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RESEARCH

A Study on SP-A Expression in Rat Testis during Postnatal Development

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Abstract

Background Surfactant proteins A (SP-A) is integral components of the host defense system, known for their significant antimicrobial properties and diverse immunomodulatory functions, particularly within the lung alveolus. Although SP-A is expressed in the testis, its specific roles in testicular cells, especially in relation to spermatogenesis, remain inadequately characterized.

Objective This study addresses the gap by investigating the localization and expression of SP-A in rat testis tissue at 5, 20, 50, and 70 days of postnatal development. Additionally, it examines the localization of SP-A in developing spermatid to spermatozoa within seminiferous tubules.

Materials and Methods Wistar albino rats were divided into four groups (N = 6 per group) based on distinct postnatal developmental days (PNDs): 5, 20, 50, and 70. The expression level of SP-A was estimated using Western blotting, and immunohistochemistry analysis was conducted.

Results At PND 50, SP-A immunoreactivity was present in elongating spermatids (steps 9–15) and increased at PND 70. Notably, SP-A signals in elongated spermatids (steps 16–19) at PND 50 intensified and peaked at step 16 in early-stage I-II at PND 70 and began to decrease in steps 17–19 in stages IV-XIV at PND 70. Western blot analysis of testis tissue extracts also confirmed the gradual increase in SP-A expression corresponding to postnatal development days.

Conclusion These findings suggest that SP-A may serve similar functions in rat testicular tissue as in lung alveoli, including roles in innate immunity, host defense, modulation of the immune response, regulation of inflammation, and protection against infections also may play a role in spermatogenesis and steroidogenesis.

Keywords Surfactant Protein A · Testis · Immunohistochemistry · Spermatids · Spermatozoa

1 Introduction

The mammalian testis is an immune privileged tissue coordinated by endocrine and paracrine systems. It is maintained by physical and immunological mechanisms, including the blood-testis barrier and immunosuppressive molecules secreted from resident testicular cells; Sertoli, peritubular myoid, Leydig, and macrophages. The testis exhibits effective local innate immunity against pathogens [1].

Pulmonary surfactant is a mixture primarily composed of phospholipids and proteins that cover the alveolar space. Four surfactant proteins have been identified and characterized in the lung: surfactant protein-A (SP-A), surfactant protein-B (SP-B), surfactant protein-C (SP-C), and surfactant protein-D (SP-D) [2]. Each of these proteins has distinct biological functions. SP-A and SP-D are hydrophilic, whereas SP-B and SP-C are hydrophobic. SP-A is a major hydrophilic surfactant glycoprotein with a molecular mass

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of 28–36 kDa, playing a variety of roles within the lung alveolus [3]. As a member of the C-type lectin (collectin) family, SP-A consists of collagenous carbohydrate-binding proteins that function as opsonins against pathogens through the carbohydrate recognition domain (CRD) [4]. SP-A plays a pivotal role in innate host defense by binding to pathogens via the CRD, resulting in the aggregation of bacteria and neutralization of viruses [5–12]. Additionally, SP-A facilitates the clearance of apoptotic and necrotic cells, reduces allergic responses, and promotes the resolution of inflammation [13].

Moreover, SP-A is an important pattern recognition protein in innate immunity and the regulation of inflammatory processes, enhancing phagocytosis by alveolar macrophages within the alveolar space [14, 15]. Studies using SP-A transgenic mouse models to determine the role of SP-A in host defense have demonstrated that SP-A knockout mice exhibit increased susceptibility to bacterial and viral infections [16, 17]. SP-A has an integral role in regulating innate host defense within lung.

SP-A protein is primarily present on the mucosal surface of the lung alveolus; however, it is also found in several extrapulmonary organs, including those of the male genital tract, prostate, spermatozoic secretions, spermatogonia, spermatocytes, Sertoli cells, and Leydig cells of healthy human testis [18]. It has been previously demonstrated that SP-A protein, as well as other collectin family members such as SP-D and Mannan Binding Lectin-A (MBL-A), increase in murine testis at puberty. This increase is regulated by testosterone and modulated by lipopolysaccharide (LPS), indicating a role for these proteins in infection and inflammation in the testis. [19].

