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ARTIFACTS OF CLASSICAL HISTOLOGICAL TECHNIQUES AT THE TESTICULAR SECTIONS IN THE WISTAR RAT

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ABSTRACT

The objective of this study was to identify the artifacts of classical methods of tissues study at the testicular sections in adult rat. 4 testes were taken from 2 adult Wistar rats at 12 weeks of age. Samples were taken under ether inhalation anesthesia. The gonads were fixed in 10% saline formalin and dehydrated in alcohol and then embedded in paraffin. The transverse sections made by a microtome were spread out on slides. The histologycal sections were colored by hematoxylin-eosin and then covered with coverslips before optical microscope analysis. Observation of the histological sections showed different types of degradation caused by the technique. These are the formation of artificial voids in the seminiferous tubules, the tearing of the tunica albuginea, the destruction of part both interstitial tissue and peritubular sheath of the seminiferous tubules. Dye deposits were also observed. From the collection of tissue samples to observation, there are many steps that are likely to generate alterations. Therefore, proper handling as well as prompt fixation and careful tissue processing will reduce these artifacts.

KEYWORDS: Artifacts, Histological testicular sections, Optical microscope, Wistar rats.

INTRODUCTION

Classical histological techniques are used for preparatory treatments that make possible microscopic observation for the morphological and functional study of tissue elements. The classic methods for studying tissues (sampling, fixation, inclusion, sectioning, staining, mounting) aim to obtain thin, transparent histological sections that can be observed under an optical microscope.[1,2]

Artifacts refer to certain types of image degradation generally directly related to the type of technique used during routine histological preparation of sections. In other words, they are histological alterations inherent in the methods of tissues study. The problem is to recognize them when they occur and not to confuse them with normal tissue components or pathological changes. In certain situations, the presence of an artifact can compromise the interpretation or even a precise diagnosis.[1,3,4,5,6] In a previous study, artifacts were observed on testicular sections 5 micrometers thick in pups.[7]

The objective of this study was to identify the artifacts of the classical methods of tissues study at the testicular sections in the adult rat.

MATERIAL AND METHODS

Biological material

The biological material concerned 4 testes of 2 adult Wistar rats at 12 weeks of age, from the animal facility of the Physiology, Pharmacology and Pharmacopoeia laboratory of the Nangui Abrogoua University of Abidjan in Côte d'Ivoire. Good laboratory practices and the various experimental protocols were followed in accordance with the instructions for the protection of experimental animals of the European Legislation Council 87/609/EEC.[8]

Sample

The dissection was performed under general ether anesthesia by inhalation under a bell. The testes were removed after an inguinal incision.

Histological processing

The collected gonads were fixed in 10% saline formalin for 72 hours. After fixation, the testes have been dehydrated in alcohol baths of increasing concentrations (70%, 95%, 100%), clarification in toluene, impregnation and inclusion in paraffin (melting point: 55- 57°C). Five micron thick sections were made with a paraffin microtome (Leica). They were spread on slides (76 x 26mm, Fisher). Hydration was performed in three alcohol baths of decreasing degrees (100%, 90% and 70%), followed by washing with distilled water.

Histological sections were colored by hematoxylin-eosin for 5 minutes. They were then rinsed with running water and then immersed in the 1% eosin solution for 7 minutes. They were finally rinsed with running water.

Immediately after staining, the slides are soaked in three baths of absolute alcohol then in two baths of toluene (dehydration).

The assembly was carried out between slide and coverslip with Eukitt.[2,9,10]

Optical microscope observation and photos taking

The sections were observed under an optical microscope. The photomicrographs were taken using a digital camera (Canon 12 Megapixels).

RESULTS

The results focused on the different types of alterations observed on testicular histological sections and their locations.

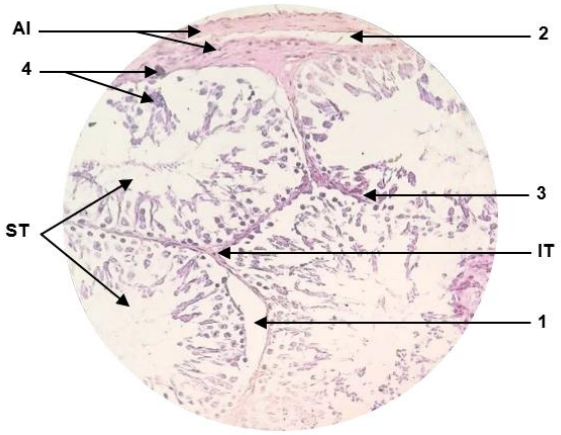

Figure : Transverse histological section at the testis of 12-week-old rat. (Hematoxylin-eosin, OM x 40). Albuginea (Al), seminiferous tubule (ST), Interstitial tissue (IT) between the seminiferous tubules. Histological alterations: (1) = Artificial void in the seminiferous tubule, (2) = Tearing of the tunica albuginea, (3) = Destruction of part both interstitial tissue and peritubular sheath of the seminiferous tubules, (4) = Dye deposits at the tunica albuginea and the seminiferous tubule.

