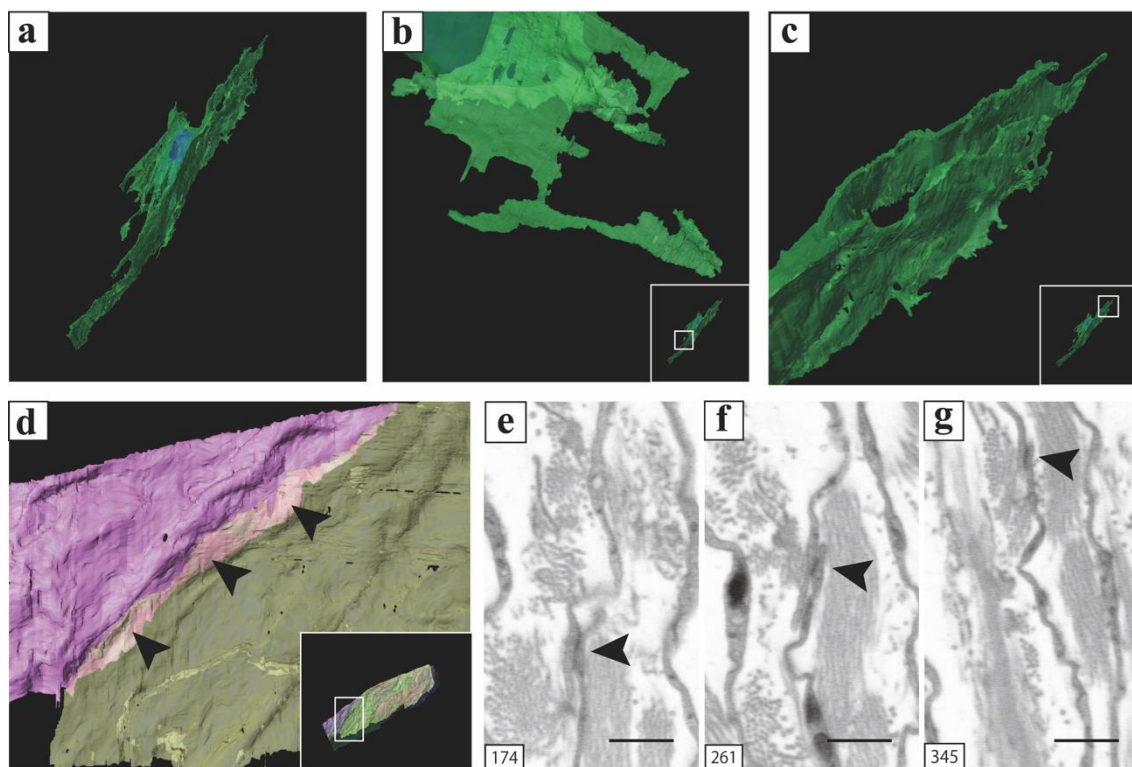
Figure 9. 3D reconstructed image of PICs in the lamina propria and 3D reconstruction of the interfaces between PICs [131]. (**a**) 3D reconstructed image of a flattened, sheet-like PIC with multiple cellular processes. (**b**) Higher magnification of the flattened, sheet PIC processes. (**c**) Higher magnification of the elongated, rod-shaped PIC processes. (**d**) Higher magnification of the white square area in the inset showing the 3D reconstruction of interfaces between PICs (black arrows). (**e–g**) 2D digital slices including the areas indicated by black arrowheads in (**d**) extracted from the sequential images. High electron density was observed in the areas indicated by black arrows in (**d**) (black arrows in (**e–g**)). Images shown are taken from a paper [131].

12. Conclusions

To date, numerous morphological studies on interstitial cells have been reported. These studies represent the initial step in elucidating their functional roles, given that the characteristic morphology of these cells may influence smooth muscle movement. The morphological investigations of PICs in male reproductive organs offer intriguing possibilities, indicating that PICs might participate in sperm transport mechanisms within the male reproductive tract. Further functional research on PICs in the male reproductive tract holds the potential for a breakthrough in ejaculatory disorders, for which effective therapeutic strategies have not yet been established.

Author Contributions: T.H. revised the manuscript. K.-I.U., K.-I.N. and T.I. contributed to the data interpretation and revised the manuscript. All authors reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

