Al-Qadisiyah Journal of Pure Science
ISSN(Printed): 1997-2490 ISSN(Online): 2411-3514
DOI: 10.29350/jops
Al-Qadisiyah Journal of Pure Science

Molecular Expression of ADAM28 Gene in Epididymis tissue of Wister Albino Rat

Manar Mousa Alhussein[1], Eman Faisal Albaghdady[2]

[1]Department of Pathological Analysis. College of science, University of AlQadisiyah, Al-Qadisiyah, Iraq

[2]Department of Anatomy and Histology, College of Veterinary Medicine, University of AlQadisiyah, Al-Qadisiyah, Iraq

Abstract

Objective: For the molecular expression of the ADAM28 gene in the epididymis tissue of rat aged 66 days.

Methods: At 66 days of age, five mature male rats were obtained; caput, corpus, and cauda epididymal regions (right and left) side were separated based on morphological analysis. Right regions were kept in 10% buffered neutral formalin for microscopic studies and 50 mg of each region in left side was mixed with 1 µl of the TRIzol® for quantitative real-time PCR (qRT-PCR) using reagent kit for the isolation of total RNA (Bioneer, Korea).

Results: Histologically, each segment: the initial, caput, corpus, and cauda have its own distinctive cell kinds, duct diameter, epithelial height. The initial segment has a significant difference ($P < 0.05$) by decreasing tubular diameter when compared with other segments. The caudal has thicker, muscle layers compared to other segments. The expression of ADAM28 gene along the segments of epididymis was demonstrated at different levels. also, we found that the ADAM28 gene in corpus was higher than that of other segments and in the cauda it was greater than that in caput without significant differences ($P < 0.05$).

Conclusion: The ADAM28 gene found along the epididymis segments of rat.

Keywords: rat, epididymis, gene expression, ADAM28

1-Introduction

The epididymal mammalian, which resembles a highly convoluted tube and promotes conduction of the testis with the ductus deferens, appears as a complex organ, according to [1] the organ, known as the epididymis, has many segments. Four primary anatomical regions have been identified by some research: the beginning part, caput, corpus, and cauda [2] Like in laboratory rodents [3] mention, the globular proximal caput, the extended corpus, and engorged distal cauda are the three regions that make up the epididymis, according to the majority of researchers; the epididymis contains morphological traits unique to its species [4].

The cauda keeps spermatozoa in a quiescent condition and aids in sperm preservation; the proximal regions—the first part and caput —facility sperm development [1] [5]. In addition, each region is structured into intraregional subdivisions surrounded by loose connective tissue septa (CTS), which support the organ itself [5]. Therefore, the researchers believe the epididymal regions are grouped as

smaller organs located side by side because each segment appears to have an advanced complication to the typical of rat epididymal segments like choosy gene and protein expression, signal pathway transduction inside lobules, and contributes specifically within the epididymal lumen milieu with a wide variety of changes occurring in it, like fluid, ion, and antioxidant uptake and release [6-8]. One possible cause of male infertility is segment loss [9].

An extremely specific epididymal luminal environment is necessary for epididymal function, and it is created and preserved by the coordinated cellular activity of the epididymal epithelial lining cells. The distal region where spermatozoa are stored may become significantly more acidic due to the presence of clear cells, which are expressively more common in the epididymal cauda than in the caput region and contribute to net proton secretion [10].

The epithelial cells are distributed in different subdivisions of the epididymal epithelium to perform various functions like absorption, secretion, endocytosis, luminal fluid acidification, immune protection, phagocytosis, and the creation of antioxidants. These cells primarily consist of principal cells (PCS), which are nearly (85%), narrow (establish merely in the initial part), and basal cells, with other specialized cells such as apical, halo, and clear cells accompanying them [11]. The epididymal fluid protein profile is significantly altered as a result of coordinated secretions and endocytosis, exposing the gametes to substances that will alter the physiology of the sperm. Furthermore, target proteins, miRNAs, and lipids are delivered to spermatozoa by the apocrine secretions of principle cells [10]. Because of the epididymis's intricate activities and compartmentalization, there are a number of possible causes of epididymal failure that can lead to problems with male fertility and even the condition of the progeny [12].

Recent studies have shown that extracellular vesicles (epididymosomes) are complex and variable protein structures secreted by the epididymal epithelium, leading to the physical roles of proteins and mRNAs in epididymal activities [10] [13]. These transfers to spermatozoa in cauda altering the sperm membrane by interacting with proteins that are essential for sperm to enter through the oocyte wall, then bind with the oolemma [10] [14] [15].

The discovery of a surface protein known as PH-30 (ADAM1) led to the discovery of the disintegrin and metalloproteinases (ADAMs) family [16] [17]. The ADAMs family is classified as a secreted enzyme and has multiple domains, such as a pro-domain (metalloprotease, disintegrin (integrin ligand), cysteine-rich region, epidermal growth factor-like domain, transmembrane, and cytoplasmic cauda) that includes several chemokines and cytokines. Some of these enzymes also break down other extracellular matrix components, such as the ADAM proteins related to matrix metalloproteinases (MMPs), which consider highly conserved domains [18] [19]. The extracellular matrix (ECM) is assembled and broken down during morphogenesis, growth, morphogenesis, tissue remodeling, and reparation by secreted zinc metalloproteinase family known as a disintegrin and metalloproteinase with thrombospondin (ADAMTSs) [20].

Male reproduction has some functions dependent on proteolytic enzymes and metalloproteinases family proteins, which regulate testicular development and sliding into the scrotum, sperm maturation, and sperm-egg binding (Hongmei and Khalil,2022); in addition, problems with spermatogenesis, male infertility, aging, and testicular cancer have all been connected to it [17] [22] [23].

Expression the ADAM family is made up of at least 34 members that are varieties with different mammalian organs. These individuals can be categorized into three evolutionary groupings (I, II, and III). Group I consists of 11 ADAMs that are expressed in the testis, while Group II has five ADAMs expressed in the germ cells of the testes. These groups each display distinct structures [24]. Integral membrane proteins belonging to phylogenetic group III, A disintegrin and metalloproteinases 7 (ADAM7) and A disintegrin and metalloproteinases 28 (ADAM28), in rodents like rats, are produced by the epididymis

duct and exhibit high regional expression with varying amounts along distinct parts of the epididymis [1] [14]. According to [24], the two types exhibit a great degree of amino acid sequence identity (53%).

In human, disintegrin and metalloproteinase 28 are expressed primarily by lymphatic and epithelial cells; also they are expressed by immune-localized epithelial cells in various tissues in mice, like epididymal epithelial cells and thymus- cells. ADAM28 is related to snake venom metalloproteases (SVMPs) and is expressed by tissues that are derivative from the foregut in embryological state [16] [24] [25]. Additionally, it can be released as soluble through the proteolytic cleavage of certain proteinases, such as ADAM 7[25].