DISCUSSION

A set of particularities related to the rat made it possible to adopt it for this study. Anatomically, the rat testis has a similarity to that of human.[11] The rat gonad is used for studies in the biomedical field.[12,13,14]

From the removal of the testes to the observation, there are many steps that are likely to generate alterations or degradation of the tissue.[15,16] In this work, several types of degradation were localized at the testicular histological sections. These are the formation of artificial voids in the seminiferous tubules, the tearing of the tunica albuginea, the destruction of part both interstitial tissue and peritubular sheath of the seminiferous tubules. Dye deposits were also observed.

To explain the appearance of these alterations, data reported that during the sampling, several artifacts resulting from the gesture and the mechanical effects (manipulation of the organs with pliers and scissors) could have caused crushing and holes.[3,7,16,17]

The possible retraction of the tissues or cells due to the passage of the tissue samples in the solutions (formaldehyde, alcohol, toluene) was noted during the fixation, dehydration and clarification stages; at impregnation it was caused by heat. This retraction would be responsible for the artificial voids.[3]

The gonads removed in this work were immediately placed in a sufficient quantity of 10% saline formalin for fixation and to prevent post-mortem decomposition.[15,18] However, fixing defects with formalin could also be explained by the fact that this compound contains non-coagulant agents making the fixing of the pieces progressive and requiring longer fixing times[3]. In addition, the quality of fixation with formalin is often judged to be poor for certain tissues, such as muscle, bone and testis, the architecture of which often appears to be altered after cutting.[19] This disadvantage has also been observed in the case of the rat testis. Unlike formalin, Bouin's liquid is a fixative which contains coagulating agents allowing rapid fixing of the parts, which moreover explains the rapidity of obtaining a good consistency of samples is manifested an another study.[18]

The tearing of the tunica albuginea and the destruction of part of the peritubular sheath to the seminiferous tubules and then of the interstitial tissue resulted from the cutting step. They would be due to the microtome blade.[7,16,20] Nevertheless, the importance of the paraffin microtome lies in its usefulness, as it is an instrument that makes sections from tissues embedded in the paraffin blocks. This produces ribbons of thin sections, which are spread on glass slides. The thicknesses of the sections are variable according to the needs of the study.[7,16]

The dye deposits represent clumps of stain particles visible on the slide.[7,16] Other types of hematoxylin-eosin staining artifacts have been reported, namely too pale staining of the nuclei and cytoplasm of cells.[3] Staining is an important step in the histological technique. Its purpose is to highlight the tissue elements and to differentiate between them.[1,10] It gives the possibility of studying the whole structure of the tissue or the

architecture of the lesions and, is the most commonly used stain.[3]

It is convenient to note that histology is interested in the microscopic structure of the tissues and cells composing an organism, in order to better understand and analyze their functioning. It thus allows the observation of the effects of certain parameters on the histology of the testis such as pathologies, for example varicocele, factors related to lifestyle, namely tobacco, alcohol, drugs and an unbalanced diet. Added to this is exposure to environmental pollutants.[21] These anomalies should not be confused with artifacts.

This work has shown artifacts inherent to conventional histological techniques in testicular sections in adult rats from 12-week-old.

To avoid the histological alterations observed by this work, during the removal, it is recommended to handle the organ carefully and not to expose the tissues to mediocre fixatives.[3,7,16] Fixation by perfusion would improve the conservation quality of the constituents (proteins in particular)[22,23]. It is very important to carry out the dehydration quickly, because too long a stay in alcohol would give too pale a color.[1] A damaged microtome blade should be replaced as it will not produce good cuts and a reduction in the cutting speed of the microtome is required.[7] Never let sections dry out when staining.[1]

CONCLUSION

From the collection of tissue samples to observation, there are many steps that are likely to generate alterations. Therefore, proper handling as well as prompt fixation and careful tissue processing will reduce these artifacts.

Conflict of interest

The authors declared no conflict of interest.

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