It has been previously reported that SP-A is present in the midpiece, the tail, and sometimes at the equatorial region of human spermatozoa (Kankavi et al. 2008) [20]. The presence of SP-A in the male reproductive tract of humans, rodents, and stallions has been reported [18, 20, 21]. In contrast to Kankavi et al. [20], Beileke et al. [18] did not detect SP-A at the protein level in human spermatozoa. However, the primary findings of Kankavi et al. [20] supported those of Rokade and Madan [22], who showed that SP-A and SP-D proteins were localized on murine sperm head and tail.

Lamellar bodies (LBs) are subcellular structures that can be demonstrated by electron microscopy. Originally, LBs were believed to be present only in the lungs. These LBs are located in type II cells of the lung alveoli, forming an integral part of the lung surfactant system. However, their presence is not restricted to the lungs [23]. The extrapulmonary presence of SP-A protein, as well as annulated lamellae (AL) in Leydig cells [24], raises questions about their functional roles in these cells, which remain unanswered.

As supported by large number of studies, the expression of SP-A has been shown in the testis of rat, mice, human and stallion. However, there is no data concerning the investigation of SP-A in postnatal morphogenesis in rat testis and exact cellular immunolocalization in the testis remains unclear. Specific localization and the presence of SP-A in the different steps and stages of postnatal developments, which suggests a possible role of SP-A in testicular steroidogenesis and germ cell proliferation in the rat testis and as well as in the Leydig cell.

In the present study, our objective was to establish a concrete expression pattern of SP-A in the rat testis, with particular interest in spermatids and their transition to spermatozoa during postnatal development. The localization of SP-A in the rat testis tissue was determined using immunohistochemistry, while changes in SP-A protein levels were examined through Western blotting at different PNDs. The results may provide insights into how SP-A is involved in the development and function of the testis.

2 Material and Methods

2.1 Developmental Time Periods

The rats were categorized into four developmental time periods, designated by postnatal days (PNDs), which are established for male rats and are used to characterize microscopic changes. These periods are defined as neonatal (PND 5), infantile (PND 20), postpubertal (PND 50), and mature (PND 70) for Wistar albino rats, as previously described by Ojeda et al. (1980) [25].

2.2 Methods

Wistar albino rats were housed under 12-h light–dark cycles with food and water provided ad libitum. The study protocols were approved by the Experimental Animal Ethics Committee at Burdur Mehmet Akif Ersoy University, Turkey (Approval No. 2024–02-14/1270). Rats were divided into four groups based on distinct developmental days as PND 5, PND 20, PND 50, and PND 70. Each group comprised six animals. After euthanasia, tissue specimens were obtained for immunohistochemical examination and Western blotting. For internal control, bronchoalveolar lavage (BAL) fluid was obtained and processed as described previously [26]. Samples for western blotting were preserved at -80 °C, while those for immunohistochemistry were fixed in Bouin's solution at ambient temperature. Following fixation, the tissues were rinsed overnight in 70% alcohol, then underwent a sequence of washes in graded alcohols (80%, 96%, and 100%). The materials were further cleared in benzene and methyl benzoate solutions prior to being embedded in paraplast.

2.3 Western Blotting

20–30 µg of pooled testis tissue protein homogenate and BAL samples were mixed with Laemmli sample buffer. SDS-PAGE and Western blotting of BAL and testis tissues were performed as previously described [27]. Anti-rat SP-A polyclonal IgG (rabbit IgG) (5.76 mg/ml) was used at a 1:500 dilution, along with a secondary antibody (anti-rabbit horseradish peroxidase-conjugated; A-0545, Dako Japan, Kyoto, Japan) and GAPDH antibody (PA1-987, Invitrogen). Blots were imaged using the GeneGnome XRQ system (Syngene, Seoul, Korea).

