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Developmental switch in immunolocalization of phosphodiesterase 7A protein in testicular cells of mice, rats, and camels

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ABSTRACT

Background: Spermatogenesis is a complex process of cell differentiation preceded and accompanied by distinct gene expressions that lead to a variety of cellular and physiological changes. By degrading cyclic adenosine 3′,5′-monophosphate (cAMP), some phosphodiesterases contribute to spermatogenesis by modulating signal transduction across various physiological processes. Although phosphodiesterase 7A (PDE7A) degrades cAMP; its function in the testis as a regulator remains unclear. Furthermore, the exact cell types that express PDE7A at different stages of testicular development remain unknown.

Aim: This study aimed to study the immunohistochemical localization of the PDE7A protein throughout normal testicular morphogenesis in immature and mature stages in mice, rats, and camels.

Methods: Testes from immature and pubertal animals were examined to evaluate testicular morphology and the cellular distribution of PDE7A. Immunohistochemistry was performed using a monoclonal antibody directed against the C-terminal portion of PDE7A, and routine hematoxylin and eosin staining was used to assess overall testicular histology.

Results: In all species, PDE7A protein is successively expressed in specific spermatogenic stages of the immature testis, such as spermatogonia and primary spermatocytes, as well as in the interstitial tissue: Leydig cells, blood vessels, and myoid cells. In mature testes, the PDE7A localization was noted specifically within the round and elongated spermatids of seminiferous tubules, as well as in the endothelial cells lining the blood vessels and peripheral nerve fibers.

Conclusion: PDE7A expression in the postnatal testis is supported by new evidence in this paper, including the first description of it in camels and its first expression in peripheral nerves. Following meiosis, the enzyme is mostly expressed in germ cells and exhibits stage-specific localization. These findings may point to PDE7A's regulatory function in spermatogenesis.

Keywords: Cyclic adenosine monophosphate, Immunohistochemistry, Phosphodiesterase 7A, Spermatogenesis, Testis.

Introduction

The testis has a variety of specialized cell types that function together to perform reproductive functions, such as making male sex hormones and the gametes required for fertilization. It consists of seminiferous tubules and interstitial spaces, where spermatogenesis occurs and steroidogenesis takes place, respectively, both of which are essential for mammalian fertility. A defect in spermatogenesis can result in infertility, reproductive disorders, or cancer (Khawar *et al.*, 2019). The cyclic adenosine monophosphate (cAMP)/protein kinase A signaling pathway is one of the important molecular regulators of testicular function, responsible for regulating endocrine functions by phosphorylating target proteins to initiate cellular responses (Liu and Y, 2022; Kang *et al.*, 2023).

cAMP levels are strictly controlled by coordinated actions of adenylate cyclases, which synthesize cAMP, and phosphodiesterases (PDEs), which catalyze its hydrolysis (Yamamoto *et al.*, 2021). PDEs are the enzymes that regulate the processes of termination of cyclic nucleotide signals within cells, which are essential to cell growth, differentiation, and gene transcription (Park *et al.*, 2003). PDE 4, PDE 7, and PDE 8 are cAMP-specific families out of a total of 11 PDE families. Phosphodiesterase 7A (PDE7A) is a member of the seventh family of PDEs that have a single gene with three splice variants (PDE7A1, PDE7A2, and PDE7A3) (Szczypka, 2020). This enzyme exhibits distinct kinetic and pharmacological characteristics, attributable to its unique amino acid composition, and demonstrates a markedly higher affinity for cAMP (Km ≈ 0.02 μM) compared with

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cGMP (Km ≈ 3.9 μM) (Calamera *et al.*, 2022). PDE7A has widespread expression across the body, notably in skeletal muscle, pancreas, and kidney medulla and testes (Zuo *et al.*, 2019), as well as populations of immune and inflammatory cells (Szczypka, 2020), and at low abundance in the brain (Schröder *et al.*, 2016). Despite its wide tissue distribution, its own specific functions in the brain and testes remain poorly understood (Aftanas *et al.*, 2023). There is experimental evidence that relates PDE7A activity to aspects of cognition (Liu *et al.*, 2023) and the pathophysiology of neurodegenerative disorders (Khan *et al.*, 2022). In addition, improper regulation of PDE7A has been associated with tumor biology and has been implicated in studies of preclinical pharmacological inhibition of this enzyme to decrease cancer cell metastasis (Hao and Yu, 2017). Increased expression levels of PDE7A protein have also been found in placental trophoblast cell lines after acute and chronic air pollution exposure to particulate matter, indicating perhaps that it may be a biomarker of pollutant exposure in reproductive tissues (Familari *et al.*, 2019). To the best of our knowledge, however, no study has so far examined the potential role of PDE7A during testis development.

Determining the precise subcellular localization of PDEs is crucial, as their spatial distribution directly influences cyclic nucleotide signaling and, consequently, their specific physiological outcomes (Lorigo *et al.*, 2021). The co-existence of multiple isoforms within the same cellular compartment has been shown to enable coordinated regulation of local signaling domains, making them attractive targets for pharmacological intervention, for example, the cardiac PDE4 isoforms—PDE4A, PDE4B, and PDE4D—have been implicated in the fine-tuning of cardiac function and identified as promising therapeutic targets in heart failure (Sherstnev *et al.*, 2025). Similarly, the spatial localization of PDE7 isoforms in the testis may hold similar importance for the regulation of testicular cell functions and associated reproductive disorders.

Although transcriptomic profiling has demonstrated moderate expression of PDE7B in normal human testis, with PDE7A largely undetectable, murine studies reveal high PDE7B transcript levels in spermatocytes, suggesting a role in germ cell differentiation and testicular physiology (Campolo *et al.*, 2023). Two independent studies investigated the presence of PDE7A1 mRNA in mouse testes. One study used the polymerase chain reaction) PCR((Wang *et al.*, 2000), while another, using RNase protection analysis, found weak signals (Bloom and Beavo, 1996; Miró *et al.*, 2001). Additionally, the localization of PDE7A mRNA was examined by Miró *et al.* (2001) in the mature rat testis, revealing a strong signal in the Leydig cells. However, the low or absent detection of PDE7A in transcriptomic datasets does not rule out its physiological significance, highlighting the need for further targeted studies to elucidate its presence at the protein level and thus its role in reproductive biology.