References

1. Babakhanzadeh, E.; Nazari, M.; Ghasemifar, S.; Khodadadian, A. Some of the Factors Involved in Male Infertility: A Prospective Review. *Int. J. Gen. Med.* **2020**, *13*, 29–41. [CrossRef]
2. Irvine, D.S. Epidemiology and aetiology of male infertility. *Hum. Reprod.* **1998**, *13*, 33–44. [CrossRef] [PubMed]
3. Mahat, R.K.; Arora, M.; Bhale, D.V.; Holkar, S.; Kumar, S.; Yadav, T. Risk Factors and Causes of Male Infertility-A Review. *Biochem. Anal. Biochem.* **2016**, *5*, 1–5.
4. Luconi, M.; Forti, G.; Baldi, E. Pathophysiology of sperm motility. *Front. Biosci. Landmark* **2006**, *11*, 1433–1447. [CrossRef] [PubMed]
5. Ellis, L.C.; Farr, G.C.; Tesi, R.J. Contractility of seminiferous tubules as related to sperm transport in the male. *Arch. Androl.* **1981**, *6*, 283–294. [CrossRef] [PubMed]
6. Soler, C.; Yeung, C.H.; Cooper, T.G. Development of sperm motility patterns in the murine epididymis. *Int. J. Androl.* **1994**, *17*, 271–278. [CrossRef] [PubMed]
7. Axnér, E.; Linde-Forsberg, C.; Einarsson, S. Morphology and motility of spermatozoa from different regions of the epididymal duct in the domestic cat. *Theriogenology* **1999**, *52*, 767–778. [CrossRef] [PubMed]
8. Yeung, C.-H.; Cooper, T.G. Acquisition and Development of Sperm Motility Upon Maturation in the Epididymis. In *The Epididymis: From Molecules to Clinical Practice: A Comprehensive Survey of the Efferent Ducts, the Epididymis and the Vas Deferens*; Springer: Cham, Switzerland, 2002; pp. 417–434.
9. Fleck, D.; Kenzler, L.; Mundt, N.; Strauch, M.; Uesaka, N.; Moosmann, R.; Bruentgens, F.; Missel, A.; Mayerhofer, A.; Merhof, D.; et al. ATP activation of peritubular cells drives testicular sperm transport. *Elife* **2021**, *10*, e62885. [CrossRef]
10. Elfgen, V.; Mietens, A.; Mewe, M.; Hau, T.; Middendorff, R. Contractility of the epididymal duct: Function, regulation and potential drug effects. *Reproduction* **2018**, *156*, R125–R141. [CrossRef]
11. Koslov, D.S.; Andersson, K.E. Physiological and pharmacological aspects of the vas deferens-an update. *Front. Pharmacol.* **2013**, *4*, 101. [CrossRef]
12. Klinge, E.; Sjöstrand, N. Smooth muscle of the male reproductive tract. In *Pharmacology of Smooth Muscle*; Springer: Berlin/Heidelberg, Germany, 1994; pp. 533–573.
13. Courtois, F.; Carrier, S.; Charvier, K.; Guertin, P.A.; Journel, N.M. The control of male sexual responses. *Curr. Pharm. Des.* **2013**, *19*, 4341–4356. [CrossRef]
14. Vanderwinden, J.-M. Role of Interstitial Cells of Cajal and their relationship with the enteric nervous system. *Eur. J. Morphol.* **1999**, *37*, 250–256. [CrossRef]
15. Hirst, G.D.; Ward, S.M. Interstitial cells: Involvement in rhythmicity and neural control of gut smooth muscle. *J. Physiol.* **2003**, *550*, 337–346. [CrossRef]
16. Brading, A.F.; McCloskey, K.D. Mechanisms of Disease: Specialized interstitial cells of the urinary tract—An assessment of current knowledge. *Nat. Clin. Pract. Urol.* **2005**, *2*, 546–554. [CrossRef]
17. Hashitani, H. Interaction between interstitial cells and smooth muscles in the lower urinary tract and penis. *J. Physiol.* **2006**, *576*, 707–714. [CrossRef]
18. Sanders, K.M.; Ward, S.M.; Koh, S.D. Interstitial cells: Regulators of smooth muscle function. *Physiol. Rev.* **2014**, *94*, 859–907. [CrossRef]
19. Heinrich, A.; DeFalco, T. Essential roles of interstitial cells in testicular development and function. *Andrology* **2020**, *8*, 903–914. [CrossRef]
20. Sanders, K.M.; Ward, S.M. Interstitial cells of Cajal: A new perspective on smooth muscle function. *J. Physiol.* **2006**, *576*, 721–726. [CrossRef]
21. Ward, S.M.; Sanders, K.M.; Hirst, G.D. Role of interstitial cells of Cajal in neural control of gastrointestinal smooth muscles. *Neurogastroenterol. Motil.* **2004**, *16*, 112–117. [CrossRef]
22. Koh, B.H.; Roy, R.; Hollywood, M.A.; Thornbury, K.D.; McHale, N.G.; Sergeant, G.P.; Hatton, W.J.; Ward, S.M.; Sanders, K.M.; Koh, S.D. Platelet-derived growth factor receptor-α cells in mouse urinary bladder: A new class of interstitial cells. *J. Cell Mol. Med.* **2012**, *16*, 691–700. [CrossRef]
23. Rumessen, J.J.; Vanderwinden, J.M. Interstitial cells in the musculature of the gastrointestinal tract: Cajal and beyond. *Int. Rev. Cytol.* **2003**, *229*, 115–208.
24. Blair, P.J.; Rhee, P.L.; Sanders, K.M.; Ward, S.M. The significance of interstitial cells in neurogastroenterology. *J. Neurogastroenterol. Motil.* **2014**, *20*, 294–317. [CrossRef]
25. Daniel, E.; Berezin, I. Interstitial cells of Cajal: Are they major players in control of gastrointestinal motility? *Neurogastroenterol. Motil.* **1992**, *4*, 1–24. [CrossRef]
26. Burns, A.J.; Herbert, T.M.; Ward, S.M.; Sanders, K.M. Interstitial cells of Cajal in the guinea-pig gastrointestinal tract as revealed by c-Kit immunohistochemistry. *Cell Tissue Res.* **1997**, *290*, 11–20. [CrossRef]

