OPEN ACCESS Freely available online

Andrology-Open Access

Research Article

Accurate Epididymal Anatomy is the Basis for the Study of Epididymal Function

Jun Zhao*, **Tie Chong**

Department of Urology, The Second Affiliated Hospital, Xi'an Jiaotong University, Shaanxi, Xi'an 710016 P.R. China

ABSTRACT

The epididymis is a tiny but critical organ that connects the testicles to the vas deferens, mediating the discharge of mature sperm from the testes. Sperm maturation processes in the epididymis are essential for natural reproduction. The anatomy of the epididymis has been controversial, with differing epididymal compartmentalization schemes and testing indicators leading to different results. Stable and reproducible partitioning methods and sensitive, high-specificity technologies are needed to clarify the sperm-regulating mechanisms of the epididymis.

Keywords: Epididymal; Proteomic; Sperm; Testes; Anatomy

INTRODUCTION

Mammalian spermatozoa are released from the testes to the epididymis in an immature immobile state. As they travel through the epididymis, they undergo a series of well-orchestrated biochemical modifications, which are necessary for achieving *in vivo* fertilization, under the regulation of epididymal proteins. Underlying sperm maturation processes, there is a segment-specific epididymis regulated profile wherein each segment exhibits a combination of distinct and common regulators of gene and protein expression and signaling events [1-3]. Consequently, elucidating epididymal function requires accurate and reproducible epididymis partitioning methods.

The classical anatomic division of the epididymis yields three or four major segments, a testis-proximal segment known as the caput or efferent duct and caput, an elongated corpus segment, and a distal segment known as the cauda. Mouse and rat epididymides have been further divided into 10 and 19 segments, respectively [1]. Recommended partitions of the human epididymis have varied across studies. Some partitioning dividing the human epididymis into the efferent duct, caput, corpus, and cauda regions, with subtle differences in zone boundaries being suggested across research groups [4,5]. Other researchers adopt a simpler scheme, dividing the human epididymis into caput, corpus, and cauda regions without providing clear descriptions of how these three segments were defined [6-8]. This variability has been attributed to the fact that the inter-segment septa are largely incomplete with substantial inter-individual variability among specimens. The lack of clear inter-segment divisions in the human duct has made functional analyses challenging. Different ways of thinking about epididymal functions may provide new testable hypotheses about the sperm maturation process [9].

MATERIALS AND METHODS

Proximal epididymis research and controversy

Whereas the initial segment, caput, corpus, and cauda of the epididymis are separated by obvious septa in rodents, the human duct does not have clear divisions between successive epididymal regions. The aforementioned 10 identified mouse epididymal regions and 19 identified rat epididymal regions have septa that are well organized with divisional locations along the organ that are consistent across individuals. In contrast, the human epididymis septa are not organized in a manner that permits unambiguous definition of epididymal segments. For the most part, they are incomplete, and show great variability from one specimen to another.

To compensate for the lack of distinct anatomic divisions in the human epididymis, Sullivan et al. have used immunohistochemistry in longitudinal sections to distinguish between cell types of the efferent duct and cell types of the caput epididymis epithelia. They divided the epididymis into eight segments based on microarray and gene ontology analyses.

Correspondence to: Jun Zhao, Department of Urology, The Second Affiliated Hospital, Xi'an Jiaotong University, Shaanxi, Xi'an 710016 P.R. China, Tel: 86-18182661736; E-mail: zhjdoc@xjtu.edu.cn

Received: 20-Sep-2023, Manuscript No. ANO-23-27065; **Editor assigned:** 22-Sep-2023, PreQC No. ANO-23-27065 (PQ); **Reviewed:** 06-Oct-2023, QC No. ANO-23-27065; **Revised:** 10-Jan-2025, Manuscript No. ANO-23-27065 (R); **Published:** 17-Jan-2025, DOI: 10.35248/2167-0250.25.14.338

Citation: Zhao J, Chong T (2025) Accurate Epididymal Anatomy Is the Basis for the Study of Epididymal Function. Andrology. 14:338.

Notably, the proximal epididymis is composed of efferent ducts that exhibit a specific cellular signature that is distinct from that observed in the adjacent epididymis tubule. The gene expression pattern of the caput segment of the human epididymis is homogeneous and less segmented than that documented in rodent species.