2.4 Immunohistochemistry

The immunohistochemical staining was conducted using the protocol established by Özbek et al. [28]. A rotary microtome (RM2125RT; Leica) was employed to section paraffin-embedded tissues into 5 µm-thick slices. Dewaxing was performed with xylene, followed by dehydration via a sequence of graded alcohol solutions. The slides were washed with phosphate-buffered saline (PBS) and subsequently treated with a 3% hydrogen peroxide (H_2O_2) solution in distilled water for 20 min. Following rinsing with PBS, the sections underwent antigen retrieval with citrate buffer (10 mM, pH 6.0), while the slides were heated in a microwave for 20 min. Following the encirclement of the sections with DakoPen, a 10% solution of normal goat serum blocking agent (Ultra V Block®; Thermo Fisher Scientific) was administered for 10 min after a wash with PBS. The slides were subsequently treated overnight with primary antibody anti-rat SP-A polyclonal IgG (rabbit IgG) (5.76 mg/ml). Following a rinse with PBS, the sections were incubated with a secondary antibody (biotinylated goat anti-rabbit IgG; Thermo Fisher Scientific) for 30 min at ambient temperature. After an additional wash in PBS, the slides were treated with enzyme-conjugated streptavidin (streptavidin–horseradish peroxidase; Thermo Fisher Scientific) for 30 min. 3-Amino-9-Ethylcarbazole (AEC) served as the chromogen for immunostaining visualization, while the slides were counterstained with Gill's hematoxylin. Immunohistochemical staining was assessed utilizing an Olympus BX51 light microscope fitted with a digital camera (DP74; Olympus). Images were obtained with a digital camera in conjunction with CellSens software. All immunostaining processes were performed within a humidity chamber. Positive and negative controls were incorporated to validate the specificity of the immunohistochemical reaction. In the negative control, the primary antibody was substituted with PBS, and nonspecific rabbit serum (sc-2027; Santa Cruz Biotechnology) served as the isotype control.

2.5 Semi-quantitative Assessment of Immunoexpression

In the PNDs of 50 and 70 days, a total of 14 distinct stages were identified within the seminiferous tubules, assessed according to the various cell types present at these stages [29, 30]. Transverse sections of testicular tissue from each rat were first analyzed at low magnification (×10) to evaluate the quality of immunolabeling, changes in histomorphology, and the patterns of cellular labeling during the postnatal development. Due to the lack of standardized protocols or optimized scoring algorithms for immunohistochemical markers, this study utilized a semiquantitative approach to assess the staining intensity based on a density score (IS), which reflects the strength of the immunoreaction. Immunostaining in testicular tissue was classified by intensity levels as follows: negative indicates the absence of visible staining, even under high magnification (40×); weak denotes staining that is discernible only at high magnification (40×); moderate signifies staining that is clearly visible at low magnification (10×); and strong indicates staining that is prominently apparent at low magnification (10×), consistent with prior methodologies. The evaluation of staining intensity was carried out by two independent researchers (M.Ö., O.K.), and the average values from both assessments were calculated. Immunohistochemical reactions were analyzed separately across distinct postnatal days (PND 5, PND 20, PND 50, and PND 70).

2.6 Statistical Analysis

GraphPad Prism (Version 8.0.2, GraphPad Software, San Diego, CA, USA) was used for statistical analysis. Differences between groups were evaluated using one-way ANOVA followed by Tukey's multiple comparisons test. A p-value of < 0.05 was considered the threshold for statistical significance.

3 Results

3.1 Western Blot

The expression SP-A in the testis during different postnatal development periods was further confirmed at protein level by Western blot. The rabbit anti-rat polyclonal SP-A antibody was used for the detection of SP-A in testicular tissue extracts and BAL samples as positive control. The specific immunoreactive band of molecular weight ~ 34 kDa was detected by chemiluminescence using ECL reagents (Amersham). The target protein of the groups was divided by housekeeping protein (GAPDH) and the protein amounts were measured using Image Studio to determine the protein

amount (Fig. 1.a). At PND 5, there were no/ or undetectable positive immunoreactive band that corresponded to the known molecular weight of SP-A. SP-A protein levels were first detectable in rat testis tissue extracts at PND 20, albeit at the lowest expression level. Expression levels increased significantly at PND 50 and peaked at PND 70 (Fig. 1.a). The same results were observed by three independent blotting as well. Visual inspection of the Western blots shows increased band intensity at PND 50 and PND 70, likely reflecting an increase in SP-A protein depending on the PNDs. These results are further summarized in the associated quantitative analysis and visual representations (Fig. 1.b), providing a clear correlation between the postnatal days and SP-A expression levels.