The ADAM28 protein exists in two distinct forms: the transmembrane or prototype (ADAM28m) and the shorter (ADAM28s). The first form consists of various functional domains, including prodomain, metalloproteinase, and disintegrin. Cysteine-rich. Epidermal growth factor-like domain, a transmembrane, and a cytoplasmic tail. On other hand, (ADAM28s) form has a structure similar to the first form, but the final three parts are absent [16] [26].

The proteolytic activity of ADAM28 has effects that encourage the formation of tumors, [17] [27], such as in human lung and breast malignancies. Through their binding to integrin a4B1, they also regulate cell proliferation of osteoblast-like cells, have directly influence on the regulation of airway remodeling and have a significant impact on cell adhesion [19] [28].

With the ability to use epididymal spermatozoa—rather than testicular spermatozoa—to obtain a greater live birth ratio by sperm injection through the intracytoplasmic, the epididymis may be crucial to sperm maturation [29].

The molecular and biochemical mechanisms by which post-testicular sperm mature, the role of epididymal fluid of the cauda in keeping spermatozoa latent for prolonged periods, and the causes of epididymal dysfunction resulting from differences in the luminal fluid composition along the human epididymis that separate fertile from infertile individuals are all unknown. Male infertility diagnosis and therapy are limited by these reasons [30].

The majorities of proteins in the epididymal tubule are specific for each species and vary in different regions of the organ [10]. Consequently, molecular instruments have been created to gauge the expression levels of specific sperm proteins that serve as indicators of male fertility. The ADAM family is among the principal helpful models for examining the coordinated parameters and structure of sperm surface proteins [31] [32]. In an effort to fill in the knowledge gap regarding this protein, the aim of this work was to use qPCR techniques for molecular detection of the ADAM28 gene in epididymis rats throughout puberty (age 70 days). This may potentially lead to the discovery of new markers for fertility and semen quality and provide additional information about the protein alterations in the epididymis.

Ethical approval

The study was performed under the Ethical Standards Approved by the scientific board of College of Veterinary Medicine, University of Al-Qadisiyah (committee approval number: 1890 / 22/11/2022

2. Materials and methods

2.1. Experimental animals housing and sacrificing

Five mature male rats, at aged 66 days, with a mean weight of around (183±7.810) gm were acquired from the animal household in University of Al- Qadisiyah, which is part of the college of veterinary medicine. The rats housed in a laboratory environment with controlled normal settings and were raised in an air-conditioned room. Keeping a constant temperature of 23 ±2ºC and relative humidity of 66-71% while utilizing 12-hour light and dark cycles. Each was put in a separate 47 x 34 x 18 cm plastic cage (England) that had wood chips inside [33]. Supplied by Animal House. Before any surgery, the animals were given 10 days to be comfortable [34], as well as unlimited access to water and pelleted standardized food (commercial rodent chow) [35]. In order to prevent any physiological or biological alterations in the

organs and tissue of the rats, only healthy rats were used in this investigation. To expose the testes and separate the epididymis duct from the testis, longitudinal excisional skin cuts were made in the scrotum wall while the rat was under general anesthesia and receiving an IM injection of the Xylazine (10 mg/kg of the body weight) and ketamine (80 mg/kg of the body weight). Based on gross anatomy and location of each epididymis (left and right) was separated into the caput, corpus, and cauda regions. Every epididymal specimens were split into two groups. The specimens from the first group (right side) were fixed in 10% neutral buffered formalin (NBF) for histological research. Meanwhile, the specimens from the second group about (50mg) were stored in 1µl in a TRIzol® reagent kit to enable molecular expression of ADAM 28 gene. As per the organization's guidelines, the samples were handled at 4°C. For using in the Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR), by using the total RNA extraction reagent.

2.2. Histological process of tissue

The epididymis was rinsed with normal saline solution (0.9% NACL). After being cleared of adhering fat and divided into the caput, corpus, and cauda, it was preserved in 10% NBF for duration of 28 hours. The specimens were prepared for processing by first being cleaned in tap water, then being moved to 70% ethanol for a full day. They subsequently went through the typical histological preparation procedures (dehydration, clearing, impregnation, and paraffin embedding), after which the tissues were sectioned then staining with Harris Hematoxylin and Eosin (H&E) to demonstration general histological features, and special stains (Masson trichrome and Wiegert's elastic stain) for collagen fibers demonstration and Periodic acid–Schiff (PAS) is a staining technique used to distinguish polysaccharides [36].For obtaining the morphometric estimation for each specimens (epithelium height and the diameter of the epididymal tubule of ducts), five slide of each specimen were measure (Twenty-five tubules outcome from each specimen). The data was recorded using an ocular micrometer set to 40X. Sections were statistically analyzed using light microscope (Olympus, Japan) [34].

2.3. Molecular analysis of ADAM28 gene in epididymis segments

The total RNA from epididymis tissue (caput, corpus, and cauda) was extracted using the AccuZol® kit, and the M-MLV Reverse Transcriptase kit (Bioneer, Korea) was used for producing cDNA. qRT-PCR was utilized to quantify the specific gene in this study (ADAM28) and the results were normalized using the GAPDH gene as a housekeeping gene. The process followed the method previously reported in a study by [14]. The qRT-PCR data was analyzed using the ΔCT procedure for ratio expression of target genes and GAPDH, following the procedure specified in a publication by [37].

(Table 1): Displayed Primers using for RT-qPCR.

Gene	Primer Sequence (5'-3')		Product size	Accession number
ADM28 gene	F	AAAGCAGGTTTCCAGTGTGC	83bp	NM_001394671
	R	TTTGCTGCTCTGCACACAAC		
GAPDH gene	F	ATGCCCCCATGTTTGTGATG	136bp	NM_017008.4
	R	TCCACGATGCCAAAGTTGTC		

(ADAM28 gene A disintegren and metalloprotanase gene 28 GAPDH=Glyceraldehyde-3-phosp-hate dehydrogenase, F: Forward Primer-R: Reverse Primer)

2.4. Statistical analysis

Version 24 of the SPSS® software was used to analyze the data. Using the one-way analysis of variance and the post Hoc analysis, comparisons done across several locations of the epididymal rats as means ± the standard errors. Value was measured as significant at $P<0.05$ [38].

3-Results and discussion

This investigation used thirty specimens from three locations (caput, corpus, and cauda) of adult rats' epididymal tissue. Fifteen specimens were obtained for the histological analysis and the same number for the molecular expression of the ADAM28 gene in segmental epididymis tissue.

Many studies have been carried out over the span of more than 60 years to gain a better understanding

of the mechanisms required for the epididymis to produce functional gametes, which is vital for managing post-gonadal spermatozoa in all species [4].

3-1-Histological investigations

The study looks at the histological features of the epididymal segments of the caput, corpus, and cauda rats. It finds well-organized epididymal tubules and unique tubular lumens that are packed with sperms and wrapped in tunica albuginea and adipose tissue. Segments and sub-segments of the epididymis were created by connective tissue septa. Each segment corresponds to a unique physiological compartment with particular microenvironments that either directly or indirectly affect sperm maturation [39]. The intraluminal compartment protein composition changes along the epididymis, with unique proteome and secretome properties for each region [40, 41], which secreted by the epididymal cells. According to study by [6], paracrine signals do not cross the septal border when transitioning between segments because they are restricted by septa inside certain segments.