Considering the diversity in the structure of the human proximal epididymis, and the difficulty inherent in making a precise dissection of the caput tissue, Leir et al. have used single cell RNA-sequencing to differentiate the roles of principal, apical, narrow, basal, clear, halo, and stromal cells in the epididymis. However, because cells interact with neighboring and downstream cells *via* paracrine mechanisms, researchers need to identify regulatory mechanisms by which different cell types within each region communicate or cross-talk with one another to elucidate functional biology.

RESULTS AND DISCUSSION

With the aim of establishing a simple method for partitioning the human epididymis that is easy to implement and provides a consistent research substrate, we reconstructed 3-D epididymal tubules from 7 μm-thick transverse serial sections of an epididymis. Based on the reconstructed results and armed with the surgical tabletop microscope, 20 subsegments pre epididymis were divided and same subsegment tissues from eight human epididymites were analyzed by non-labeled sequential window acquisition of all theoretical spectra mass spectra (SWATH MS) inclusive of all potential fragment divisions [10]. To ensure the quality of protein analysis, each epididymal sample was divided into 20 segments under a surgical tabletop microscope; the resultant tissue specimens coming from the same segment were submitted to SWATH MS analysis. Some variation in the same segment was observed across specimens, including variation in the clarity of inter-segment septa (e.g. clarity of the distal end of the first segment), variation in tissue quantities within locations, and variation in completeness of connective tissue wrapping. Notwithstanding, inter-segment divisions could be identified consistently under a surgical tabletop microscope, even in the proximal epididymis. We found that accurate and reproducible epididymal zonation could be achieved with a surgical microscope.

In order to understand the anterior-posterior sequence of the different segments of the epididymis, the distance to this segment was determined by the amount of up or down-regulated protein change in the same sub-segment of epididymal protein compared to other epididymal sub-segmental proteins. The criteria for a protein to be considered an inter-segment DEP were a ≥ 1.5-fold greater quantity to be considered up-regulated and a ≤ 0.67-fold lesser quantity to be considered down-regulated (Figure 1A).

Inter-segment variation within the proximal epididymis was highly variable. DEPs were identified by comparing each of seven segments (I–VII) of the proximal epididymis with each of the other six segments; the quantities obtained for each comparison are shown in Figure 1C. A smaller number of DEPs between segments was assumed to be an indicator of segment adjacency. Accordingly, our data indicate that spermatozoa discharged from the testis flow from the caput region labeled as segment II (Cap-II) to Cap-V, then to Cap-III and IV, and then to Cap-I and VI, and finally to Cap-VII, after which they would be expected to proceed to the most proximal segment of the corpus (Figure 1B). For example, there are fewer DEPs between Cap-II and Cap-V (n=103, including 60 up-regulated and 43 down-regulated) than between Cap-II and any of the other caput segments, leading to the inference that Cap-II is adjacent to Cap-V. Meanwhile, Cap-V had even fewer DEPs with Cap-III (n=78) and Cap-IV (n=67) than with Cap-II, leading to the inference that Cap-V is also adjacent to Cap-III and IV. The inferred most likely progression of flow is shown with arrows and numbers in Figure 1B; for validation, the ordering inferred here should be compared to results from further studies employing histological, genetic, and other methods.

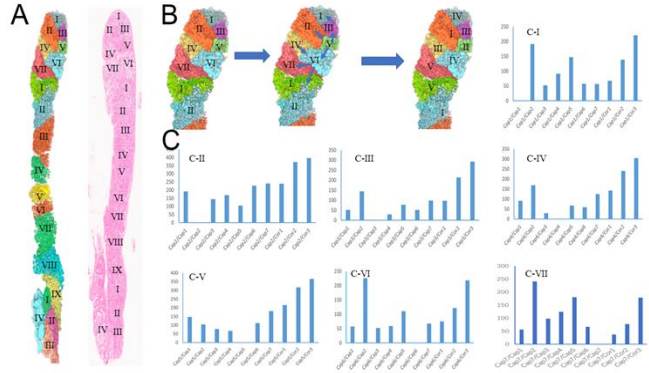

Figure 1: A. Immunohistochemical section of human epididymis (right) and pseudo-colored 3-D reconstruction of epididymal tubules (left). B. Inferred order of spermatozoa flow through the proximal epididymis following discharge from the testes determined based on quantitation of differentially expressed proteins (DEPs) between segments. C. Comparisons of DEPs between proximal epididymis segments. Each bar graph shows the quantity (y-axis) of DEPs (*i.e.* proteins that are differentially expressed, up-regulated or down-regulated) between pair-wise comparisons of segments. The quantities of DEPs between segment I of the caput and each of the other segments of the caput is shown in graph C-I, and so on, for each segment.

