

## Evaluation of the efficiency of two fasteners used in the preservation of the testicular parenchyma

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For a more detailed histological evaluation of the structures is necessary to an adequate tissue fixation. The main aims of fixing include the inhibition of tissue autolysis, tissue hardening, preservation of the different cellular components, improvement in the optical differentiation of tissues. The purpose of this study was to evaluate the efficiency of two types of fixatives (Formaldehyde and Bouin) in the preservation of buffaloes testes, as well as its influence on the following parameters: height of the seminiferous epithelium, diameter of seminiferous tubules and histological characterization of the seminiferous tubules. Testes fragments were placed in formaldehyde 4% and bouin solution for 24 hours. Thereafter they were dried and diaphanous in xylene and embedded in paraffin. Sections of 5 µm thick were stained with hematoxylin-eosin (HE). The testicular parenchyma of testes fixed with Bouin showed a lower tissue retraction. No significant difference was observed between the two fasteners on the values of the diameters of the seminiferous tubules, only the height of the seminiferous epithelium. The height of the seminiferous epithelium fixed by Bouin had a lower height ( $26.51 \pm 5.56$ ) compared to the fixed with formaldehyde ( $44.43 \pm 7.16$ ). According to the results, the solution formaldehyde can be used as a fixative with restrictions on histomorphometric analysis of buffaloes testes, and Bouin's solution best suited for these evaluations in the buffalo testis.

**Keywords** buffaloes; fixers; histomorphometry; testis

### 1. Introduction

The setting is one of the most important stages of histological technique, an inhibitory factor of cellular metabolism, which tends to stabilize the structures and biochemical intra- and extracellular components, preserving the tissue elements, but also allow the penetration of other substances following the fixing [1]. They act as coagulating protein and prevents cell apoptosis, which would damage the tissues for microscopic examination. They also have an antiseptic action, eliminating bacteria and other potential disease-causing agents, which would eventually threaten the health of handling such tissues [2]. The fasteners are the most commonly used buffered formalin and Bouin, both lay down the protein preventing its degradation. Formaldehyde, more accessible, is the most widely used fixative in histological techniques, although their results are mostly not satisfactory [3].

The choice of a fixation depends on the nature of the pathological process in this tissue, cell structure, or biochemical nature of the element to be preserved. However, one must know the different types of fasteners and to verify its compatibility with the various staining methods, with the property of the element tissue antigen [1].

Considering the study to assess possible changes in the processes involved in spermatogenesis [4], the analysis of morphological and morphometric parameters of the tubular housing in the testis appear to be a simple strategy for an effective diagnosis [5]. However, fixing the biological material is one of the most important steps in histological preparation, especially in the testis. In mammals, the testis is surrounded by a layer of dense connective tissue and the presence of lymphatic space hinder tissue fixation [6], which demand a specific methodology for fixing to a preservation and minimize artifacts posteriormente [7]. In consideration, this study aimed to test two types of fasteners, evaluating the effectiveness of each of the preservation of testicular structure and its influence on testicular morphometry of Murrah buffaloes, pondering the seminiferous tubule diameter and height of seminiferous epithelium.

### 2. Material and Methods

Murrah buffalo bulls were divided into two groups: G1 contained four 18-month-old males and G2 consisted of three 24-month-old males. The animals were obtained from the Department of Animal Production, Faculty of Veterinary Medicine, UNESP-Botucatu/SP and, raised under extensive management conditions. Buffaloes were fed *Brachiaria sp.* and mineral salt "ad libitum" and supplemented with corn silage in dry periods. The animals were, vaccinated and treated with vermifuge regularly.

Prior to collecting the testes, the animals were physically restrained and anesthetized [8] according to the ethical principles for animal experimentation as recommended by the Brazilian College of Animal Experimentation [9] and weighted in a mechanical balance (AÇORES® - ACR 1500, 4000 kg, Brazil).

Each testis was sectioned into three regions (the capitata, middle and end caudata portions) for tissue fixation. The middle portions of testicular parenchyma were later placed in Formaldehyde and Bouin's solution for at least 24 h. After dehydration in increasing concentrations of alcohol, followed by xylenes infiltrations, the tissue samples were embedded in paraffin [10]. A microtome (Leica RM2145, Berlin, Germany) was used to sections the tissue into slices 5-mm thick, and these were stained with hematoxylin- and eosin (HE). The samples were analyzed under a light microscope (Leica DM 2500)

Descriptive analyses of the mean and standard deviation for each testicular parameter were performed with the GraphPad Prism4 program (GraphPad Software Inc, USA).

### 3. Results and Discussion

The testicular samples were fixed by formaldehyde found empty spaces between the seminiferous tubules mostly, but also noted a tissue retraction of tubules in both ages analyzed, resulting in a decrease in the light of the seminiferous tubules (Figure 1a, 1c). The occurrence of the tissue retraction probably occurred by poor installation compared to tissue fixed in Bouin's solution (Figure 1b, 1d). According to France and Russell [7], the linear shrinkage in the inclusion of tissue is around 15% when the testes are embedded in paraffin and 3-5% when the material is fixed in plastic resin. The use of two fixatives showed that tissue fixation influences the retraction of the testicular parenchyma in Buffalos.