Every epididymal segment has smooth muscle fibers that envelope the pseudostratified columnar epithelium, which was composed of principal(PCs), basal, and clear cell epithelial cells. This epithelium lines the epididymal tubules. The principal cells had columnar appearances and short stereocilia extending from the cauda to the initial area. The cells that sit along the basement membrane are called basal cells, and they have an oval shape. Apical cells were rounder and more prominent in the beginning and caput, whereas narrow cells were thin with an apically spherical nucleus, and the cauda included a significant number of transparent, vacuolated cells. Also tiny halo cells were extant at the base of the epithelial lining of epididymal tubule (Fig. 1, 2, 3, and 4). The epithelial lining appears positive reaction with PAS stain (Fig.5). These cell types have unique physiological role [11] and every type of cell helps to create and maintain a distinct luminal environment where spermatozoa mature and storage [42].

One notable variation is the amount of lipid droplets that are exclusive to the corpus epididymis, indicating substantial regional specialization throughout the epididymis [44]. the principal cells (PCs) made a complicated and varied contribution to the creation of an acid luminal milieu in the epididymis, depending on the place in different regions of the epididymis, like PCs in initial segment reabsorb bicarbonate through a variety of transporters and enzymes that the kidney's proximal tubules also use; while PCs in the cauda are capable of secreting protons or bicarbonate based on physiological inputs. The presence of luminal sodium in the cauda epididymis of rats and mice is necessary for luminal acidification [45], additionally; apical cells that were engaged in acidification are mainly present in the caput and initial segment epithelium [46]. All epididymal segments in our study have basal cells, which suggest that may produce more vesicles or dynamic membrane remodeling [44]. The e significant impact of epididymal function on the results of male fertility, and the congenial luminal milieu created by the excurrent duct's lining epithelial cells is mostly responsible for this function. Ca2+homeostasis and acidity are two significant elements of the luminal microenvironment that are tightly controlled by a web of signaling pathways and cell-cell interactions [47].

In our study, the adult rat cauda epididymis contained clear cells. These cells of the cauda epididymis facilitate the transfer of several proteins required for the development of sperms [48]. Moreover, clear cells exhibit high levels of expression of genes associated with the synthesis of metabolic energy, (high ATP levels) that required driving the acidity of the epididymal lumen. Unique of the most prominent roles of the epididymis is to maintain an acid luminal state, which prevents sperms from becoming active to the mate [49].

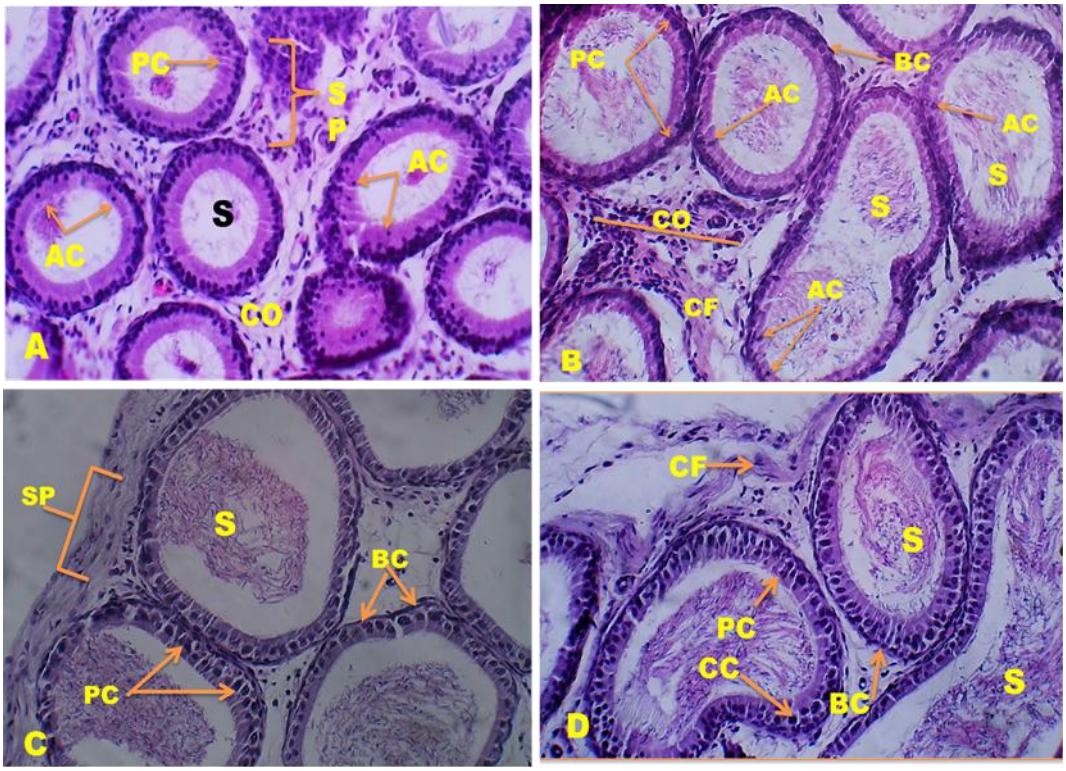

Fig.1. A photomicrograph section of initial part (A), caput (B), corpus (C), and cauda (D) of adult rat epididymal at age 66- day showing luminal tubule lined with pseudostratified columnar epithelial sounded with smooth muscles (SM) and consist of principle columnar cell with stereocilia (PC), (basal cell resting on the basal lamina(BC), these duct full with sperm(S), between tubules there are loose connective tissue septa (SP contain (CF)). (H&E, 400X)