The classification of reproductive maturity is a key consideration in comparative reproductive studies, as it reflects major anatomical and functional transitions in gonadal development. In the present work, the immature category refers to animals that fall within early developmental phases, which may include the neonatal stage (immediate postnatal period), the juvenile stage (characterized by rapid somatic growth and incomplete gametogenic activity), or the prepubertal stage (preceding reproductive capability) (Plant, 2015; Robertson *et al.*, 2021). The mature category (pubertal) refers to animals that have reached sexual maturity, which may include the postpubertal stage (characterized by established spermatogenesis and endocrine regulation) or the adult stage (characterized by fully developed and stable reproductive function) (Al-Saaidi and J, 2013).

While the expression of PDEs in rodent testes has been well-described, this research addressed critical gaps in knowledge with two important novelties: (i) the first study of PDE7A protein expression, distribution, and localization during the testicular developmental stages (immature *vs.* mature); and (ii) pioneering analysis of testicular PDEs in camels, which represent reproductive adaptations that are unique and have thus far been totally unexamined in terms of PDE biology. Accordingly, we aimed to describe the spatiotemporal expression of PDE7A within mouse, rat, and camel testes in order to understand its role in testicular maturation and spermatogenesis. Overall, this comparative developmental approach provides innovative insight into the physiological roles of PDE7A and highlights potential targets for the management of male reproductive pathologies.

Materials and Methods

Study design and area

This experimental study was conducted on a total of 30 animals of three types of mammals: Albino mice, Sprague–Dawley albino rats, and camels (*Camelus dromedarius*). The animals were divided into two age groups: immature (4–7 days, 3–8 weeks, and 18–30 months in mice, rats, and camels, respectively; $n = 5$) and mature (2–6 months for mice and rats, 3–5 years in camels; $n = 5$). From each species, five immature and mature testicular samples were obtained. The study was carried out between July 2021 and September 2022 in the physiology laboratory of the faculty of veterinary medicine, University of Tripoli, Libya.

Tissue collection

The testicles of the camel were collected from the slaughterhouse and transported to the laboratory in a refrigerated box within 3 hours of slaughter. Camel age and sexual maturity were assessed using the classical criteria of Wahby (1938), which are consistent with recent theriogenology reports (Rashad *et al.*, 2022;

Ali *et al.*, 2025). Rats and mice were obtained from a veterinary faculty animal house, housed with food and water provided *ad libitum*, and euthanized via decapitation after ether anesthesia. Testis tissues from all species were then isolated and fixed in formalin.

Tissue processing

After dissection, the testes were cut into pieces measuring 5 × 5 mm and fixed at 4°C overnight with either 10% formalin or Bouin's fixative solution, as determined by the intended use of the analysis. Bouin's solution was used for sections intended for histological examination using hematoxylin and eosin (H&E) stains since it provides crisp morphological detail of the seminiferous epithelium and interstitial tissue (Waleed Aziz *et al.*, 2023). Conversely, formalin was used with specimens to be processed for immunohistochemistry (IHC) for its recommended role in Chu *et al.* (2005).

Bouin's fixed tissue was initially washed with 10% neutral buffered formalin prior to any coloration; the yellow coloration could be reduced. All tissues, including formalin-fixed tissues, were then dehydrated through a graded series of ethanol concentrations (70% to absolute) and subsequently embedded in paraffin wax (Histoplast, Shandon Scientific Ltd., Pittsburgh, USA). Sections of 4–5 µm thickness were prepared from Bouin's fixed tissue blocks and stained with H&E following standard protocols (Bancroft and Gamble, 2008). Tissue sections were evaluated according to developmental stage: sections with more than 70% of seminiferous tubules containing spermatogonia or primary/secondary spermatocytes were classified as immature. In contrast, mature sections displayed a full spermatogenic cycle, with spermatogonia developing into elongating spermatids or spermatozoa in the cauda epididymis. Cell types—including spermatogonia, round and elongated spermatids—were identified according to standardized morphological criteria described in testicular histology atlases (Russell *et al.*, 1993).

Immunohistochemistry

The primary antibody used for immunohistochemical staining to detect PDE7A proteins was the anti-PDE7A mouse monoclonal IgG2a (kappa light chain) (Santa Cruz Biotech, Santa Cruz, CA), which is specifically suggested for detecting PDE7A1 and PDE7A2 in mouse, rat, and human tissues. This antibody does not recognize the PDE7A3 isoform; therefore, the findings of the present study are restricted to PDE7A1 and PDE7A2. The immune specificity of the antibody was confirmed earlier by Bloom and Beavo (1996).

The immunohistochemical analysis (peroxidase enzyme-conjugated secondary antibody method (indirect peroxidase) was carried out according to Butler and Heidenreich (2019). Samples were sectioned, using a Leica microtome, into 5 µm slices and mounted on histological Superfrost® glass slides (Thermo Scientific, Menzel-Gläser, Braunschweig, Germany). The slides were incubated in an oven (Gallenkamp®, England) at 60°C for 60 minutes, then deparaffinized in xylene and rehydrated in a graded ethanol series.

Endogenous peroxidase activity was blocked by incubating the deparaffinized sections in 3% hydrogen peroxide in methanol for 10 minutes. The sections were then washed 2× with Phosphate Buffered Saline (PBS), and the antigens were retrieved by heating the sections for 10 minutes at 95ºC in 0·01 M citrate buffer (pH 6·0) in a steam cooker, consistent with established protocols (Shi *et al.*, 1997; Ramos-Vara, 2005). Then sections were washed in 0.05% Tween 20 (Santa Cruz Biotechnology, CA) in PBS, before being incubated for 30 minutes with UltraCruz Blocking Reagent (Santa Cruz Biotechnology, CA) to minimize non-specific binding.

