Morphology of Rat Testis Preserved in Three Different Fixatives

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Summary: Histopathological examination of testes is important in assessing spermatogenesis and testicular function. Modified Davidson’s fluid (mDF) has been proposed as a superior substitute for Bouin’s fluid (BF) for fixation of adult animal testes. Besides, 4% paraformaldehyde (PFA) has been commonly used to fix testes with convenience. We compared the morphology of the rat testis fixed in 4% PFA, mDF, or BF using hematoxylin and eosin (HE)-stained sections. Fixation in 4% PFA resulted in obvious tissue shrinkage artifacts, especially between seminiferous epithelium cells. Shrinkage artifacts were also observed in the central area of the testes fixed in BF. Use of mDF did not cause shrinkage artifacts between seminiferous tubules, though a small amount can be observed in seminiferous tubules between germ cells. Clarity of nuclear detail in testes fixed in mDF and BF is better compared to 4% PFA. Our study demonstrated that fixation in mDF provided better morphologic details in the rat testis as compared with 4% PFA and BF.

Key words: fixative; testis; HE staining

Histopathological examination of testes is important in assessing spermatogenesis and testicular function, both in clinical practice and laboratory experiments. Before histopathological examination is conducted, testes should be properly fixed. Clinically, it is relatively easy to fix testes as only a small volume is involved, while to fix bulky samples from the laboratory is much more difficult. The reason is that the testis cannot be trimmed to allow optimal penetration of a fixative and complete stabilization of histological features has been a challenging task, especially for animals with big testes, such as rats. Hence, an appropriate fixative is desirable. Generally, Bouin’s fluid (BF) is recommended as the fixative of choice [1]. However, modified Davidson’s fluid (mDF) has been proposed as a choice superior to formalin. Bouin’s fixatives for adult animal testes [2, 3]. Besides, 4% paraformaldehyde (PFA), a commonly used fixative, has been employed to fix testes with convenience. Perfusion fixation is ideal for preserving the morphology of testes, but is of limited use. In this study, we compared morphology of the rat testis fixed in 4% PFA, mDF, or BF by using hematoxylin and eosin (HE) stained sections, in order to find a better fixative for adult animal testes.

1 MATERIALS AND METHODS

1.1 Animals

Adult male Sprague-Dawley rats, weighing 250±50 g, were provided by the Animal Center of Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. The experimental protocols were approved by the Animal Care and Use Committee of Tongji Medical College, Wuhan, China.

1.2 Methods

After anaesthetized with an ip injection of pentobarbital (at 40 mg/kg), 10 rats were castrated and each testis was cut into two halves. One of the four pieces from one animal was used for other studies, while the other three were fixed in 4% PFA (4 g PFA + 100 mL pH 7.2–7.4 0.1 mol/L PBS), mDF (30% formaldehyde+15% ethanol+5% glacial acetic acid+50% distilled water, as been described previously[2]) and BF (75% picric acid+25% formaldehyde+5% glacial acetic acid), respectively for 24 h. Trimming was performed for the testes fixed in 4% PFA and mDF at the midpoint of the fixation. BF-fixed testes were trimmed after picric acid was removed by 70% ethanol rinses. After fixation, the carcasses were processed through graded alcohols, cleared in xylene, and embedded in paraffin. Five micrometer sections were obtained, de-paraffinized, and HE-stained for overall morphological evaluation.

2 RESULTS

2.1 Macroscopic Views

Testes fixed in 4% PFA were flesh-red-like, and the seminiferous tubules at the cutting edge were floating in the fixative. Trimming was hard as the testes were very soft even after 12 h fixation. However, testes fixed in mDF and BF turned white quickly after being put in the fixative and the seminiferous tubules were not floating, but frozen-like. Besides, it was much easy to trim testes to a proper shape. And the testes fixed in BF were in a unique yellowish color, which made them easy to be
distinguished (fig. 1).

Fig. 1 Macroscopic views of testes after different fixations with, from left to right, 4% PFA, BF and mDF

2.2 Microscopic Views

Grossly, testes fixed in 4% PFA had obvious tissue shrinkage artifacts, especially between seminiferous epithelium cells, which made interstitial tissues isolated (fig. 2). Shrinkage artifacts were also observed in BF-fixed testes, but they were mostly located in the central area of the specimen but not at the periphery (fig. 2). Broad interstitial spaces accompanied by small diameter tubules with numerous tubes lacking patent lumens characterized the shrinkage artifacts. There was no obvious shrinkage artifacts present in mDF-fixed testes (fig. 2).

Shrinkage artifact of seminiferous tubules and germ cells was found in all testes at different levels (fig. 2). Moreover, testes fixed in 4% PFA had a sharp shrinkage artifact, while with BF-fixation, the artifact was much less, and the artifact with mDF fixation was somewhere between them. Clarity of nuclear details in testes fixed in 4% PFA was relatively poor, which was improved in testes fixed in BF and mDF. Interstitial cells between seminiferous epithelium were clearly visible in 4% PFA fixed testes. But this was not usually true with testes fixed in BF and mDF due to the residual eosin stain.

Fig. 2 Rat testes preserved in different fixatives (HE)

Fixation with 4% PFA resulted in obvious tissue shrinkage artifacts, so did BF. mDF had a small amount of shrinkage artifacts in seminiferous tubules between germ cells, but had an overall better morphologic detail preservation compared to 4% PFA and BF.

3 DISCUSSION

To preserve testis, which is structurally different from some other tissues, it was necessary to fix it as a whole to maintain the delicate intra- and intertubular cellular relationships. Therefore, the penetrating nature of fixative is of prime importance. Out of the experiment requirements, we cut the testes in half and fixed them in 4% PFA, mDF and BF, respectively. Our results showed