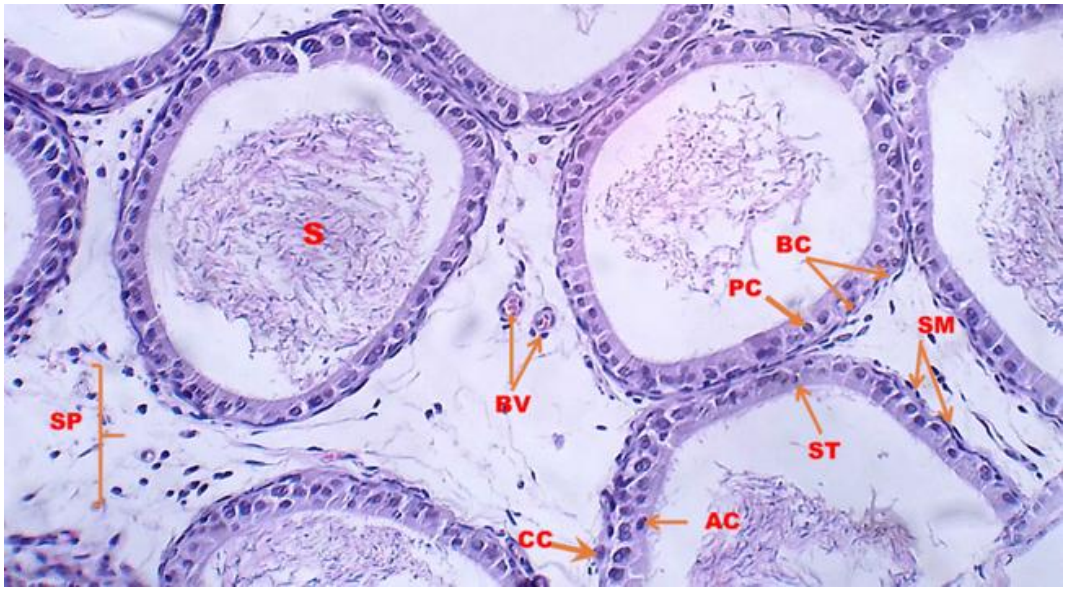

Fig. 2. A photomicrograph of section of corpus segment of epididymis of rat show were full with sperm(S), and line by several type of cells: principle cell(PC), basal cell(BC), clear cell(CC), apical cell (AC) and surrounded by and smooth muscles. Between ducts loose connective tissue as speta that contain blood vessel (BV), (SM) H&E, 400X

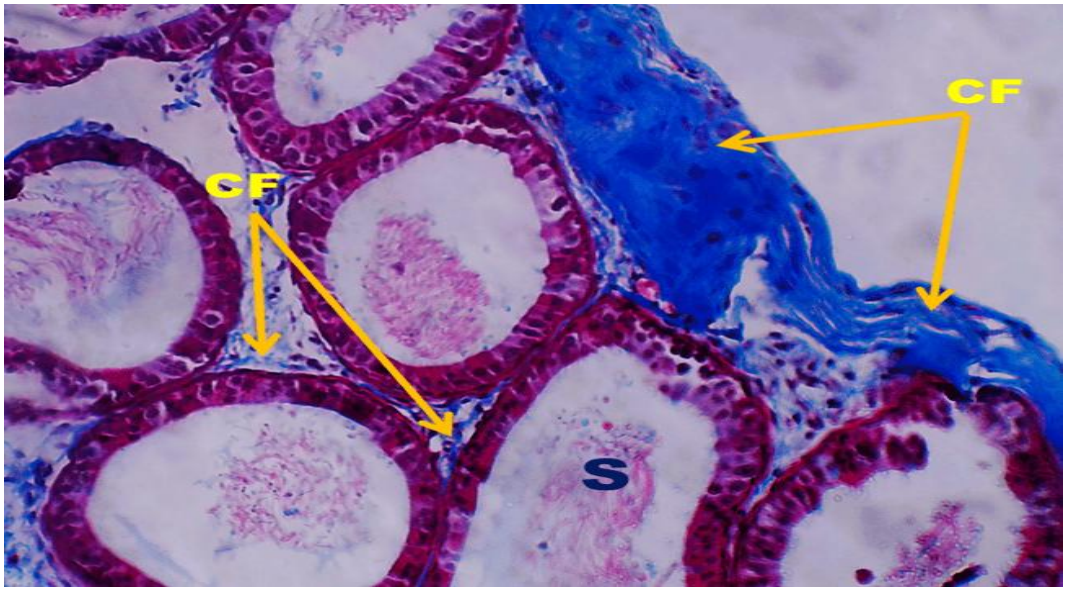

Fig.3. A Photomicrograph of cauda segment of epididymis full with sperm (S). Showing collagen fiber that was (CF). (Masson trichrome 200X)

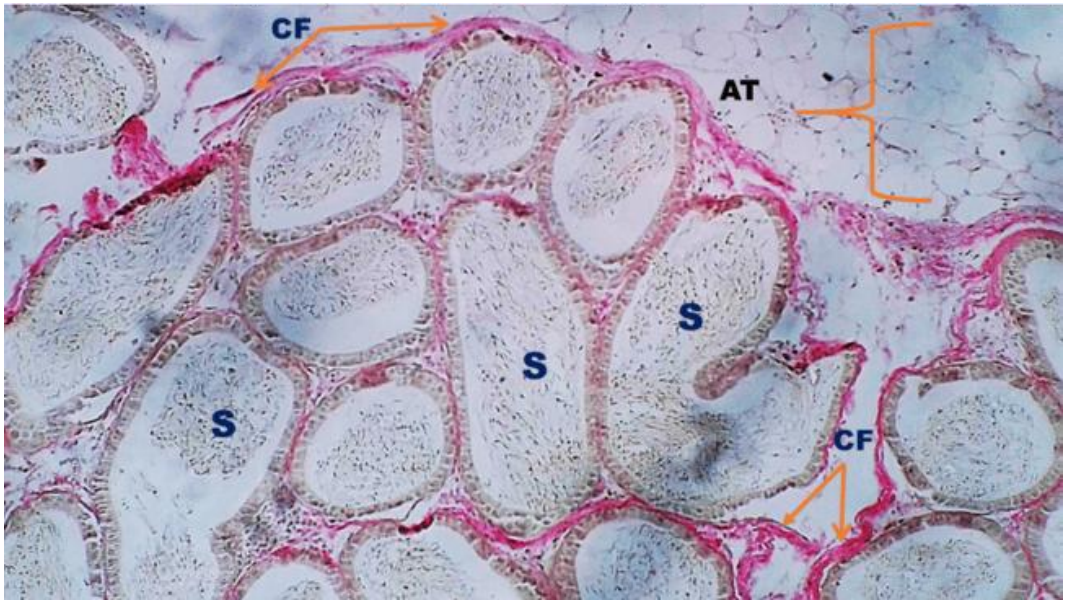

Fig. 4. A photomicrograph of section of cauda segment of epididymis of rat, that full with sperm (S) and covered with layers of adipose tissue (AT), between tubular duct there were collagen fibers (CF), 100X, (Wiegert's elastic stain)