Next, the sections were incubated overnight at 4°C in the blocking reagent with the diluted primary antibody, anti-PDE7A antibody (B-11), and mouse monoclonal IgG2a. Primary antibody optimization was performed at 1:50 to 1:500 dilutions, according to the manufacturer's guidelines (Santa Cruz Biotechnology), with 1:50 providing the optimal balance between strong specific staining and minimal background. Following incubation with the primary antibody, the sections were washed three times with PBS and incubated for 1 hour with a secondary antibody, mouse IgG2a binding protein conjugated to horseradish peroxidase (HRP) (m-IgG2aBP-HRP: sc-542731) (Santa Cruz Biotech, Santa Cruz, CA), diluted 1:100 in blocking reagent.

The sections were then washed three times in PBS and stained with diaminobenzidine (DAB) (0.05%) DAB in Tris/HCl, pH 7.6, and 0.03% H_2O_2 (Santa Cruz Biotechnology, CA) until a precipitate formed for a maximum of 20 minutes. The stained sections were rinsed in PBS and water and counterstained for 10 seconds in Mayer's hematoxylin (Santa Cruz Biotechnology, CA). Finally, the sections were washed for 10 minutes in running tap water and subsequently dehydrated in a graded ethanol series followed by xylene and mounted in Depex Mounting Media (Park Scientific Limited, Northampton, UK).

Photomicrography and evaluation of IHC slides

Micrographs were captured using a light microscope equipped with a digital camera (AMscope MD500, Irvine, CA, USA). Immunohistochemical staining was assessed independently by two blinded observers. The intensity and distribution of PDE7A staining were evaluated using a semi-quantitative scoring scale: − (no staining), 1+ (mild staining), 2+ (moderate staining), and 3+ (strong intense brown staining). To control for non-specific staining, (1) the primary antibody was replaced with IgGs from the same species at the same concentration, and (2) sections were incubated with DAB reagent alone to rule out endogenous peroxidase activity. To ensure the reliability of our technique, mouse and rat testes were used as internal positive controls. Previous research reports of PDE7A mRNA expression in Leydig cells of these species (Miró *et al.*, 2001). This pattern of known expression served as the

basis for validating the primary antibody. However, the monoclonal antibody used has been previously validated (Redondo *et al.*, 2012), supporting the reliability of the immunohistochemical procedure.

Ethical approval

The use of testicular tissues for this study was approved by a bioethics committee of the Libyan Biotechnology Research Center (BEC-BTRC) (permit no. BEC-BTRC 25-2022).

Results

Histological results

This study examined 30 male individuals from mice, rats, and camels. Testes were collected, and histological morphology was evaluated at both immature and mature stages. Most stained samples exhibited a normal histological architecture (Fig. 1). At 400× magnification, analysis of immature testes revealed the presence of spermatogonia, Sertoli cells, and primary and secondary spermatocytes. Figure 1A shows immature Sertoli cells arranged in a pseudo-stratified order within the seminiferous tubules and actively proliferating to enhance the growth of the testis. Some gonocytes remained centrally located within the seminiferous tubules (Fig. 1C), whereas others had migrated toward the basement membrane, differentiating into spermatogonia (Fig. 1B). In pubertal testes, seminiferous epithelium exhibited the full complement of germ cell types (Fig. 1D–F). Testicular growth progressed over time, with fully differentiated Leydig cells closely associated with enhanced spermatogenic activity (Fig. 1F). Collectively, these observations indicate that the testicular samples analyzed in this study represent two key phases of postnatal development across the three examined species.

Table 1 summarizes the positive findings for PDE7A immunoreactivity. The immunohistochemical analysis revealed that PDE7A expression was clearest in spermatogonial cells and primary spermatocytes in prepubertal testis tissues, with lower expression observed in the cytoplasm of Leydig cells. Sertoli cells, on the other hand, lacked any significant expression (Fig. 2).

In contrast, in the testis of the pubertal mammalian species, Leydig cells, myoid cells, or spermatogonia exhibited no appreciable signal, while the area of the developing acrosome of spermatids at the onset of the maturity stage displayed only modest cytoplasmic staining, as shown in the main image and inset (Fig. 3H and I). In round and elongating spermatids, the cytoplasmic expression was most prominent (Fig. 3).

However, no immunoreactivity was observed when the primary PDE7A antibody was omitted in control sections (Fig. 3E, J, and O). PDE7A immunoreactivity was detected in the endothelial lining of lymphatic vessels, venules, arteries, and veins across all examined species (Fig. 4). Notably, consistent immunolabeling was also observed in a cross-section of peripheral nerve fibers (Fig. 4C).

The general patterns of PDE7A immunoreactivity in all testicular cells were similar among all species. The intensity of PDE7A was approximately equal in all species. PDE7A was strongly expressed in the cytoplasm of round and elongated spermatids.

Discussion

This study demonstrates that the PDE7A protein is conserved and highly expressed in the testis across different mammalian species. Cellular PDE7A staining was localized in both germ cells and most interstitial cells of the testis before puberty. However, during puberty, after undergoing post-meiotic differentiation: PDE7A expression is predominantly localized in germ cells in mice, rats, and camels. Testicular PDE7A protein expression was also demonstrated in the endothelial cells lining the blood vessels and peripheral nerve fibers surrounded by Schwann cells in the testicular interstitium.

To the best of our knowledge, this study represents the first comprehensive investigation into the distribution of the PDE7A protein within testicular cells across different mammalian species. Previous studies have partially explored this topic at the mRNA level. Only faint PDE7A signals were detected in mouse testes by RNase protection assays (Bloom and Beavo, 1996), although subsequent PCR-based screening confirmed the presence of PDE7A1 in mouse testis (Wang *et al.*, 2000). Similarly, PDE7A expression in rat testis was verified using *in situ* hybridization and Reverse Transcription (RT)-PCR (Miró *et al.*, 2001), but it remained undetectable in human testis using microarray analysis (Azevedo *et al.*, 2014). These discrepancies likely reflect differences in tissue source, sample handling, detection sensitivity, or methodological approaches.