Although there were substantial quantities of DEPs in the proximal epididymis segment compared to those in the corpus and caudal segments, there were relatively few DEPs among the segments of the corpus. It is noteworthy that DEP quantities among segments of the efferent ducts, caput, and corpus differed from DEP quantities obtained for caudal segment comparisons, suggesting that the cauda epididymis is not likely to be a sperm storage site [11].

CONCLUSION

The human epididymis differs from that of laboratory rodents in that it lacks an initial segment and has a proximal region that is occupied by efferent ducts and an adjacent caput epididymis segment. Using a surgical tabletop microscope, septa between human epididymis segments were easily recognized. Our ability to obtain consistent segmentation across samples from eight

individuals indicates that our procedure is reproducible. By analyzing DEPs between segments, we can infer the order of sperm flow through the epididymal tubules. There remains a need for sensitive and efficient methods for the detection of DEPs of more subtle distinction across segments.

FINANCIAL DISCLOSURES

This work was supported by social development science and technology research project (2023-YBSF-614).

FUNDING/SUPPORT AND ROLE OF THE SPONSOR

None.

ACKNOWLEDGMENT STATEMENT

None.

AUTHOR CONTRIBUTIONS

Jun Zhao had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Analysis and interpretation of data: Jun Zhao, Tie Chong.

Drafting of the manuscript: Jun Zhao.

Critical revision of the manuscript for important intellectual content: Jun Zhao, Tie Chong.

Obtaining funding: Jun Zhao.

Administrative, material support and supervision: Tie Chong.

REFERENCES

1. Jelinsky SA, Turner TT, Bang HJ, Finger JN, Solarz MK, Wilson E, et al. The rat epididymal transcriptome: comparison of segmental gene expression in the rat and mouse epididymides. Biol Reprod. 2007;76(4):561-570.
2. Lagarrigue M, Lavigne R, Guevel B, Palmer A, Rondel K, Guillot L, et al. Spatial segmentation and metabolite annotation involved in sperm maturation in the rat epididymis by MALDI imaging mass spectrometry. J Mass Spectrom. 2020;55(12):e4633.
3. Legare C, Sullivan R. Differential gene expression profiles of human efferent ducts and proximal epididymis. Andrology. 2020;8(3): 625-636.
4. Leir SH, Yin S, Kerschner JL, Cosme W, Harris A. An atlas of human proximal epididymis reveals cell-specific functions and distinct roles for CFTR. Life Sci Alliance. 2020;3(11):e202000744.
5. Sullivan R, Legare C, Lamontagne-Proulx J, Breton S, Soulet D. Revisiting structure/functions of the human epididymis. Andrology. 2019;7(5):748-757.
6. Dube E, Chan PT, Hermo L, Cyr DG. Gene expression profiling and its relevance to the blood-epididymal barrier in the human epididymis. Biol Reprod. 2007;76(6):1034-1044.
7. Thimon V, Koukoui O, Calvo E, Sullivan R. Region-specific gene expression profiling along the human epididymis. Mol Hum Reprod. 2007;13(10):691-704.
8. Zhang JS, Liu Q, Li YM, Hall SH, French FS, Zhang YL. Genome-wide profiling of segmental-regulated transcriptomes in human epididymis using oligo microarray. Mol Cell Endocrinol. 2006;250(1-2):169-177.
9. Domeniconi RF, Souza AC, Xu B, Washington AM, Hinton BT. Is the Epididymis a Series of Organs Placed Side By Side?. Biol Reprod. 2016;95(1):10.
10. Zhao J, Zhai X, Ma Y, Zhang T, Wang Z, Chong T. Anatomic characteristics of epididymis based on histology, proteomic, and 3D reconstruction. Andrology. 2020;8(6):1787-1794.
11. 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. Int J Mol Sci. 2020;21(15): 5377.