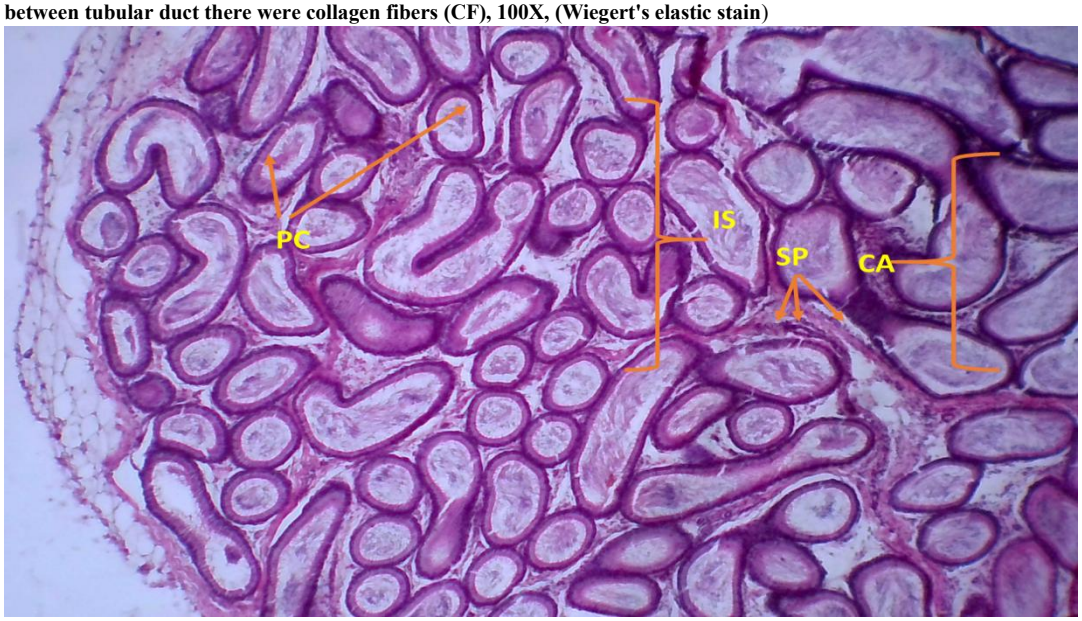

Fig.5.A photomicrograph of section of caput segment (CA) and initial segment (IS) of epididymis of rat, that full with sperm (S), the lining epithelial almost principle cells (PC) appear positive reaction with PAS stain. Between intersegmental tubule there was loose connective tissue as septa (SP). 40X, (PAS stain)

The caput lined with the taller epithelial columnar cells with the base site of the nucleus and the lowest luminal diameter of tubules; while the cauda lined with the shorter columnar epithelium or cuboidal cells with the round to flattened nuclei with a larger diameter. This agreement with [50].

The results of the investigation showed that the tube lumens of the rat epididymis segments differed in form. Compared to the slightly uneven caput, the tubular lumens of the first segment were regular; the lumens of the corpus segment were spherical, longer, and narrower than those of the other segments. The cauda's tube lumens were greatly expanded and densely packed with sperm (Fig. 1). Additionally, there was a notable variation in the average tube diameter across all adult rat epididymis segments. As compared to tubular diameter in caput, corpus, and cauda (186.06±4.95, 195.24±9.2, 221.36±14.13) µm, respectively), there was a significant difference in the initial segment (90.54±3.27) µm, and there was a significant difference in tubular diameter in caput and corpus when compared with cauda (Table.2).

(Table 2). Histological dimension of epididymis segments (Epithelial height (EH), and tubular diameter (TD) (µm) of the epididymis segments of adult male rats at age 66 days. (Mean ± SE) * mean significant at p≤0.05

Epididymis segments	TD (µm)	EH (µm)
Initial segment	90.54±3.27*	26.87±1.70
Caput	186.06±4.95*	14.38±0.49
Corpus	195.24±9.28*	18.37±0.76
Cauda	221.36±14.13*	19.11±0.77

In this study, when compared between the caput, corpus and cauda segments, the mean epithelial height and stereocilia length decrease from the caput to the corpus segment. The initial segment's epithelia height mean was (26.87±1.70) µm and higher than that of the other segments (caput, corpus and cauda) segments as (14.38±0.49, 18.37±0.76, 19.11±0.77) µm respectively, however there was no statistically significant difference between them at that point. (Table 2). This was in line with [43] observation that the epithelial height, shape, and cell types of the epididymis segments of greater cane rat.

Additionally, smooth muscle encircling the epididymal epithelium was increasingly thicker towards the cauda epididymis and is thinnest at the caput. Actually, there are two distinct smooth muscle layers surrounding the cauda and one layer encapsulating the caput [8]. The epididymal fluid flow and smooth muscles contractions that surrounding the epididymal tubule allow the gametes to go toward the organ's terminal region, the cauda, or the cauda of the epididymis, where they are stored in between ejaculations [29].

3-2-Gene expression of the ADAM28

The current study showed that RT-qPCR analysis revealed expression of the ADAM28 gene across all epididymal regions (caput, corpus, and cauda). Thus, threshold cycle (Ct) values varied between regions (range, CT 34.99; CT 40.15; CT 34.73; CT 40.93; CT 34.71; CT 42.95) (Figure 6). Also, melting peak analysis confirmed the specificity of the RT-qPCR primers, demonstrating no amplification of non-specific products (Fig.7).

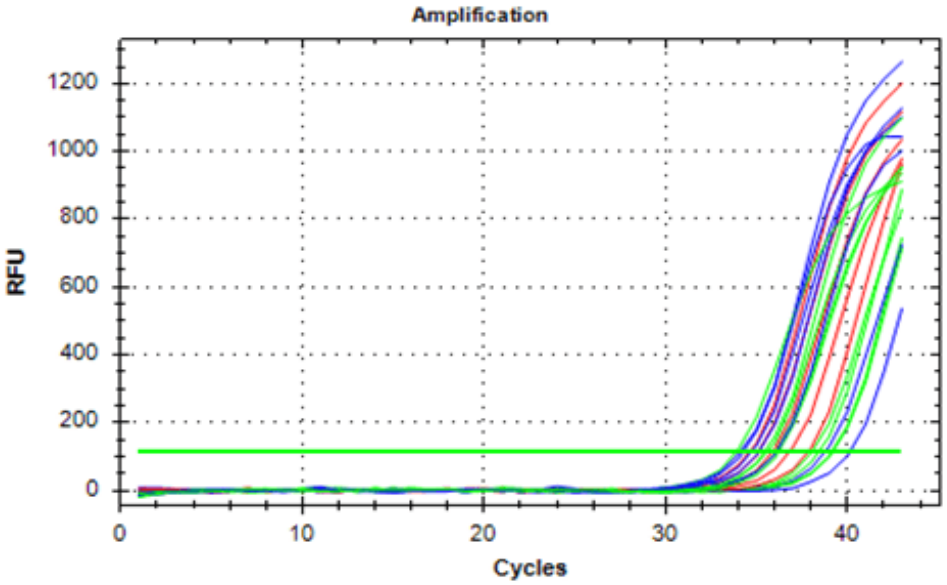

Fig. 6. the Real Time amplification plots for ADAM28 gene in experimental rat epididymis tissue. The green plots: Caput tissue samples , the blue plots: Corpus tissue samples, and The red plots: Cauda tissue samples