27. Hudson, N.P.; Pearson, G.T.; Kitamura, N.; Mayhew, I.G. An immunohistochemical study of interstitial cells of Cajal (ICC) in the equine gastrointestinal tract. *Res. Vet. Sci.* **1999**, *66*, 265–271. [CrossRef]

28. Horowitz, B.; Ward, S.M.; Sanders, K.M. Cellular and molecular basis for electrical rhythmicity in gastrointestinal muscles. *Annu. Rev. Physiol.* **1999**, *61*, 19–43. [CrossRef]

29. Lee, J.C.; Thuneberg, L.; Berezin, I.; Huizinga, J.D. Generation of slow waves in membrane potential is an intrinsic property of interstitial cells of Cajal. *Am. J. Physiol.* **1999**, *277*, G409-23. [CrossRef]

30. Sanders, K.M.; Koh, S.D.; Ward, S.M. Interstitial cells of cajal as pacemakers in the gastrointestinal tract. *Annu. Rev. Physiol.* **2006**, *68*, 307–343. [CrossRef]

31. Barajas-Lopez, C.; Berezin, I.; Daniel, E.E.; Huizinga, J.D. Pacemaker activity recorded in interstitial cells of Cajal of the gastrointestinal tract. *Am. J. Physiol.* **1989**, *257*, C830–C835. [CrossRef] [PubMed]

32. Torihashi, S.; Fujimoto, T.; Trost, C.; Nakayama, S. Calcium oscillation linked to pacemaking of interstitial cells of Cajal: Requirement of calcium influx and localization of TRP4 in caveolae. *J. Biol. Chem.* **2002**, *277*, 19191–19197. [CrossRef] [PubMed]

33. Sanders, K.M.; Koh, S.D.; Ördög, T.; Ward, S.M. Ionic conductances involved in generation and propagation of electrical slow waves in phasic gastrointestinal muscles. *Neurogastroenterol. Motil.* **2004**, *16*, 100–105. [CrossRef]

34. Evans, E.D.; Mangel, A.W. Depolarization-stimulated contractility of gastrointestinal smooth muscle in calcium-free solution: A review. *ISRN Gastroenterol.* **2011**, *2011*, 692528. [CrossRef] [PubMed]

35. Ward, S.M.; Baker, S.A.; De Faoite, A.; Sanders, K.M. Propagation of slow waves requires IP3 receptors and mitochondrial Ca2+ uptake in canine colonic muscles. *J Physiol* **2003**, *549*, 207–218. [CrossRef] [PubMed]

36. Rømert, P.; Mikkelsen, H.B. c-kit immunoreactive interstitial cells of Cajal in the human small and large intestine. *Histochem. Cell Biol.* **1998**, *109*, 195–202. [CrossRef] [PubMed]

37. Gevaert, T.; Ridder, D.D.; Vanstreels, E.; Daelemans, D.; Everaerts, W.; Aa, F.V.D.; Pintelon, I.; Timmermans, J.P.; Roskams, T.; Steiner, C.; et al. The stem cell growth factor receptor KIT is not expressed on interstitial cells in bladder. *J. Cell Mol. Med.* **2017**, *21*, 1206–1216. [CrossRef]

38. Clayton, D.R.; Ruiz, W.G.; Dalghi, M.G.; Montalbetti, N.; Carattino, M.D.; Apodaca, G. Studies of ultrastructure, gene expression, and marker analysis reveal that mouse bladder PDGFRA. *Am. J. Physiol. Renal. Physiol.* **2022**, *323*, F299–F321. [CrossRef]

39. Andrae, J.; Gallini, R.; Betsholtz, C. Role of platelet-derived growth factors in physiology and medicine. *Genes Dev.* **2008**, *22*, 1276–1312. [CrossRef]

40. Bergsten, E.; Uutela, M.; Li, X.; Pietras, K.; Östman, A.; Heldin, C.H.; Alitalo, K.; Eriksson, U. PDGF-D is a specific, protease-activated ligand for the PDGF beta-receptor. *Nat. Cell Biol.* **2001**, *3*, 512–516. [CrossRef] [PubMed]

41. Fredriksson, L.; Li, H.; Eriksson, U. The PDGF family: Four gene products form five dimeric isoforms. *Cytokine Growth Factor Rev.* **2004**, *15*, 197–204. [CrossRef]