The cellular localization of PDE7A in our study was consistent with previously reported findings showing that mRNA expression was predominantly found in the testis, along with cross-species conservation validation from bioinformatics (El Osta *et al.*, 2024). The expression of PDE7A in human samples has been poorly reported in the literature, perhaps because the gene is inherently less expressed than in other mammals (Cardoso-Moreira *et al.*, 2020), high mRNA or protein turnover (Yang *et al.*, 2003), or there may be structural alterations to the PDE7A molecule that affect the ability of the antibody to bind to the target of interest. In addition, the maturation stage in tissues and their quality and fixative conditions might limit the sensitivity of the immunostaining (Libard *et al.*, 2019).

In the present study, a specific mouse monoclonal antibody was employed to detect PDE7A protein. This antibody recognizes an epitope located in the C-terminal region of PDE7A and is suitable for identifying both PDE7A1 and PDE7A2 isoforms in mice, rats, and humans. Despite the fact that no tissue is described in the scientific publications as a positive control standard

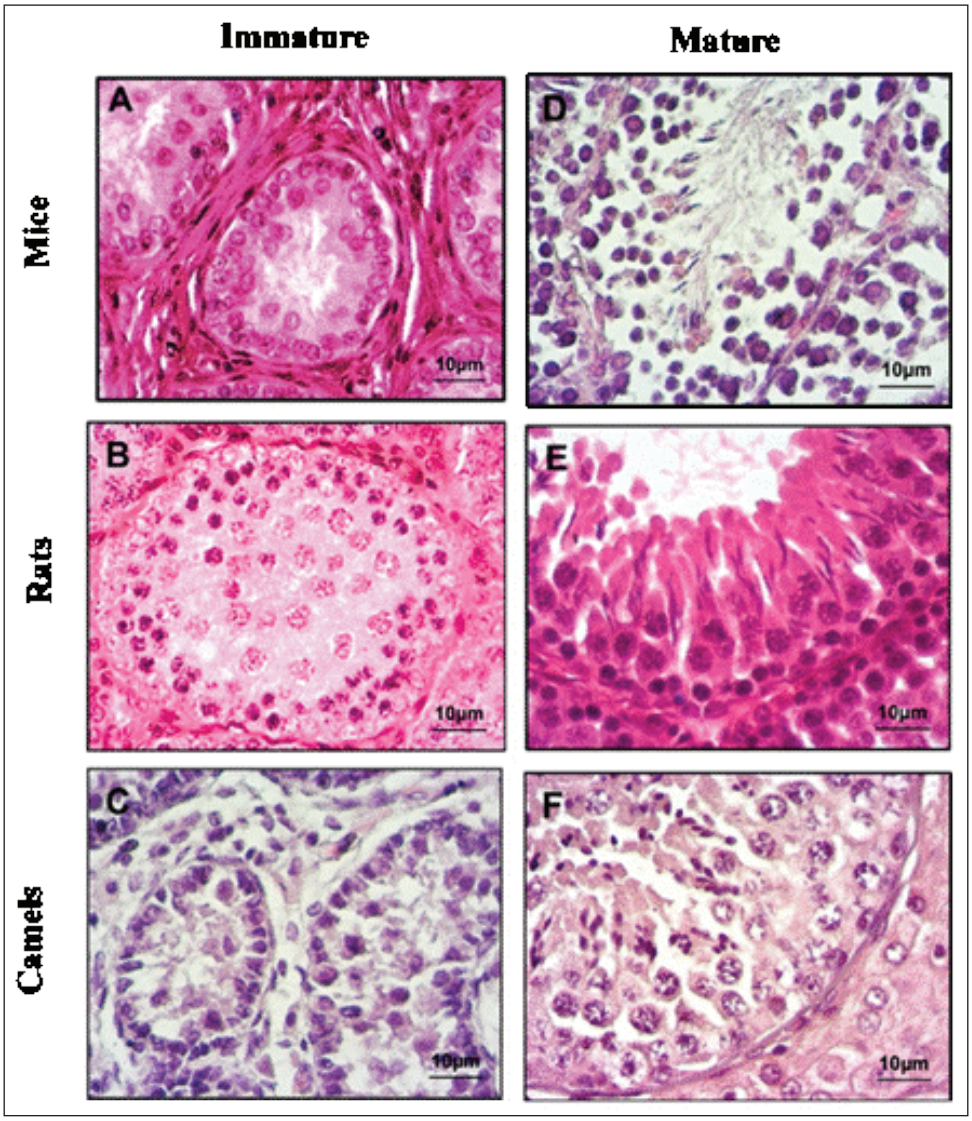

Fig. 1. Histological features of prepubertal and pubertal testes stained with H&E. Panels (A & D): mouse; (B & F): rat; (C & F): camel. (A–C) Cross-sections of prepubertal testes reveal seminiferous tubules enclosed by a basement membrane and myoid cells, lined internally with Sertoli cells, and containing stratified germinal epithelium composed of spermatogonia, spermatocytes, and gonocytes. (D–F) Sections of pubertal testes demonstrate more advanced stages of germ cell development. Both round and elongated spermatids are arranged along the luminal surface of the seminiferous epithelium, reflecting active spermatogenesis. Leydig cells are clearly visible within the interstitial connective tissue surrounding the tubules. At higher resolution, the seminiferous epithelium shows characteristic features: spermatogonia with spherical nuclei positioned on the basal membrane; spermatocytes have a distinctive chromatin pattern. Young spermatids are distinguished by their modest size, transparent cytoplasm, and spherical nuclei. The nuclei of adult spermatids are elongated and primarily located at the apex. The nuclei of Sertoli cells are triangular and have large nucleoli. Original magnification: ×400 (scale bar represents 10 µm).

for PDE7A in the testis, rat and mouse testes were used as internal positive controls based on mRNA expression data published in the literature, namely in Leydig cells (Miró *et al.*, 2001). Our protein staining matches this profile. The change in expression between pre- and post-pubertal testes not only pinpointed the age-related specificity of PDE7A expression but also demonstrated the effectiveness and specificity of the primary antibody. Moreover, the detected expression in peripheral nerve fibers of the testis, although not identified before, is anatomically in agreement with the function of PDE7A as a regulator of cAMP signaling in neurons (Miró *et al.*, 2001; Reyes-Irisarri *et al.*, 2005), and thus it can be considered as an additional indication of its location in the peripheral nervous system (PNS). Our findings are further supported by

Table 1. Testicular anatomical distribution of PDE7A immunoreactivity in mouse, rat, and camel testis.