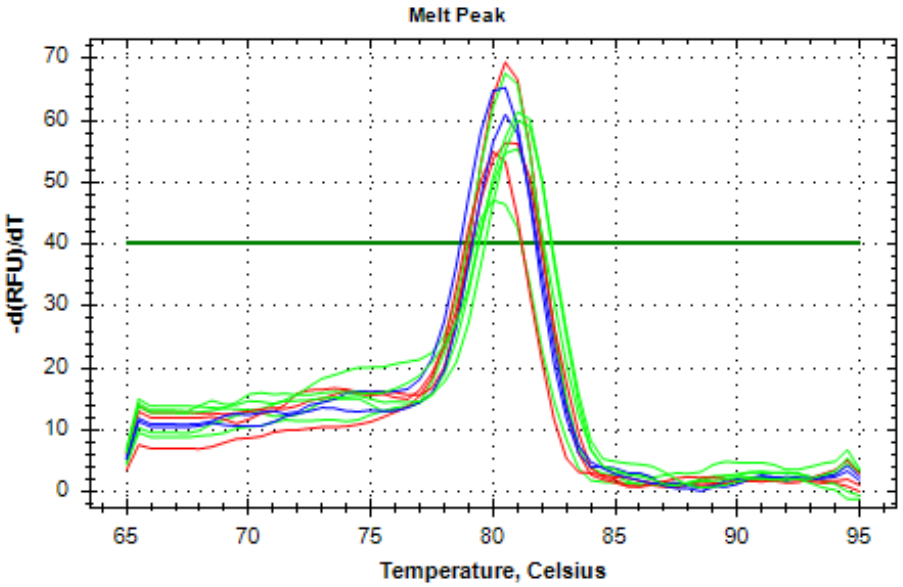

Fig . 7. The Real Time melting curve for ADAM28 gene in experimental rat epididymis tissue that showed qPCR product specify melting peak at Tm: 80.50°C.

The RNA transcription levels of the ADAM28 gene were measured in the tissues of the rat epididymis segments (caput, corpus, and cauda) at 66 days of age. We were able to demonstrate in this study that the ADAM28 protein is present in the epididymis regions at different concentration levels, with mean values of 0.16±0.06; 0.48±0.22; and 0.21±0.09 in the caput, corpus, and cauda, respectively (Table 3). On the other hand, prior research conducted by [14] [24] revealed that ADAM28 gene expression was limited to the epididymal caput

Our study's findings, which demonstrate that ADAM28 was found in every segment, suggests that it may function as an active regulator influencing the majority of cells in the epididymis segment's proliferation and specific differentiation [26]. Different epididymal sub-compartments exhibit varying levels of protein expression, indicating that each sub-region may have a unique regulatory effect on spermatozoa maturation [4]. This finding comparable with [51] who shown the epididymis segment specific genes

(Table 3). Displayed RT-qPCR Analysis of the ADAM28 gene in epididymis segment. At P < 0.05 significant level.

Experimental sample	Target ADAM28	HKG GAPDH	ΔCT	Gene expression ratio (ΔCT method)	Mean and St. error for Exp. Sample
Caput 1	36.06	34.31	-1.75	0.297	0.16±0.06
Caput 2	34.99	33.39	-1.60	0.330	
Caput 3	40.15	32.58	-7.57	0.005	
Caput 4	36.43	33.80	-2.63	0.162	
Caput 5	37.86	32.52	-5.34	0.025	
Corpus 1	35.93	33.72	-2.21	0.216	0.48±0.22
Corpus 2	34.73	33.38	-1.35	0.392	
Corpus 3	34.90	35.32	0.42	1.338	
Corpus 4	36.49	35.39	-1.10	0.467	
Corpus 5	40.93	32.84	-8.09	0.004	
Cauda 1	34.71	33.51	-1.20	0.435	0.21±0.09
Cauda 2	35.44	32.90	-2.54	0.172	
Cauda 3	34.93	33.83	-1.10	0.467	
Cauda 4	37.72	32.23	-5.49	0.022	
Cauda 5	42.95	32.84	-10.11	0.001	

However, the ratio in the corpus was higher than that in the cauda and caput, respectively, was no significant difference in ADAM28 expression levels between the three regions of the epididymis at ($P < 0.05$); whereas the mean expression of ADAM28 protein in the epididymal cauda, was higher than that in caput (Table 3). The function of each segment may be related to this variance in ADAM28 protein concentration. The early and late stages of sperm maturation are attributed to the caput and corpus, respectively. The corpus form two thirds of the epididymis duct that is necessary for late sperm maturation, whereas proteins important in adhesion pathways and cell motility are found in the cauda of the epididymis [4]. Additionally, the primary purpose of the efferent duct and epididymal caput segment, according to certain research, to absorb the protein secreted by the seminiferous tubules in the testes [52], nevertheless, there was a noticeable variation in the quantity of upregulated and downregulated proteins between intersegments since the proteins were not stable in the caput [4]. Other research suggests that since water is absorbed by the epididymal duct, lower protein concentration in the caput epididymis would have been estimated [53]. Additionally, the cauda epididymis contains between 50 -80 percent of the sperm in the epididymal lumen, where functionally mature sperm cells are kept following early maturation in the caput. The release of chemicals by the cauda's epithelial cells aids in maintaining the ultimate luminal environment for sperm quiescence during storage, this helps to explain why the corpus and cauda exhibit higher levels of ADAM28 detection than that caput. These variables influence the regulation of luminal pH, and certain proteins and enzymes may have a comparable effect [8].

In humans, ADAM28 was primarily expressed by lymphocytes and epithelial cells; in mice, however, it is expressed as immune localize epithelial cells in various tissues like epididymal and thymic epithelial cells [25] and According to [54], the amino acid sequence in the metalloprotease active site of ADAM 28 is suitable for the protein's functional protease activity; as a result, it has defense capabilities that keep sperm safe during development. [55] mention the immunoglobulins which are heterodimeric, membrane-bound, or secreted glycoproteins that are mostly expressed in the vicinity of the corpus are produced by B lymphocytes, this may be explain why the mean expression of ADAM28 protein in the epididymis corpus was higher than that of other segment in our study.

4. Conclusion

The current study showed the ADAM28 gene was found in every epididymis segments (caput, corpus and cauda) and the corpus was a higher ADAM28 gene expression ratio than that in the cauda and caput, with no significant difference between the three epididymis regions.

Acknowledgement

The authors thank the Department of Anatomy and Histology, College of Veterinary Medicine, University of Al-Qadisiyah, for its assistance with this research.

Conflict of interest

Regarding the current work, the authors declare that there is no conflict of interest.

References

[1] Zhou W, De Iuliis GN, Dun MD, Nixon B. Characteristics of the epididymal luminal environment responsible for sperm maturation and storage. Front Endocrinol. (Lausanne) 2018; 28:9:59.

[2] Gervasi MG, Visconti PE. Molecular changes and signaling events occurring in spermatozoa during epididymal maturation. Androl. 2015;5 (2):204-218. Doi: 10.1111/andr.12320.

[3] Elfgen V, Mietens A, Mewe M, Hau T, Middendorff R. Contractility of the epididymal duct: function, regulation and potential drug effects. Reproduction 2018; 156(4): R125-R141. Doi: 10.1530/REP-17-0754.

[4] Zhao J, Zhai X, Ma Y, Zhang T, Wang Z, Chong T . Anatomic characteristics of epididymis based on histology, proteomic, and 3D reconstruction. Androl 2020 8:1787–1794. https://doi.org/10.1111/andr.12842.