The analysis of morphometric parameters of the testis are extremely important because confirm quantitatively the morphological characteristics. Morphometric evaluations of testicular parameters as the diameter of the seminiferous tubules is a good indicator to determine the quality of ficadores. In Table 1 it can be observed using two fasteners two different ages and the height of the seminiferous epithelium. Although, as demonstrated in Table 1, the diameter of the seminiferous tubules did not differ significantly for the material fixed in formaldehyde solution in relation to the solution and Bouin. Several authors have analyzed the parameter settings morfom'etricos the testis as an important indicator of testicular development utilizadno different types of fasteners: pigs [11, marsupials [12; 13], cats [14]. These authors evaluated the cats and for larger diameter tubular material fixed in formaldehyde.

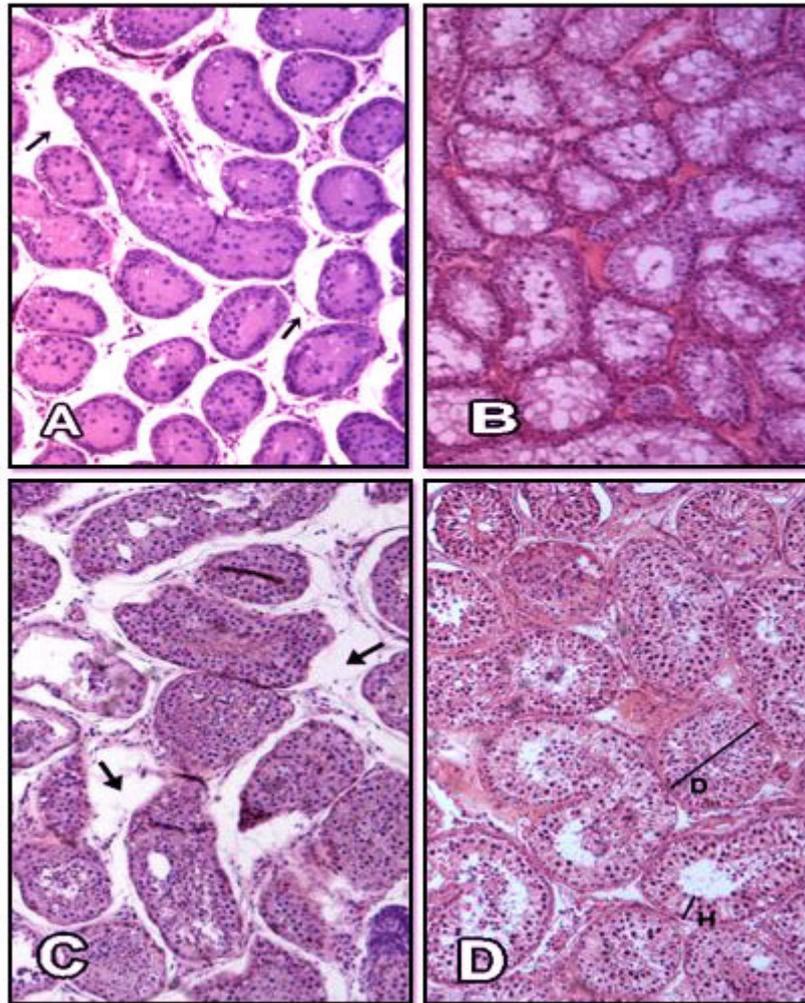
Data measured the height of the epithelium corroborate the findings in cats [14], whose material fixed in formaldehyde showed significant differences with those fixed in Bouin's solution (Table 1). The sample had a mean height of the seminiferous epithelium of  $44.43 \pm 7.16$  to  $26.51 \pm$  Formaldehyde and  $5:56$  for fixed in Bouin. França and Cardoso [11] report a range from 60 to 100  $\mu\text{m}$  at studies in domestic animals.

A height of the seminiferous epithelium, when using formaldehyde, can be explained by a decrease of testicular parenchyma, which causes a decrease in light of the seminiferous tubules and thereby increases its height in relation to the seminiferous epithelium tecidofixado with Bouin, as observed by Soares [14].

**Table 1.** Tubular diameter and height of the seminiferous epithelium of Murrah buffaloes for both types of fasteners.

Testicular parameters	G1		G2	
	Formaldehyde	Bouin	Formaldehyde	Bouin
diameter of seminiferous tubules	$79.56 \pm 7.87^a$	$77.60 \pm 6.54^a$	$150.28 \pm 14.76^a$	$153.06 \pm 16.11^a$
heigh of seminiferous epithelium	---	---	$44.43 \pm 7.16^a$	$26.51 \pm 5.56^b$

Different letters in the same row indicate significant difference ( $P < 0,05$ ).



**Fig 1.** Photomicrograph of the right testis (A, C) fixed with formaldehyde and left testis (B and D) fixed with Bouin, buffalo 18 months (A and B) and 24 months of age (C and D) in cross section. It is observed in the empty spaces (arrows), diameter (D) and height (H). HE staining, 100x.

#### 4. Conclusion

The results obtained in morphometric analysis using the two types of fasteners, it was demonstrated that there was no significant difference in parameter values for the diameter of the seminiferous tubule. The height of the seminiferous epithelium values of two types of fixing devices differ significantly. According to the results obtained, the formaldehyde solution can be used as fixing constrained to morphometric analysis of testes from buffaloes, Bouin's solution and the most suitable for these evaluations in buffalo testis and which can be used to fix other parenchymatous tissues of mammals.

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