3.2 Immunohistochemical Studies

3.2.1 Localization of SP-A protein in the Testes of Rats at Various Postnatal Days

In rat testicular tissue sections, moderate immunoreactive signals of SP-A was observed in the Leydig cells of the seminiferous intertubular area and the connective tissue as early as at PND 5 (Fig. 2.a). At PND 20, moderate SP-A immunoreactivity was apparent exclusively in the Leydig cells of the intertubular area but not in the connective tissue and seminiferous tubules (Fig. 2.b). The positive SP-A immunostaining of Leydig cells also present at PND 50 (Fig. 2.c, d, f, g) and 70 (Fig. 1.h, l, j, k) as well. Morphologically, the seminiferous tubules were categorized as described previously: early-stage (stages I-VI), mid-stage (stages VII-VIII), and late-stage (stages IX-XIV) in rats [29, 30]. The distribution of brown or dark brown immunohistochemical staining for SP-A varied across different stages (Fig. 2). Immunohistochemistry revealed that SP-A was more prominent in step 16 elongated spermatids within seminiferous tubules, particularly during the early-stage at PND 70 (Fig. 2.h). The SP-A expression was lower in subsequent stages within the seminiferous tubules at PND 70 (Figs. 2.g and 2.l). At PND 70, spermatogenetic cell types that correspond to physiological spermatogonial stages of rats and significant changes became more evident. Positive SP-A immunoreactive signals were observed in elongating spermatids, the intertubular matrix, and Leydig cells at PNDs 50 and 70 (Fig. 2.c-g). The immunoreactivity of SP-A was less intense in elongating spermatids at PND 50 (steps 9–15) compared to elongated spermatids at PND 70. Additionally, the immunoreactivity of SP-A in elongated spermatids at PND 70 (step 16) was more intense than in elongating spermatids at PND 50. Notably, at PND 70 and step 16 spermatids within the seminiferous tubules exhibited strong positive staining with the SP-A antibody and decreased slightly in the subsequent steps, reaching the final step of 19. (Fig. 2.i).

4 Discussion

This study demonstrated the expression and localization of surfactant protein A (SP-A) within rat testis tissue and highlights the PND-dependent variations in its expression. We observed moderate immunopositivity for SP-A in Leydig cells at all examined postnatal days (PNDs). Our findings suggest that SP-A protein is involved in the development of rat testis tissue at different PNDs, particularly from PND 50 to PND 70. Immunoblot analysis revealed a significant increase in SP-A expression at PND 50 and PND 70, while the lowest expression was first observed at PND 20. At