[5] Tavares DAF. The epididymis as a target of endocrine disruption: a metabolic perspective (Doctoral dissertation). Universidade da Beira Interior, Covilhã. PhD diss. 2022. 30 /06 https://ubibliorum.ubi.pt/bitstream/10400.6/12538/1/9178_19547.pdf

[6] Turner TT, Johnston DS, Finger JN, Jelinsky SA. Differential gene expression among the proximal segments of the rat epididymis is lost after efferent duct ligation. Biol Reprod 2007;77:165-171. DOI 10.1095/biolreprod.106.059493.

[7] Sullivan R, Légaré C, Lamontagne-Proulx J, Breton S, Soulet D. Revisiting structure/functions of the human epididymis. Androl 2019;7 (5):748-757. Doi: 10.1111/andr.12633

[8] James ER, Carrell DT, Aston KI, Jenkins TG, Yeste M, Salas-Huetos A. The role of the epididymis and the contribution of epididymosomes to mammalian reproduction. International Journal of Molecular Sciences 2020;21(15):5377. https://doi.org/10.3390/ijms21155377

[9] Domeniconi RF, Souza AC, Xu B, Washington ANM, and Hinton BT. Is the epididymis a series of organs placed side by side? Biol. of Reprod. 2016; 95(1): 10.https://doi.org/10.1095/biolr eprod.116.138768.

[10] Souza APB, Schorr-Lenz AM, Lucca F, Bustamante-Filho C. The epididymis and its role on sperm quality and male fertility. Anim. Reproduction 2017;14 (Suppl.1):1234-1244. DOI: 10.21451/1984-3143-AR955

[11] Breton, S, Ruan, YC, Park, Y, Kim B. Regulation of epithelial function, differentiation, and remodeling in the epididymis. Asian J. Androl 2016;18 (1): p 3-9.DOI: 10.4103/1008-682X.165946

[12] Chan JC, Morgan CP, Adrian Leu N, Shetty A, Cisse YM, Nugent BM, Morrison KE, Jašarević E, Huang W, Kanyuch N, Rodgers AB, Bhanu NV, Berger DS, Garcia BA, Ament S, Kane M, Neill Epperson C, Bale TL. Reproductive tract extracellular vesicles are sufficient to transmit intergenerational stress and program neurodevelopment. Nat Commun 2020;11 (1):1499. Doi: 10.1038/s41467-020-15305-w.

[13] Barrachina F, Battistone MA, Castillo J, Mallofr C, Jodar M, Breton S, and Oliva R. Sperm acquire epididymis-derived proteins through epididymosomes. Human Reprod 2022; 37(4):651-668. Doi: 10.1093/humrep/deac015.

[14] Oh J, Woo J-M, Choi E, Kim T, Cho B-N, Park ZY, Kim YC, Kim DH, Cho C. Molecular, biochemical, and cellular characterization of epididymal ADAMs, ADAM7 and ADAM28. Biochem Biophys Res Commun 2005;331 (4):1374-83. Doi: 10.1016/j.bbrc.2005.04.067.

[15] Chen H, Alves MBR, Belleanne´e C. Contribution of epididymal epithelial cell functions to sperm epigenetic changes and the health of progeny. Human Reprod Update 2022; 28(1):51–66. doi:10.1093/humupd/dmab029

[16] Hubeau C, Rocks N, Cataldo D. ADAM28: Another ambivalent protease in cancer. Cancer Lett 2020;21:494:18-26. Doi: 10.1016/j.canlet.2020.08.031

[17] Li Y, Chen Y, Wu W, Li N, Hua j. MMPs, ADAMs and ADAMTSs are associated with mammalian sperm fate. Theriog., 2023;1:200:147-154. Doi: 10.1016/j.theriogenology.2023.02.013.

[18] Kumper M, Steinkamp J, Zigrino P. Metalloproteinases in dermal homeostasis. Am J Physiol Cell Physiol 2022;323 (4): C1290–C1303, doi:10.1152/ajpcell.00450.2021

[19] Bendavid G, Hubeau C, Perin F, Gillard A, Nokin M-J, Carnet O, Gerard C, Noel A, Lefebvre P, Rocks N, Cataldo D. Role for the metalloproteinase ADAM28 in the control of airway inflammation, remodeling and responsiveness in asthma. Front Immunol 2023;5:13:1067779. Doi: 10.3389/fimmu.2022.1067779.

[20] Lin Z, Wu T, Wang L, Dang S, Zhang W. ADAMTS18 deficiency leads to preputial gland hypoplasia and fibrosis in male mice. Reprod Biol 2021;21(3):100542. Doi: 10.1016/j.repbio.2021.100542

[21] Hongmei Qu, Khalil RA. Role of ADAM and ADAMTS disintegrin and metalloproteinases in normal pregnancy and preeclampsia Bioch Pharm 2022;206:115266. https://doi.org/10.1016/j.bcp.2022.115266

[22] Björkgren I, Sipilä P. The impact of epididymal proteins on sperm function. Reproduction 2019;158(5): R155-R167. Doi: 10.1530/REP-18-0589.

[23] Łukaszewicz-Zajac M, Dulewicz M, Mroczko B. Disintegrin and Metalloproteinase (ADAM) Family: Their Significance in Malignant Tumors of the Central Nervous System (CNS). Int. J. Mol. Sci., 2021;22(19):10378. https://doi.org/ 10.3390/ijms221910378

[24] Cho C. Testicular and epididymal ADAMs: expression and function during fertilization. Nat Rev Urol 2012;9(10):550-60. Doi: 10.1038/nrurol

[25] Lee JY, Park SW, Chang HK, Kim HY, Rhim T, Lee JH. A disintegrin and metalloproteinase 33 protein in patients with asthma: Relevance to airflow limitation. Am J Respir Crit Care Med 2006;173 (7):729-35. Doi: 10.1164/rccm.200409-1175OC

[26] Zhao Z, Wang Y, Wang D, Liu H. The Regulatory Role of a Disintegrin and Metalloproteinase 28 on the Biologic Property of Human Periodontal Ligament Stem Cells. J Periodontol 2010;81 (6):934-44. Doi: 10.1902/jop.2010.090703

[27] Yin Q, Gu J, Qi Y, Lu Y, Yang L, Liu J, Liang X. ADAM28 from both endothelium and gastric cancer cleaves von Willebrand Factor to eliminate von Willebrand Factor-induced apoptosis of gastric cancer cells. Europ J of Pharm 2021; 898: 173994.https://doi.org/10.1016/j.ejphar.2021.173994

[28] Ozeki N, Kawai R, Yamaguchi H, Hiyama T, Kinoshita K, Hase N, Nakata K, Kondo Q, Mogi M, Nakamura H. Retraction notice to “IL-1β-induced matrix metalloproteinase-13 is activated by a disintegrin and metalloprotease-28-regulated proliferation of human osteoblast-like cells “. Exp. Cell Res 2020; 397(1): 112339. https://doi.org/10.1016/j.yexcr.2020.112339

[29] Drevet JR. Epididymal approaches to male contraception. Basic and Clinical Androl 2018; 28:12. https://doi.org/10.1186/s12610-018-0078-y

[30] Yunaini L, Pujianto DA. Conditioned medium and secretome from epididymal epithelial cell cultures improve sperm kinetics and capacitation. Vet World 2023;16 (6): 1325–1332. Doi: 10.14202/vetworld

[31] Ashrafzadeh A, Karsani SA, Nathan S. Mammalian sperm fertility related proteins. Int J Med Sci. 2013;10 (12):1649-57. Doi: 10.7150/ijms.6395

[32] Kathrins M. Original descriptions of the relationship between epididymal function and sperm morphology. Fertil Steril 2017;108 (1):45-46. Doi: 10.1016/j.fertnstert.2017.05.023.