42. Hoch, R.V.; Soriano, P. Roles of PDGF in animal development. *Development* **2003**, *130*, 4769–4784. [CrossRef]

43. Iino, S.; Horiguchi, K.; Horiguchi, S.; Nojyo, Y. c-Kit-negative fibroblast-like cells express platelet-derived growth factor receptor alpha in the murine gastrointestinal musculature. *Histochem. Cell Biol.* **2009**, *131*, 691–702. [CrossRef]

44. Monaghan, K.P.; Johnston, L.; McCloskey, K.D. Identification of PDGFRα positive populations of interstitial cells in human and guinea pig bladders. *J. Urol.* **2012**, *188*, 639–647. [CrossRef]

45. Gevaert, T.; Vanstreels, E.; Daelemans, D.; Franken, J.; Van Der Aa, F.; Roskams, T.; De Ridder, D. Identification of different phenotypes of interstitial cells in the upper and deep lamina propria of the human bladder dome. *J. Urol.* **2014**, *192*, 1555–1563. [CrossRef]

46. Lee, H.; Koh, B.H.; Peri, L.E.; Sanders, K.M.; Koh, S.D. Functional expression of SK channels in murine detrusor PDGFR+ cells. *J. Physiol.* **2013**, *591*, 503–513. [CrossRef]

47. Kurahashi, M.; Mutafova-Yambolieva, V.; Koh, S.D.; Sanders, K.M. Platelet-derived growth factor receptor-α-positive cells and not smooth muscle cells mediate purinergic hyperpolarization in murine colonic muscles. *Am. J. Physiol. Cell Physiol.* **2014**, *307*, C561-70. [CrossRef]

48. Horiguchi, K.; Komuro, T. Ultrastructural observations of fibroblast-like cells forming gap junctions in the W/W(nu) mouse small intestine. *J. Auton. Nerv. Syst.* **2000**, *80*, 142–147. [CrossRef]

49. Mantani, Y.; Haruta, T.; Nishida, M.; Yokoyama, T.; Hoshi, N.; Kitagawa, H. Three-dimensional analysis of fibroblast-like cells in the lamina propria of the rat ileum using serial block-face scanning electron microscopy. *J. Vet. Med. Sci.* **2019**, *81*, 454–465. [CrossRef]

50. Schneider, P.; Meier, M.; Wepf, R.; Müller, R. Serial FIB/SEM imaging for quantitative 3D assessment of the osteocyte lacuno-canalicular network. *Bone* **2011**, *49*, 304–311. [CrossRef]

51. Guérin, C.J.; Kremer, A.; Borghgraef, P.; Shih, A.Y.; Lippens, S. Combining serial block face and focused ion beam scanning electron microscopy for 3D studies of rare events. *Methods Cell Biol.* **2019**, *152*, 87–101.

52. Smith, D.; Starborg, T. Serial block face scanning electron microscopy in cell biology: Applications and technology. *Tissue Cell* **2019**, *57*, 111–122. [CrossRef]

53. Takeya, M.; Hashitani, H.; Hayashi, T.; Higashi, R.; Nakamura, K.I.; Takano, M. Role of mucosa in generating spontaneous activity in the guinea pig seminal vesicle. *J. Physiol.* **2017**, *595*, 4803–4821. [CrossRef]

54. Neuhaus, J.; Schröppel, B.; Dass, M.; Zimmermann, H.; Wolburg, H.; Fallier-Becker, P.; Gevaert, T.; Burkhardt, C.J.; Do, H.M.; Stolzenburg, J.U. 3D-electron microscopic characterization of interstitial cells in the human bladder upper lamina propria. *Neurourol. Urodyn.* **2018**, *37*, 89–98. [CrossRef]

55. Stanfield, P.R.; Nakajima, Y.; Yamaguchi, K. Substance P raises neuronal membrane excitability by reducing inward rectification. *Nature* **1985**, *315*, 498–501. [CrossRef]

56. Benham, C.D.; Bolton, T.B.; Lang, R.J. Acetylcholine activates an inward current in single mammalian smooth muscle cells. *Nature* **1985**, *316*, 345–347. [CrossRef]

57. Wiesenfeld-Hallin, Z. Substance P and somatostatin modulate spinal cord excitability via physiologically different sensory pathways. *Brain Res.* **1986**, *372*, 172–175. [CrossRef]

58. Faussone-Pellegrini, M.S. Relationships between neurokinin receptor-expressing interstitial cells of Cajal and tachykininergic nerves in the gut. *J. Cell. Mol. Med.* **2006**, *10*, 20–32. [CrossRef]

59. Zhu, M.H.; Sung, I.K.; Zheng, H.; Sung, T.S.; Britton, F.C.; O'Driscoll, K.; Koh, S.D.; Sanders, K.M. Muscarinic activation of Ca2+-activated Cl- current in interstitial cells of Cajal. *J. Physiol.* **2011**, *589*, 4565–4582. [CrossRef]

60. Dickson, E.J.; Falkenburger, B.H.; Hille, B. Quantitative properties and receptor reserve of the IP(3) and calcium branch of G(q)-coupled receptor signaling. *J. Gen. Physiol.* **2013**, *141*, 521–535. [CrossRef]