		Immature species			Mature species		
Region	**Tissue**	**Mouse**	**Rat**	**Camel**	**Mouse**	**Rat**	**Camel**
Seminiferous tubules	Spermatogonia	3+	2+	3+	(−)	(−)	(−)
	Sertoli Cells	(−)	(−)	(−)	(−)	(−)	(−)
	Spermatocytes	3+	3+	3+	(−)	(−)	(−)
	RSs	NF	NF	NF	3+	3+	3+
	ESs	NF	NF	NF	3+	3+	3+
Interstitial tissue	Leydig Cells	3+	2+ to 3+	3+	(−)	(−)	(−)
	Myoid Cells	2+	2+	3+	(−)	(−)	(−)

RSs, Round Spermatids; ESs, Elongated Spermatids; NF, not found cells; negative (−); mild staining (1+); moderate staining (2+); marked staining (3+).

data from the Human Protein Atlas (proteinatlas.org), which consistently highlights the expression of the PDE7A protein in testicular cell types such as Leydig cells and spermatids (Atlas, 2023; GeneCards, 2023).

The incorporation of camels in this study provides a new angle since there is relatively little testicular physiology available for dromedaries. The reproductive biology of camels is distinct from other species, including seasonal breeding, the duration of spermatogenesis cycles, and may influence the histological and immunohistochemical results we obtained. Previous authors have described structural characteristics of dromedary testes, including differences in the diameter of the seminiferous tubules, the distribution of Leydig cells and developmental stages of germ cells (Elzawam *et al.*, 2025), and the investigation of PDE7A in camel testis adds further knowledge to our understanding regarding the significance of PDE7A in camel reproduction, as well as providing clarity about PDE7A regulation in other mammals.

According to our IHC tests on immature testis sections, the PDE7A protein is found in high expression within testicular germline stem cells as well as interstitial cells. This pattern of expression is similar to that of PDE1 and PDE2 in the germinal cells of rats and PDE11A in humans (Dimitriadis *et al.*, 2022). Sasaki *et al.* (2002) reported that PDE7B transcripts were abundant in rat spermatocytes. The role that cAMP signaling plays within the spermatogenic cycle is to regulate the development of germ cells (Wang *et al.*, 2022). However, the specific functions of the PDE7A protein in the prepubertal testis are not well understood. Present findings showed PDE7A protein expression in the peripheral nerve fibers surrounded by Schwann cells in the testicular interstitium. This is consistent with the presence of PDE5 expression in the peripheral nerve fibers, which have been linked to modulating signaling pathways that affect nerve function and regeneration. It also influences processes such as protein synthesis, activation of transcription factors, and Schwann cell production of neurotrophic factors (Alhamdi *et al.*, 2025). Inhibition of PDE5 has been shown to promote nerve healing by stimulating neurovascular remodeling and neurite outgrowth, as seen in diabetic neuropathy (Wang *et al.*, 2017a,b). However, our results demonstrate for the first time the presence of PDE7A in the PNS, as it was previously known to be present in regions of the central nervous system in rodents (Chen *et al.*, 2021), and its role is linked to reducing cAMP levels, which is responsible for myelination and Schwann cell differentiation (Salzer, 2015), suggesting that PDE7A influences these processes (Chen *et al.*, 2024). Therefore, further research is needed to clarify how PDE7A is expressed and functions in Schwann cells.

Cyclic nucleotides play a role in regulating vascular tone, mediating cell cycle arrest, and inhibiting smooth muscle cell proliferation (Lorigo *et al.*, 2021). When the adenylyl or guanylyl cyclase is activated by some agents; it induces relaxation of the contracted smooth muscle cAMP-dependent and cGMP-dependent protein kinases, which are targets for cAMP and cGMP action, resulting in vasorelaxation (Pasmanter *et al.*, 2025). The endothelium maintains normal vascular function (Bkaily and Jacques, 2023). The second messenger, cAMP, has been shown to reduce intracellular Ca2+ concentration in vascular smooth muscle cells (VSMCs), affecting relaxation and reducing permeability (Lincoln and Cornwell, 1991; Suzuki, 2025). Our findings highlight PDE7A expression in testicular VSMCs, aligning with earlier studies reporting PDE7 dominance in aortic smooth muscle cells of adult rats (Zhai *et al.*, 2012; Lorigo *et al.*, 2021) and human VSMCs (Miró *et al.*, 2000).