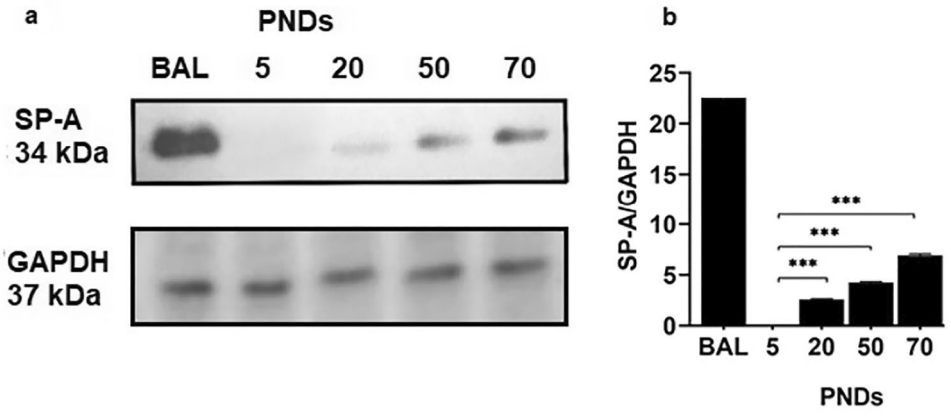
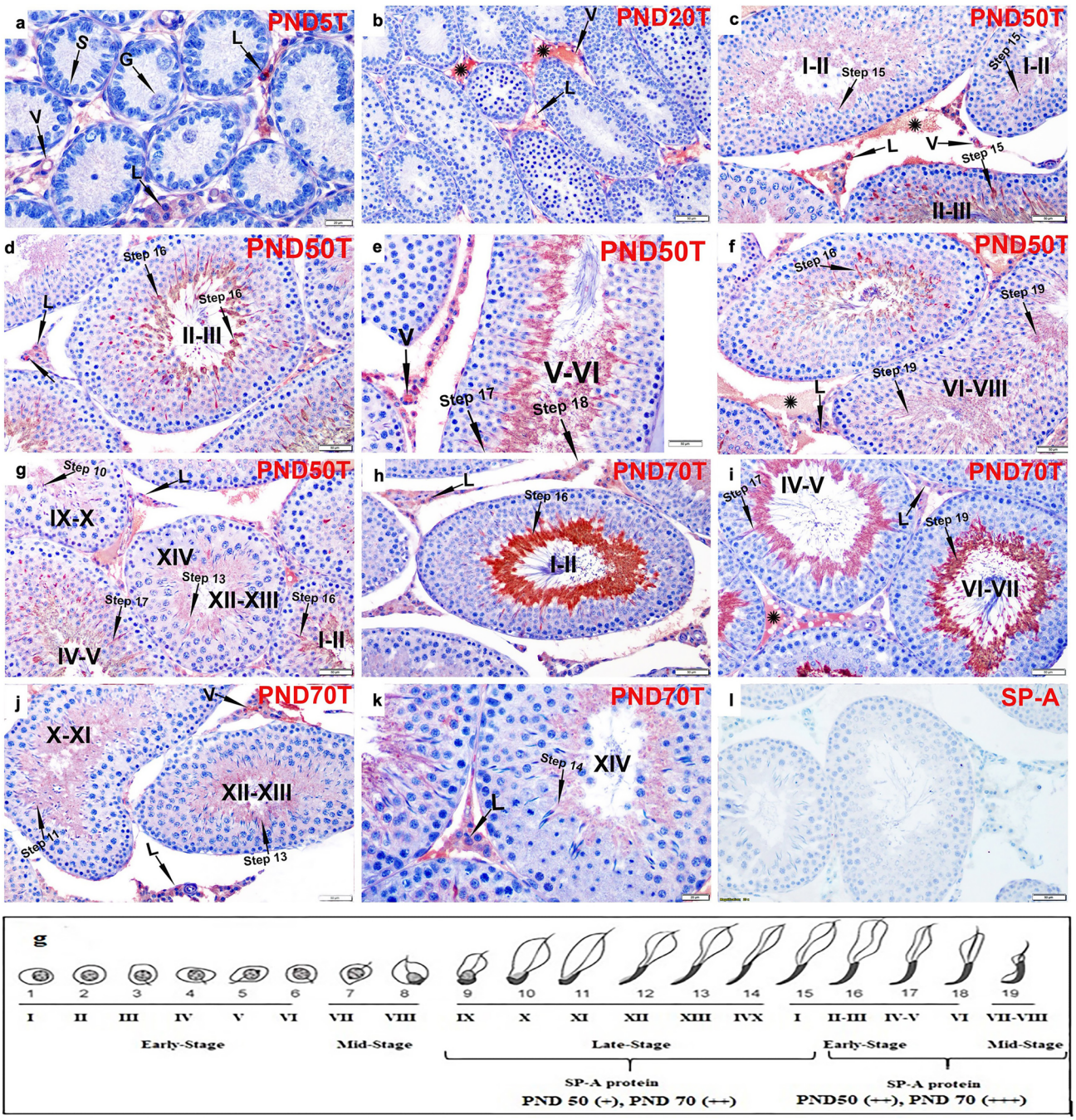