61. Hisatsune, C.; Nakamura, K.; Kuroda, Y.; Nakamura, T.; Mikoshiba, K. Amplification of Ca^{2+} signaling by diacylglycerol-mediated inositol 1,4,5-trisphosphate production. *J. Biol. Chem.* **2005**, *280*, 11723–11730. [CrossRef]

62. Thillaiappan, N.B.; Chakraborty, P.; Hasan, G.; Taylor, C.W. IP3 receptors and Ca^{2+} entry. *Biochim. Biophys. Acta Mol. Cell Res.* **2019**, *1866*, 1092–1100. [CrossRef]

63. Jin, X.; Shah, S.; Liu, Y.; Zhang, H.; Lees, M.; Fu, Z.; Lippiat, J.D.; Beech, D.J.; Sivaprasadarao, A.; Baldwin, S.A.; et al. Activation of the Cl- channel ANO1 by localized calcium signals in nociceptive sensory neurons requires coupling with the IP3 receptor. *Sci. Signal.* **2013**, *6*, ra73. [CrossRef]

64. Gerevich, Z.; Borvendeg, S.J.; Schröder, W.; Franke, H.; Wirkner, K.; Nörenberg, W.; Fürst, S.; Gillen, C.; Illes, P. Inhibition of N-type voltage-activated calcium channels in rat dorsal root ganglion neurons by P2Y receptors is a possible mechanism of ADP-induced analgesia. *J. Neurosci.* **2004**, *24*, 797–807. [CrossRef]

65. Mutafova-Yambolieva, V.N.; Hwang, S.J.; Hao, X.; Chen, H.; Zhu, M.X.; Wood, J.D.; Ward, S.M.; Sanders, K.M. Beta-nicotinamide adenine dinucleotide is an inhibitory neurotransmitter in visceral smooth muscle. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 16359–16364. [CrossRef]

66. Sperlágh, B.; Heinrich, A.; Csölle, C. P2 receptor-mediated modulation of neurotransmitter release-an update. *Purinergic Signal* **2007**, *3*, 269–284. [CrossRef]

67. Erb, L.; Weisman, G.A. Coupling of P2Y receptors to G proteins and other signaling pathways. *Wiley Interdiscip. Rev. Membr. Transp. Signal.* **2012**, *1*, 789–803. [CrossRef]

68. Lee, H.; Koh, B.H.; Peri, L.E.; Sanders, K.M.; Koh, S.D. Purinergic inhibitory regulation of murine detrusor muscles mediated by PDGFRα+ interstitial cells. *J. Physiol.* **2014**, *592*, 1283–1293. [CrossRef]

69. Kurahashi, M.; Baker, S.A.; Kito, Y.; Bartlett, A.; Hara, M.; Takeyama, H.; Hashitani, H.; Sanders, K.M. PDGFRα+ Interstitial Cells are Effector Cells of PACAP Signaling in Mouse and Human Colon. *Cell Mol. Gastroenterol. Hepatol.* **2022**, *14*, 357–373. [CrossRef]

70. Jiménez, M. Platelet-derived growth factor receptor-α-positive cells: New players in nerve-mediated purinergic responses in the colon. *J. Physiol.* **2015**, *593*, 1765–1766. [CrossRef]

71. Setchell, B.P.; Davies, R.V.; Gladwell, R.T.; Hinton, B.T.; Main, S.J.; Pilsworth, L.; Waites, G.M.H. The movement of fluid in the seminiferous tubules and rete testis. *Annal. Biol. Anim. Biochim. Biophys.* **1978**, *18*, 623–632. [CrossRef]

72. Worley, R.T.S.; Leendertz, J.A. A videomicrographic low-frequency movement analyser (VLMA) and perifusion chamber for recording and analysis of the physical behaviour of seminiferous tubules and other contractile tissues in vitro. *J. Microsc.* **1988**, *151*, 61–69. [CrossRef]

73. Clermont, Y. Contractile elements in the limiting membrane of the seminiferous tubules of the rat. *Exp. Cell Res.* **1958**, *15*, 438–440. [CrossRef]

74. Ross, M.H. The fine structure and development of the peritubular contractile cell component in the seminiferous tubules of the mouse. *Am. J. Anat.* **1967**, *121*, 523–557. [CrossRef]

75. Maekawa, M.; Kamimura, K.; Nagano, T. Peritubular myoid cells in the testis: Their structure and function. *Arch. Histol. Cytol.* **1996**, *59*, 1–13. [CrossRef]

76. Losinno, A.D.; Morales, A.; Fernández, D.; Lopez, L.A. Peritubular myoid cells from rat seminiferous tubules contain actin and myosin filaments distributed in two independent layers. *Biol. Reprod.* **2012**, *86*, 150. [CrossRef]

77. Pickering, B.T.; Birkett, S.D.; Guldenaar, S.E.; Nicholson, H.D.; Worley, R.T.; Yavachev, L. Oxytocin in the testis: What, where, and why? *Ann. N. Y. Acad. Sci.* **1989**, *564*, 198–209. [CrossRef]

78. Worley, R.; Nicholson, H.D.; Pickering, B.T. Testicular oxytocin: An initiator of seminiferous tubule movement. *Recent Prog. Cell. Endocrinol. Testis* **1985**, 205–212.