[33] Favareto AP, Fernandez CD, da Silva DA, Anselmo-Franci JA, Kempinas WD. Persistent impairment of testicular histology and sperm motility in adult rats treated with cisplatin at peri-puberty. Basic & clinical pharmacology & toxicology 2011;109 (2):85-96.DOI: 10.1111/j.1742-7843.2011.00688.x

[34] Ilbey YO, Ozbek E, Cekmen M, Simsek A, Otunctemur A, Somay A. Protective effect of curcumin in cisplatin-induced oxidative injury in rat testis: mitogen-activated protein kinase and nuclear factor-kappa B signaling pathways. Human reproduction 2009; 24(7):1717-25. DOI: 10.1093/humrep/dep058

[35] D 'Souza UJ. Toxic effects of 5-fluorouracil on sperm count in wistar rats. The Malaysian journal of medical sciences 2003;10 (1):43-5.

[36] Luna LG. Manual of Histologic Staining Methods of the Armed Forces. Institute of Pathology 3 rd. 1968.p258p. Ed.McGraw-Hill Book Company.New York

[37] Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C_T method. Nat Protoc 2008;3 (6):1101-8. Doi: 10.1038/nprot.2008.73.

[38] Reddy MV. Statistical methods in psychiatry research and SPSS.2nd Edition 2019. Oakville, pp.336 Apple Academic Press

[39] Cornwall GA. New insights into epididymal biology and function. Human reproduction update 2009;15 (2):213-27. DOI: 10.1093/humupd/dmn055

[40] Souza APB, Lopes TN, da Silva AFT, Santi L, Beys-da-Silva WO, Yates 3rd JR, Bustamante-Filho IC. Changes in porcine cauda epididymal fluid proteome by disrupting the HPT axis: Unveiling potential mechanisms of male infertility. Mol Reprod Dev 2020;87 (9):952-965. DOI: 10.1002/mrd.23408

[41] Menad R, Fernini M, Lakabi L, Smaï S, Gernigon-Spychalowicz T, Farida K, Bonnet X, Moudilou E, Exbrayat JM. Androgen and estrogen receptors immunolocalization in the sand rat (Psammomys Obesus) cauda epididymis. Acta Histochemica 2021;123 (2):151683. https://doi.org/10.1016/j.acthis.2021.151683

[42] Da Silva N, Barton CR. Macrophages and dendritic cells in the post-testicular environment. Cell and Tissue Research 2016;363:97–104. Doi: 10.1007/s00441-015-2270-0

[43] Adebayo AO, Olurode SA. The morphology and morphometry of the epididymis in the greater cane rat (Thryonomys swinderianus Temmincks). Folia Morphol (Warsz) 2010; 69(4):246–252 https://pubmed.ncbi.nlm.nih.gov/21120812/.

[44] Rinaldi VD, Donnard E, Gellatly K ,Rasmussen M ,Kucukural A ,Yukselen O, Manuel Garber M, Sharma U , Rando OJ.An atlas of cell types in the mouse epididymis and vas deferens .eLife 2020; 9: e55474. Doi: 10.7554/eLife.55474.

[45] Breton S, Nair AV, Battistone MA. Epithelial dynamics in the epididymis: role in the maturation, protection, and storage of spermatozoa. Androl 2019;7 (5):631-643. Doi: 10.1111/andr.12632.

[46] Chattaraj S, Basu S, Datta U, Ray K, Sarkar U. ROLE OF CHOLESTEROL IN REGULATION OF SPERM MATURATION IN EPIDIDYMAL MICRO-ENVIRONMENT OF LARGE WHITE

YORKSHIRE BOAR (SUS SCROFA DOMESTICUS). World Journal of Pharmacy and Pharmaceutical Sciences 2018;7 (7): 1128-1139. DOI: 10.20959/wjpps20187-11982.

[47] Shum W, Zhang BL, Cao AS, Zhou X, Shi SM, Zhang ZY, Gu LY, Shi S. Calcium Homeostasis in the Epididymal Microenvironment: Is Extracellular Calcium a Cofactor for Matrix Gla Protein-Dependent Scavenging Regulated by Vitamins. Front Cell Dev Biol 2022;10:827940. Doi: 10.3389/fcell.2022.827940

[48] Sullivan R, Frenette G, Girouard J. Epididymosomes are involved in the acquisition of new sperm proteins during epididymal transit. Asian J Androl 2007; 9:483–491. Doi: 10.1111/j.1745-7262.2007.00281.

[49] Breton S, Brown D. Regulation of luminal acidification by the V-ATPase. Physiology (Bethesda) 2013; *28*(5):318–329. Doi: 10.1152/physiol.00007.2013.

[50] Knoblaugh S, True L**.** Male reproductive system. In: Comparative Anatomy and Histology: A Mouse, Rat, and Human Atlas. 2e (eds. P.M. Treuting and S.M. Dintzis). 2018; 335 – 363. London: Academic Press **https://doi.org/10.1016/B978-0-12-802900-8.00018-**

[51] Huang Y. Li X, Sun X, Yao J, Gao F, Wang Z, Hu J, Wang Z, Ouyang B, Tu X, Zou X, Liu W, Lu M, Deng C, Yang Q, Xie Y. Anatomical Transcriptome Atlas of the Male Mouse Reproductive System During Aging. Front Cell Dev Biol 2022; 9:782824. Doi: 10.3389/fcell.2021.782824

[52] Dacheux JL, Dacheux F. New insights into epididymal function in relation to sperm maturation. Reproduction 2014;147 (2): R27-42. DOI: 10.1530/REP-13-0420

[53] Robaire B, Hinton B T. The epididymis. In Plant TM. Zeleznik AJ. (Eds.), Knobil and Neill's physiology of reproduction 2015; (pp. 691– 771). New York, NY**: Elsevier. http://store.elsevier.com/**

[54] Lin Y-C, Sun H-G, Lee Y-M, Goo Y-W, Liu, H-W. Cloning and Characterization of a Complementary DNA Encoding a Human Epididymis-Associated Disintegrin and Metalloprotease 7 Protein. Biol Reprod 2001;65 (3):944-50. Doi: 10.1095/biolreprod65.3.944.

[55] Schroeder HW Jr, Cavacini L. Structure and function of immunoglobulins. Allergy Clin Immunol 2010;125 (2 Suppl 2): S41-52. Doi: 10.1016/j.jaci.2009.09.046.