Fig. 1 **a** Western blot analysis of SP-A in BAL and rat testis tissue extracts at PNDs 5, 20, 50 and 70. Western blotting of GAPDH as a loading control. Western blot of BAL. 2. Rat testis tissue homogenates probed with an antibody against SP-A. An exposure time of 1 min and enhanced chemiluminescence were utilized. The target protein of the groups was divided GAPDH and the protein amounts were measured using Image Studio to determine the protein amount. $P < 0.05$ was accepted as the statistical significance limit (Fig. 2.b)

PND 5 (neonatal stage), no tubular stages were apparent, therefore no positive immunostaining signal was observed in the seminiferous tubules. By PND 20 (late infantile stage, corresponding to 1–23 months in humans), tubular stages began to appear, and the development of tubular lumina continued, slowly reaching sufficient maturity for SP-A protein expression. Consistent with our findings, Rokade and Madan (2019) reported the lack of SP-A immunoreactivity in testis tissue of 9- and 15-day-old mice. Interestingly, at PND 50 (peripubertal/adolescent stage, corresponding to 12–16 years in humans), spermiogenesis occurred, elongating spermatids appeared in the seminiferous tubules, and by approximately PND 52, spermatozoa were present in the epididymis. SP-A immunoreactivity was detected in the elongating spermatids of the seminiferous tubules starting at PND 50. Finally, at PND 70 (late puberty/adolescence), SP-A expression became evident. This study elucidates the changes in SP-A protein expression patterns in postnatal rat testis, with a marked increase observed from PND 50 onwards. This upregulation in SP-A protein expression may result from an increase in tissue expression and/or a rise in the number of germ cells, possibly due to their proliferative capacity before the first wave of spermatogenesis.

SP-A plays key roles in modulating both the innate and adaptive immune systems within the lung (31). This innate immune molecule is also expressed throughout the body, including the human [18, 20], mice [31], and rat [32] reproductive tract. The antimicrobial effects of SP-A and SP-D against bacteria [33, 34], fungi [35], and viruses [12] are well documented.