79. Hargrove, J.; Seeley, R.; Ellis, L. Rabbit testicular contractions: Bimodal interaction of prostaglandin E1 with other agonists. *Am. J. Physiol. Leg. Content* **1975**, *228*, 810–814. [CrossRef]

80. Filippini, A.; Tripiciano, A.; Palombi, F.; Teti, A.; Paniccia, R.; Stefanini, M.; Ziparo, E. Rat testicular myoid cells respond to endothelin: Characterization of binding and signal transduction pathway. *Endocrinology* **1993**, *133*, 1789–1796. [CrossRef]

81. Gelain, D.P.; de Souza, L.F.; Bernard, E.A. Extracellular purines from cells of seminiferous tubules. *Mol. Cell Biochem.* **2003**, *245*, 1–9. [CrossRef]

82. Marini, M.; Rosa, I.; Guasti, D.; Gacci, M.; Sgambati, E.; Ibba-Manneschi, L.; Manetti, M. Reappraising the microscopic anatomy of human testis: Identification of telocyte networks in the peritubular and intertubular stromal space. *Sci. Rep.* **2018**, *8*, 14780. [CrossRef]

83. Pawlicki, P.; Hejmej, A.; Milon, A.; Lustofin, K.; Płachno, B.J.; Tworzydlo, W.; Gorowska-Wojtowicz, E.; Pawlicka, B.; Kotula-Balak, M.; Bilinska, B. Telocytes in the mouse testicular interstitium: Implications of G-protein-coupled estrogen receptor (GPER) and estrogen-related receptor (ERR) in the regulation of mouse testicular interstitial cells. *Protoplasma* **2019**, *256*, 393–408. [CrossRef]

84. Liu, Y.; Liang, Y.; Wang, S.; Tarique, I.; Vistro, W.A.; Zhang, H.; Haseeb, A.; Gandahi, N.S.; Iqbal, A.; An, T.; et al. Identification and characterization of telocytes in rat testis. *Aging* **2019**, *11*, 5757–5768. [CrossRef]

85. Yang, P.; Ahmad, N.; Hunag, Y.; Ullah, S.; Zhang, Q.; Waqas, Y.; Liu, Y.; Li, Q.; Hu, L.; Chen, Q. Telocytes: Novel interstitial cells present in the testis parenchyma of the Chinese soft-shelled turtle Pelodiscus sinensis. *J. Cell Mol. Med.* **2015**, *19*, 2888–2899. [CrossRef]

86. Mietens, A.; Tasch, S.; Stammler, A.; Konrad, L.; Feuerstacke, C.; Middendorff, R. Time-lapse imaging as a tool to investigate contractility of the epididymal duct—Effects of cGMP signaling. *PLoS ONE* **2014**, *9*, e92603. [CrossRef]

87. Robaire, B.; Hinton, B.T. The Epididymis. In *Knobil and Neill's Physiology of Reproduction*, 4th ed.; Elsevier: New York, NY, USA, 2015; pp. 691–772.

88. Muratori, G.; Contro, S. Observations on the movements of the canal of the epididymis. *Boll. Soc. Ital. Biol. Sper.* **1951**, *27*, 538–539.

89. Knight, T.W. A qualitative study of factors affecting the contractions of the epididymis and ductus deferens of the ram. *J. Reprod. Fertil.* **1974**, *40*, 19–29. [CrossRef]

90. Hib, J. Effects of autonomic drugs on epididymal contractions. *Fertil. Steril.* **1976**, *27*, 951–956. [CrossRef]

91. Hib, J.; Caldeyro-Barcia, R. Neurohormonal control of epididymal contractions. *Basic Life Sci.* **1974**, *4*, 111–126.

92. Markkula-Viitanen, M.; Nikkanen, V.; Talo, A. Electrical activity and intraluminal pressure of the cauda epididymidis of the rat. *J. Reprod. Fertil.* **1979**, *57*, 431–435. [CrossRef]

93. Talo, A.; Jaakkola, U.M.; Markkula-Viitanen, M. Spontaneous electrical activity of the rat epididymis in vitro. *J. Reprod. Fertil.* **1979**, *57*, 423–429. [CrossRef]

94. van de Velde, R.L.; Risley, P.L. The origin and development of smooth muscle and contractility in the ductus epididymidis of the rat. *J. Embryol. Exp. Morphol.* **1963**, *11*, 369–382. [CrossRef]

95. Mitsui, R.; Hashitani, H.; Lang, R.J.; van Helden, D.F. Mechanisms underlying spontaneous phasic contractions and sympathetic control of smooth muscle in the rat caudal epididymis. *Pflug. Arch.* **2021**, *473*, 1925–1938. [CrossRef]