Leydig cell androgenesis is primarily regulated by cAMP signaling, with PDEs playing a crucial role (Stocco *et al.*, 2005). In Leydig cells, PDE4A, B, and D and PDE7A modulate the response to the luteinizing hormone receptor (LHR)–cAMP pathway, influencing testosterone secretion (Andric *et al.*, 2010). In our study, Leydig cells showed clear cytoplasmic immunoreactivity

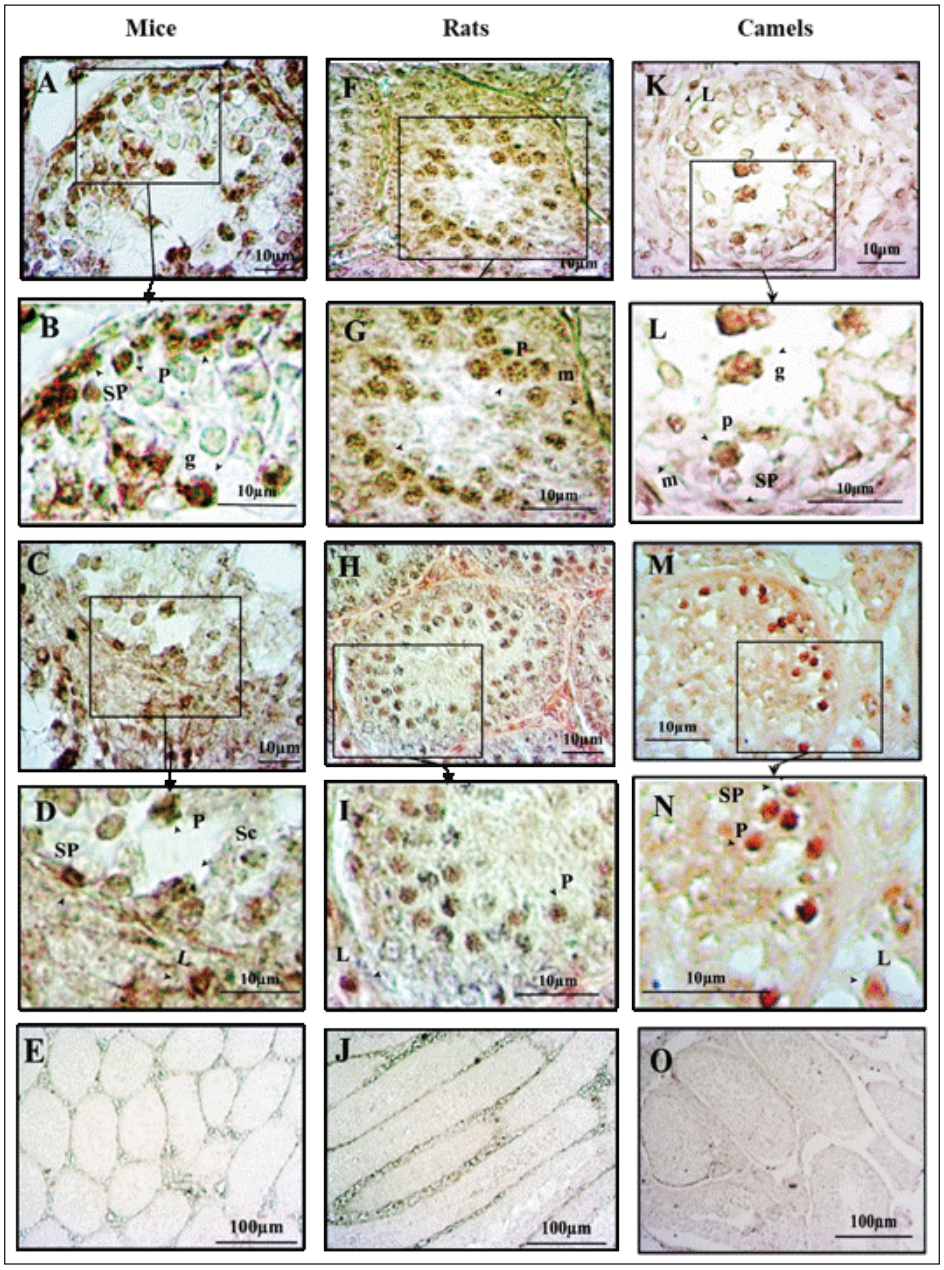

Fig. 2. Testicular expression pattern of PDE7A in premature mammalian species. Panels A–E represent seminiferous tubules from mice, F–J from rats, and K–O from camels. Positive immunoreactivity (indicated by arrowheads) is observed as a brown precipitate. (B) Higher magnification of the marked region in (A), illustrating specific staining in spermatogonia (SP), primary spermatocytes (P), and gonocytes (g) of mice. Scale bars: 10 µm for (A) and (B). (F) The inset shows a higher magnification of the boxed area in (G) to highlight the immunoreactivity in the primary spermatocytes (P), and myoid cells (m) of rats. Scale bars: 10 µm for main image and inset. (L)The inset shows a higher magnification of the boxed area in (k) to highlight the expression in the primary spermatocytes (P), and myoid cells (m), gonocytes (g), and spermatogonia (SP) of camel. Scale bars: 10 µm for main image and inset. (D) Higher magnification of the marked region in (C), illustrating specific staining in spermatogonia (SP), primary spermatocytes (P), and gonocytes (g), Leydig cells (L), and myoid cells (m) of mice. Scale bars: 10 µm for (C) and (D). (I) The inset shows a higher magnification of the boxed area in (H) to highlight the immunoreactivity in the primary spermatocytes (P), and Leydig cells of rats. Scale bars: 10 µm main image and inset. (N) Higher magnification of the marked region in (M), illustrating specific staining in spermatogonia, primary spermatocytes, and Leydig cells of the camel. Scale bars: 10 µm for (N) and (M). The identified cell types include spermatogonia (SP), primary spermatocytes (P), myoid cells (m), and Leydig cells (L). Panels E, J, and O serve as negative controls in which the primary PDE7A antibody was omitted. Overall, the distribution of PDE7A is comparable among the three species examined. Scale bars: 10 µm at ×400 magnification and 100 µm at ×100 magnification.