Spermatogenesis occurs within the seminiferous tubules, while steroidogenesis is accomplished by Leydig cells located in the interstitial spaces. It has also been suggested that Leydig cells play a role in regulating immune responses by affecting the numbers of testicular macrophages and lymphocytes [36]. In addition to these immunomodulatory effects, the expression of SP-A by Leydig cells may start as early as PND 5, contributing to the maintenance of testicular immune privilege. At the end of spermiation, spermatozoa within the lumen of seminiferous tubules showed moderate immunopositivity, indicating the completion of spermiation (Fig. 2.f). It has been shown that elongated spermatids undergo progressive changes in nuclear and cytoplasmic morphology from mid-stage VII -VIII in adult Sprague Dawley rats [37]. We observed that the degree of SP-A immunostaining was reduced in spermatozoa within the lumen compared to elongated spermatids at step 16. This reduction is likely related to the loss of cytoplasm with SP-A protein in maturing spermatozoa. Surfactant proteins, including SP-A, have been detected at the protein level in the testes, prostate, and seminal fluid [17]. Following their production by the testes, spermatozoa are stored in the epididymis. Upon ejaculation, these spermatozoa are mixed with secretions from the accessory sex glands to form the ejaculate. The secretions of the seminal vesicles, the bulbourethral (Cowper's) glands, and the prostate, collectively known as seminal plasma, contain proteins and peptides responsible for sperm protection. SP-A, identified in seminal plasma, is likely pivotal role in protecting spermatozoa from a variety of microbial pathogens and modulating the immune response within the female reproductive tract during later stages. Well-documented interactions of SP-A with innate immune system cells, including neutrophils, monocytes, and macrophages in lung alveoli [38, 39], suggest that similar mechanisms may operate in seminal plasma. The increase in SP-A protein observed at specific PNDs (50–70) in our study was reported by Rokade and Madan (2019). They demonstrated that SP-A transcript levels were significantly upregulated in the testis of post-pubertal (PNDs 30, 60, 90) mice compared to the pre-pubertal (PNDs 9, 15) group. These findings suggest that SP-A may play a critical role in the formation of spermatids and spermatozoa. Our findings also partly support those of Beileke et al. [18] who previously showed the presence of surfactant proteins A, B, C, and D in human testis, prostate and seminal fluid. They concluded that cells in the seminiferous tubule cytoplasm, spermatogonia, Sertoli, and Leydig cells, spermatocytes 1 (primary) and 2 (secondary) were positive for SP-A and SP-D, except for spermatozoa. However, we have observed immunopositive spermatozoa within lumen of seminiferous tubules in step 19 in stage VI-VII spermatids at PND 70 (Fig. 2.i). These findings are also supported by Kankavi et al. [20], who reported the presence of SP-A in the midpiece, tail, and equatorial region of mature human spermatozoa. Although Beileke et al. [18] did not detect SP-A at the protein level in spermatozoa. The novel findings of Kankavi et al. [20] are further supported by Rokade and Madan [20], who reported SP-A localization on the murine sperm head and tail. The localization of SP-A in testicular tissue could be relevant in regulating the activity of testis innate and adaptive immunity. Therefore, the presence of SP-A on spermatozoa within the seminiferous tubules could be facilitates the clearance of apoptotic and necrotic cells, reduces allergic responses, and promotes the resolution of inflammation within the testis tissue as reported in lung alveolus [13]. Phagocytic removal of the apoptotic germ cells and residual bodies is critical in maintaining testicular homeostasis and normal spermatogenesis. Decrease in SP-A expression particularly in steps 17–19 could indicate the role of SP-A in apoptosis within the seminiferous tubules. The extrapulmonary presence of SP-A protein, potentially correlated with lamellar bodies (LBs) in cells like those on mucosal surfaces, including Leydig cells, raises questions about their functional roles, which remain unanswered. The presence of lamellar cristae in Leydig cells was first reported by Reinke [40], who noted their presence in the Leydig cells of sexually active men and their absence



before puberty and in aged men. Fouquet et al. observed an increase in Leydig cells in testis tissue from birth to the infant stage [41]. Continuous research has confirmed the association of lamellar structures with human Leydig cells [42, 43]. Positive SP-A immunostaining in Leydig cells at all PNDs particularly as early as PND 5 may indicates their potential roles as candidate molecules in the early innate immunity and may source of SP-A expression of the testis. However, it has been suggested that the presence of SP-A transcript in germ cells indicates that germ cells are an important source of SP-A in murine testis [19]. Leydig cells and macrophages primarily constitute the first line of testicular defense against bloodstream pathogens [44–46]. Both SP-A and SP-D are collectin family members also known as innate immune molecules with important roles in the lung [8]. It is likely that SP-A may act synergistically with SP-D in testis tissue as described in lung alveolus. A pioneering study by Rokade et al. [47] demonstrated that another member of the surfactant and collectin family, SP-D, plays an important role in maintaining testicular immune privilege and indirectly contributes to male fertility. They also showed that SP-D knockout (SP-D -/-) male mice have significantly