96. Nouhouayi, Y.; Négulesco, I. Adrenergic innervation of the smooth muscle cells of the cauda epididymis of the mouse. *Acta Anat.* **1985**, *121*, 59–62. [CrossRef]

97. Mewe, M.; Bauer, C.K.; Schwarz, J.R.; Middendorff, R. Mechanisms regulating spontaneous contractions in the bovine epididymal duct. *Biol. Reprod.* **2006**, *75*, 651–659. [CrossRef]

98. Din-Udom, A.; Sujarit, S.; Pholpramool, C. Short-term effect of androgen deprivation on intraluminal pressure and contractility of the rat epididymis. *J. Reprod. Fertil.* **1985**, *73*, 405–410. [CrossRef]

99. da Silva e Souza, M.C.; Gimeno, M.F.; Gimeno, A.L. Spontaneous and testosterone-induced motility of isolated guinea-pig cauda epididymis. *Experientia* **1974**, *30*, 1063–1065. [CrossRef]

100. Fibbi, B.; Filippi, S.; Morelli, A.; Vignozzi, L.; Silvestrini, E.; Chavalmane, A.; De Vita, G.; Marini, M.; Gacci, M.; Manieri, C.; et al. Estrogens regulate humans and rabbit epididymal contractility through the RhoA/Rho-kinase pathway. *J. Sex. Med.* **2009**, *6*, 2173–2186. [CrossRef]

101. Mewe, M.; Bauer, C.K.; Müller, D.; Middendorff, R. Regulation of spontaneous contractile activity in the bovine epididymal duct by cyclic guanosine 5′-monophosphate-dependent pathways. *Endocrinology* **2006**, *147*, 2051–2062. [CrossRef]

102. Chuang, A.T.; Strauss, J.D.; Steers, W.D.; Murphy, R.A. cGMP mediates corpus cavernosum smooth muscle relaxation with altered cross-bridge function. *Life Sci.* **1998**, *63*, 185–194. [CrossRef]

103. Hofmann, F.; Wegener, J.W. cGMP-dependent protein kinases (cGK). *Methods Mol. Biol.* **2013**, *1020*, 17–50.

104. Shariatmadari, R.; Sipilä, P.; Vierula, M.; Törnquist, K.; Huhtaniemi, I.; Poutanen, M. Adenosine triphosphate induces Ca2+ signal in epithelial cells of the mouse caput epididymis through activation of P2X and P2Y purinergic receptors. *Biol. Reprod.* **2003**, *68*, 1185–1192. [CrossRef]

105. Belleannée, C.; Da Silva, N.; Shum, W.W.; Brown, D.; Breton, S. Role of purinergic signaling pathways in V-ATPase recruitment to apical membrane of acidifying epididymal clear cells. *Am. J. Physiol. Cell Physiol.* **2010**, *298*, C817-30. [CrossRef]

106. Hiroshige, T.; Uemura, K.I.; Hirashima, S.; Hino, K.; Togo, A.; Ohta, K.; Igawa, T.; Nakamura, K.I. Morphological analysis of interstitial cells in murine epididymis using light microscopy and transmission electron microscopy. *Acta Histochem.* **2021**, *123*, 151761. [CrossRef]

107. Da Silva, N.; Smith, T.B. Exploring the role of mononuclear phagocytes in the epididymis. *Asian J. Androl.* **2015**, *17*, 591–596. [CrossRef]

108. Breton, S.; Nair, A.V.; Battistone, M.A. Epithelial dynamics in the epididymis: Role in the maturation, protection, and storage of spermatozoa. *Andrology* **2019**, *7*, 631–643. [CrossRef]

109. Leung, G.P.H.; Cheung, K.H.; Leung, C.T.; Tsang, M.W.; Wong, P.Y.D. Regulation of epididymal principal cell functions by basal cells: Role of transient receptor potential (Trp) proteins and cyclooxygenase-1 (COX-1). *Mol. Cell Endocrinol.* **2004**, *216*, 5–13. [CrossRef]

110. Arrighi, S. Are the basal cells of the mammalian epididymis still an enigma? *Reprod. Fertil. Dev.* **2014**, *26*, 1061–1071. [CrossRef]

111. Leong, S.K.; Singh, G. Innervation of the monkey vas deferens. *J. Anat.* **1990**, *171*, 93–104.

112. Kaleczyc, J. Origin and neurochemical characteristics of nerve fibres supplying the mammalian vas deferens. *Microsc. Res. Tech.* **1998**, *42*, 409–422. [CrossRef]

113. Dixon, J.S.; Jen, P.W.; Gosling, J.A. Structure and autonomic innervation of the human vas deferens: A review. *Microsc. Re.s Tech.* **1998**, *42*, 423–432. [CrossRef]

114. Kihara, K.; Sato, K.; Oshima, H. Sympathetic efferent pathways projecting to the vas deferens. *Microsc. Res. Tech.* **1998**, *42*, 398–408. [CrossRef]

115. McGrath, J.C. Adrenergic and 'non-adrenergic' components in the contractile response of the vas deferens to a single indirect stimulus. *J. Physiol.* **1978**, *283*, 23–39. [CrossRef]