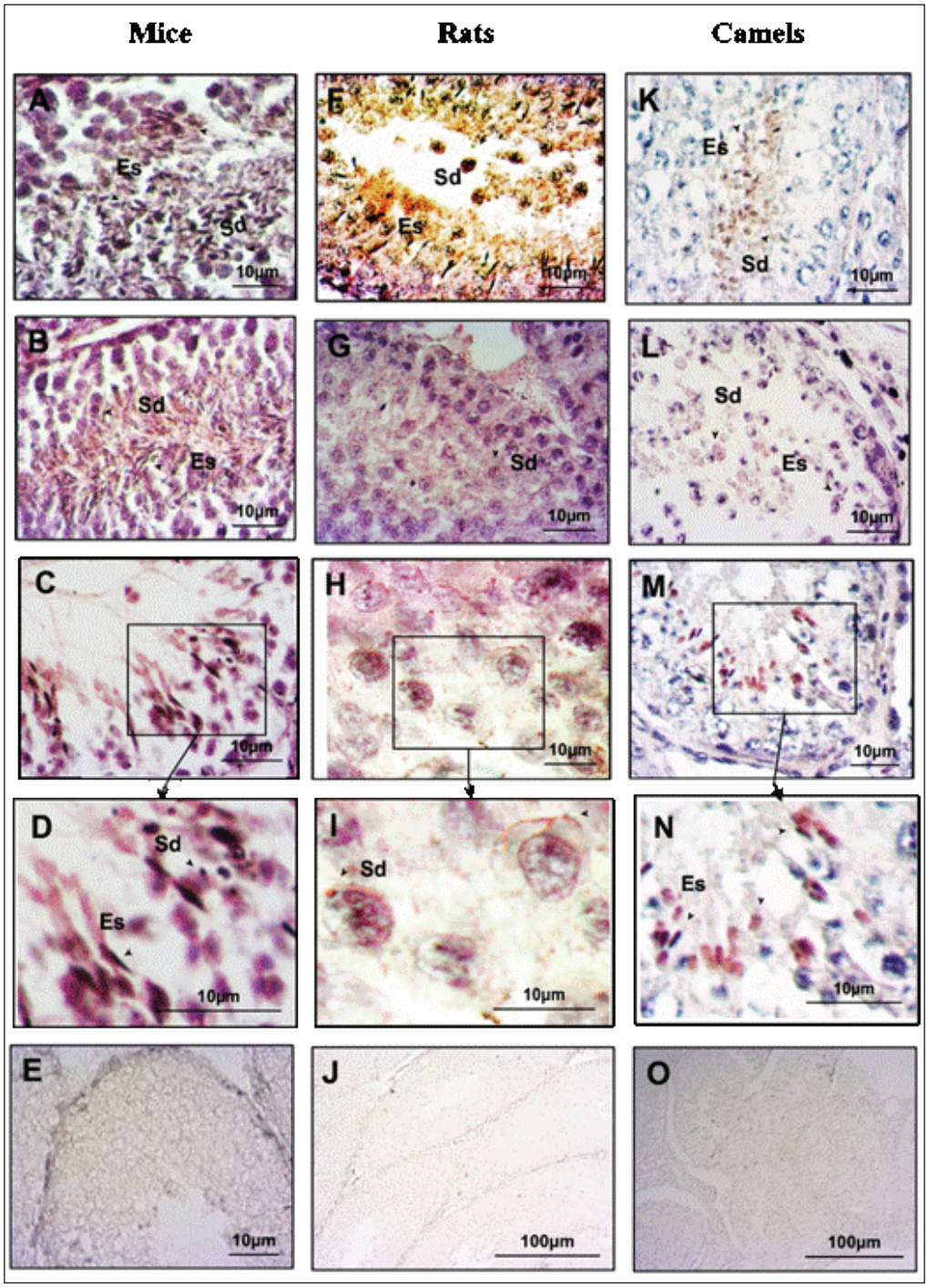

Fig. 3. Localization of PDE7A in testes of pubertal mammals. Panels A–E show seminiferous tubules from mice, F–J from rats, and K–O from camels. Positive immunoreactivity (marked by arrowheads) appears as a brown precipitate. ES indicates elongated spermatids, and Sd indicates round spermatids. Panels E, J, and O serve as negative controls, in which the primary PDE7A antibody was omitted. (D) Higher magnification of the marked region in (C), illustrating specific staining in round and elongated spermatids of mice. Scale bars: 10 µm for (C) and (D). (I) The inset shows a higher magnification of the boxed area in (H) to highlight the immunoreactivity in the area of the developing acrosome of spermatids at the onset of the maturity stage. Scale bars: 10 µm for the main image and inset. (N) Higher magnification of the marked region in (M), illustrating specific staining in round and elongated spermatids of camel. Scale bars: 10 µm for (N) and (M). All sections were processed using IHC and counterstained with Mayer's hematoxylin. Overall, PDE7A distribution is similar across the three species examined. Panels J and O are shown at ×100 magnification with a scale bar of 100 µm, while the remaining panels are at ×400 magnification with a scale bar of 10 µm.