◂**Fig. 2** Immunolocalization of SP-A in paraffin-embedded rat testicular tissue sections during different stages of postnatal development. **a** At postnatal day (PND) 5, SP-A immunopositive signal was observed in Leydig cells. **b** At PND 20, Leydig cells were displayed SP-A immunopositive signals. **c** At PND 50, elongating spermatids in early-stage tubules (stages I-II) exhibited moderate SP-A immunopositive signals, while elongating spermatids at step 15 in stages II-III showed stronger SP-A immunopositive signals. **d** At PND 50, tubules at stages II-III, elongating spermatids at step 16, SP-A immunopositive signal was present in elongated spermatids. **e** At PND 50, in stages V-VI, elongating spermatids at step 18 show strong cytoplasmic SP-A immunoreactivity. **f** At PND 50, in stages VI-VIII, elongated spermatocytes at step 19 showed moderate SP-A immunoreactivity **g** In stages IX-XIV, step 13 elongated spermatids showed moderate SP-A immunoreactivity. **h** At PND 70, tubules at stages I-II, elongated spermatocytes at step 16 showed the strongest SP-A positivity. At PND 70, in stages IV-V, elongated spermatocytes at step 19 show strong SP-A immunopositivity however, staining intensity lower than stages I-II (**i**). As shown in Fig. 1.i SP-A was specifically expressed in spermatozoa within the lumen of seminiferous tubules at stage VI-VII. At PND 70, seminiferous tubules in late-stages (X-XIII), elongated spermatocytes at steps 11–13 showed moderate SP-A immunoreactivity (**j**) at PND 70, in late-stage (XIV) at step 14, showed faint SP-A immunostaining, and Leydig cells showed SP-A immunopositivity (**k**). Testicular tissue where the primary antibody was omitted served as a negative control (**l**). Schematic summary of localization of SP-A protein during spermiogenesis (g) (partly m*odified* from Wu et al.*)* (42). Streptavidin-ABC technique with AEC as the chromogen was used for the immunohistochemical localization of SP-A in rat testes tissues. Black asterisks indicate the intertubular area matrix. L: Leydig cell, S: Sertoli cell, G: Gonocyte, V: Vessel wall, PND: Postnatal day, T: Testis. Roman numerals in Fig. 1 (c-k) represent stages of the seminiferous epithelial cycles. Bar: 20 µm (a, e, i, and k), 50 µm (b, c, d, f, g, h, i, and l). Insets in the figures show SP-A antibody reaction for this protein

reduced weights of the testis and epididymis, highlighting the importance of SP-D in the immune regulation of the testis and sperm functions.

The presence of SP-A in testicular tissue suggests their possible involvement in the acquisition of roles for this protein. To our knowledge, this study provides the first evidence of the presence of SP-A in the developing rat testis and their localization during the testis development.

The mechanism underlying the peak secretion of SP-A in the early stages of spermatogenesis likely reflects the role of SP-A in providing innate immune protection within the seminiferous tubules. During the later stages, spermatids undergo spermiation and are released from the seminiferous tubules, marking the end of protection by the blood-testis barrier. SP-A emerges as a candidate molecule providing crucial protection for spermatozoa at these later stages.

This is the first report documenting the expression and localization pattern of SP-A in the rat testis at various seminiferous developmental stages. SP-A may play roles in innate immunity, contribute to host defense mechanisms, modulate immune responses and inflammation, regulate allergic reactions, and protect against infections, similar to its functions in the lung. Additionally, SP-A may play a role in the spermatogenic process and steroidogenesis. Nevertheless, the current study provides first evidence for the involvement of SP-A in testicular development.

5 The Study Limitations

Overall, the potential importance of SP-A in male reproductive physiology needs to be clarified with further studies. A detailed understanding of the roles of SP-A in male fertility will needs future studies, ideally involving the use of SP-A knock-out animal model, histology of testis, testosterone and estradiol measurements fertility trials, sperm motility assay, and in vitro fertilization assays.

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Author contributions Orhan KANKAVİ: Writing – review & editing, Investigation, Methodology, Visualization, Validation, Data curation. Mehmet ÖZBEK: Writing – original draft, Methodology, Validation, Investigation, Data curation. Ayhan ATA: Visualization, Validation. Harun KARACA: Validation, Data curation. Mustafa ÖZTOP: Writing–original draft, Visualization. Emel ERGÜN: Visualization, Methodology.

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Data availability The data underlying this study's findings can be obtained from the corresponding author upon a justified request. No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval The study protocols received approval from the Department of the Experimental Animal Ethics Committee at Burdur Mehmet Akif Ersoy University, Türkiye (Approval No: #2024–02-14/1270.

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