116. Sneddon, P.; Westfall, D.P. Pharmacological evidence that adenosine triphosphate and noradrenaline are co-transmitters in the guinea-pig vas deferens. *J. Physiol.* **1984**, *347*, 561–580. [CrossRef]

117. Kishi, I.; Hata, F.; Takeuchi, T.; Ishii, T.; Yagasaki, O. Cooperation of ATP and norepinephrine in inducing contractile responses in guinea pig vas deferens. *Jpn. J. Pharmacol.* **1990**, *54*, 253–256. [CrossRef]

118. Sneddon, P.; Machaly, M. Regional variation in purinergic and adrenergic responses in isolated vas deferens of rat, rabbit and guinea-pig. *J. Auton. Pharmacol.* **1992**, *12*, 421–428. [CrossRef]

119. Shimodan, M.; Sunano, S. The initiation of phasic and tonic contraction by potassium and the effect of calcium, multivalent cations and Ca-antagonist on potassium contracture in guinea-pig vas deferens. *Jpn. J. Physiol.* **1981**, *31*, 15–27. [CrossRef]

120. Stone, T.W. Differential blockade of ATP, noradrenaline and electrically evoked contractions of the rat vas deferens by nifedipine. *Eur. J. Pharmacol.* **1981**, *74*, 373–376. [CrossRef]

121. Amobi, N.; Guillebaud, J.; Coker, C.; Mulvin, D.; Smith, I.C.H. Functional characterization of alpha1-adrenoceptor subtypes in longitudinal and circular muscle of human vas deferens. *Eur. J. Pharmacol.* **1999**, *367*, 291–298. [CrossRef]

122. Amobi, N.I.; Guillebaud, J.; Smith, I.C. Perspective on the role of P2X-purinoceptor activation in human vas deferens contractility. *Exp. Physiol.* **2012**, *97*, 583–602. [CrossRef]

123. Brain, K.L.; Jackson, V.M.; Trout, S.J.; Cunnane, T.C. Intermittent ATP release from nerve terminals elicits focal smooth muscle Ca2+ transients in mouse vas deferens. *J. Physiol.* **2002**, *541*, 849–862. [CrossRef]

124. Brain, K.L.; Cuprian, A.M.; Williams, D.J.; Cunnane, T.C. The sources and sequestration of Ca(2+) contributing to neuroeffector Ca(2+) transients in the mouse vas deferens. *J. Physiol.* **2003**, *553*, 627–635. [CrossRef]

125. Ohi, Y.; Yamamura, H.; Nagano, N.; Ohya, S.; Muraki, K.; Watanabe, M.; Imaizumi, Y. Local Ca(2+) transients and distribution of BK channels and ryanodine receptors in smooth muscle cells of guinea-pig vas deferens and urinary bladder. *J. Physiol.* **2001**, *534*, 313–326. [CrossRef]

126. White, C.; McGeown, J.G. Inositol 1,4,5-trisphosphate receptors modulate Ca^{2+} sparks and Ca^{2+} store content in vas deferens myocytes. *Am. J. Physiol. Cell Physiol.* **2003**, *285*, C195–204. [CrossRef]

127. Büyükafşar, K.; Levent, A.; Ark, M. Expression of Rho-kinase and its functional role in the contractile activity of the mouse vas deferens. *Br. J. Pharmacol.* **2003**, *140*, 743–749. [CrossRef]

128. Mulryan, K.; Gitterman, D.P.; Lewis, C.J.; Vial, C.; Leckie, B.J.; Cobb, A.L.; Brown, J.E.; Conley, E.C.; Buell, G.; Pritchard, C.A.; et al. Reduced vas deferens contraction and male infertility in mice lacking P2X1 receptors. *Nature* **2000**, *403*, 86–89. [CrossRef]

129. Hiroshige, T.; Uemura, K.I.; Hirashima, S.; Hino, K.; Togo, A.; Ohta, K.; Igawa, T.; Nakamura, K.I. Identification of PDGFRα-positive interstitial cells in the distal segment of the murine vas deferens. *Sci. Rep.* **2021**, *11*, 7553. [CrossRef]

130. Hiroshige, T.; Uemura, K.I.; Hirashima, S.; Hino, K.; Togo, A.; Ohta, K.; Igawa, T.; Nakamura, K.I. Three-Dimensional Analysis of Interstitial Cells in the Smooth Muscle Layer of Murine Vas Deferens Using Confocal Laser Scanning Microscopy and FIB/SEM. *Microsc. Microanal.* **2022**, *28*, 1–9. [CrossRef]

131. Hiroshige, T.; Uemura, K.I.; Hirashima, S.; Togo, A.; Ohta, K.; Nakamura, K.I.; Igawa, T. Three-dimensional analysis of interstitial cells in the lamina propria of the murine vas deferens by confocal laser scanning microscopy and FIB/SEM. *Sci. Rep.* **2022**, *12*, 9484. [CrossRef]