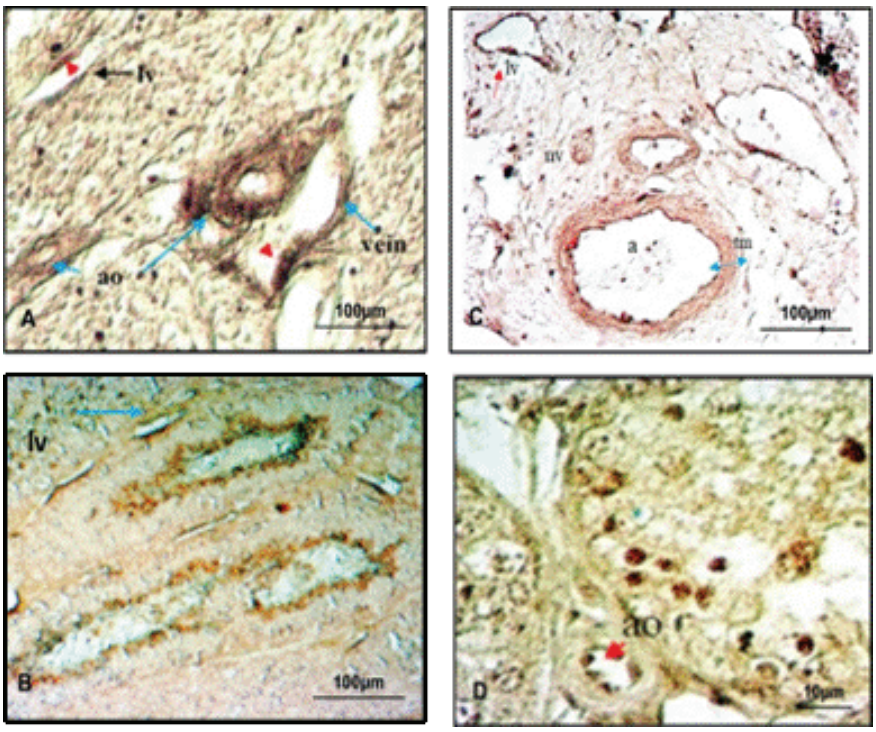

Fig. 4. Positive immunoreaction with PDE7A in endothelial cells and smooth muscle cells of the testicular blood vessels. A and B for mice testis; C and D for camels. Sections A and C are rete testis; sections B and D are seminiferous tubules. Lymphatic vessels (lv), arterioles (ao), nerve cells (nv), arteries (a), and tunica media (tm) of the artery. Endothelial cells (red arrowheads), smooth muscle cells (blue arrows). Panels A, B, and C show a scale bar of 100 µm at ×100 magnification, while Panel D shows a scale bar of 10 µm at ×400 magnification.

in prepubertal testes without postpubertal staining, indicating a consistent age dependence.

Several testicular functions depend on the activity of cAMP, including Sertoli cell function (Meroni *et al.*, 2019), Leydig cell steroidogenesis (Wang *et al.*, 2017a,b), germ cell differentiation (Bhattacharya *et al.*, 2012; Wang *et al.*, 2022), hormonal regulation (An *et al.*, 2023), and growth factor signaling (Kang *et al.*, 2022). The high expression of PDE7A that was found suggests that there is an active process of cAMP degradation going on in the prepubertal testis. However, more research needs to be done to fully understand the PDE7A regulation of cAMP in testicular development. This study also demonstrated PDE7A protein in round and elongated spermatids across mature species, consistent with the finding of PDE1A expression (Campolo *et al.*, 2023). This expression, along with its previous expression in primary spermatocytes and spermatogonia, indicates continued PDE7A activity during germ cell differentiation. At the onset of puberty, the activated form of CREM (CREMτ) is synthesized as a reaction to the stimulation of follicle-stimulating hormone and LH. These hormones attach themselves to their designated receptors located in Sertoli and Leydig cells, stimulating cAMP production and initiating transcriptional changes. Spermatids require CREMτ to undergo differentiation, which is the pivotal step in the process of spermiogenesis (Sánchez-Jasso *et al.*, 2023). Within this stage, haploid spermatids experience extensive reprogramming at the level of genes as well as cellular morphology (Meistrich, 1993; Zhang *et al.*, 2022). In the mid-phase of spermiogenesis: the nucleus of the cell elongates, the chromatin condenses, and is accompanied by the loss of the nucleosomal scaffold together with the silencing of transcription processes (Eddy, 1998; O'Donnell, 2014).

The previously reported expression pattern of PDE7A overlaps somewhat with the distribution of other testicular PDEs, suggesting that PDE7A is spatially and functionally compartmentalized within both germ and interstitial cell types in the testis, suggesting that PDE7A has unique, but partially shared, functional roles with other PDE isoforms. Furthermore, none of the investigated types of testicular cells showed nuclear or membranous localization, suggesting a very cytoplasmic distribution of PDE7A.

This trend of localization is partially coincident with reported distributions of other PDEs in the testis. PDE4 isoforms, for example, were found to localize to spermatocytes and spermatogonia with cytoplasmic and perinuclear localization (Chandrasekaran *et al.*, 2008). PDE5 was predominantly reported in the spermatid

cytoplasm (Andersson, 2018; Kaltsas *et al.*, 2025). Many cellular functions are regulated by mechanisms that control the levels of cyclic nucleotides. Cyclic nucleotides also play an important role in spermatozoal function, i.e., motility, metabolism, capacitation, and the acrosome reaction (Jin and Yang, 2017). The requirements for cAMP or cGMP in capacitation and the acrosome reaction *in vitro* varies among species, but it seems very likely that the relative amounts of each cyclic nucleotide could control these processes (Takei, 2024). Overall, the enzymatic properties of PDE7A are complex and are influenced by multiple factors, including its subcellular localization, expression level, and regulation by other signaling pathways. Further research is needed to fully understand the molecular mechanisms underlying PDE7A activity and regulation and to identify potential therapeutic targets for conditions associated with the dysregulation of PDE7A signaling.

Conclusion

This study provides the first evidence of PDE7A protein expression during testicular development postnatally, and includes characterization in camel testes. The unique expression of PDE7A in post-meiotic germ cells are an evolutionarily conserved mechanism that enables the fine-tuning of cAMP-mediated signaling pathways during spermatocyte development. Changes in PDE7A expression levels may disrupt this regulatory balance and potentially impede sperm growth, offering a scientific explanation for some forms of male infertility associated with impaired post-meiotic maturation.

PDE7A may be a promising therapeutic target in the clinical setting. Inferences into the variability of PDE7A activity may be useful for reproductive health as well as other systemic conditions in which PDE7A dysregulation is implicated, such as in cancer and neurological diseases.

Future Directions

In future studies, it will be important to further describe the molecular processes that regulate PDE7A activity and the biological role it has in different types of testicular cell types, including Leydig cells, vascular smooth muscle, and germ cells. Additional studies in model systems using targeted methods (i.e., gene knockout or knockdown model systems) will be needed to determine its biological significance in testis development, spermatid maturation, and any relevant signaling pathways.

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Conflict of interest

All authors declare that they have no conflict of interest.

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Author contributions

All authors participated in drafting and revising the manuscript, and they consented to assume responsibility for all aspects of this study.

Data availability

The majority of the images in which protein expression was demonstrated are included in this article.

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