The Methods Used in Histopathological Evaluation of Testis Tissues

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ABSTRACT

The quantitative measurements are numerical data as the outputs of various processes. Statistical analysis is employed to the collected data. Results obtained from the statistical data are considered as more reliable. Therefore, measurement processes are the basis of research sciences today. Histology is a discipline that examines the microscopic structure of cells and tissues. Morphometric measurements can be performed on cell and tissue samples through electron and light microscope. In the PubMed search engine, MEDLINE, SCIENCE DIRECT and Web of Science, Springer Link, and Ovid were scanned for the words "Testis, histopathology, quantitative, seminiferous tubule". The findings obtained through these qualitative methods provide interpretation of the changes in tissue morphology. These assessments allow researchers to identify the tissue samples and to compare the physiological variations in its morphology. In histology, the results

obtained from routine dyeing of laboratory studies are qualitative, therefore, relative differences may emerge in the interpretation of the results. In order to eliminate this risk, various quantitative measurement methods are implemented. Today, in order to employ accurate evaluations, the histometric and stereological measurement methods that are used in organs such as testicles, liver, lung, and kidney gain importance. The quantitative data obtained from these transactions is sufficient for statistical analysis. It is also important to reach a certain standardization level in the repetition of qualitative or semi-quantitative data obtained during the statistical analyses. The aim of this study is to summarize the methods used in histopathological evaluation of testicular tissues.

Key Words: Testis, Histopathology, Quantitative, Seminiferous Tubule

Testis Dokusunun Histopatolojik Değerlendirilmesinde Kullanılan Yöntemler

ÖZ

Kantitatif ölçümler, çeşitli süreçlerin çıktıları olan sayısal verilerdir. Toplanan verilerin istatiksel analizleri yapılmaktadır. Bu istatiksel verilerden elde edilen sonuçlar daha güvenilir olarak kabul edilmektedir. Bu nedenle günümüzde ölçüm işlemleri araştırma bilimlerinin temelini oluşturmaktadır. Histoloji, hücrelerin ve dokuların mikroskopik yapısını inceleyen bilim dalıdır. Testis, histopatoloji, kantitatif, seminifer tübül kelimelerini MEDLINE, SCIENCE DIRECT, Web of Science, Springer Link, Ovid, ve PubMed arama motorları kullanılarak literature taramaları yapıldı.Hücre ve doku örneklerinden elektron ve ışık mikroskobu kullanılarak morfometrik ölçümler yapılabilmektedir. Bu kalitatif yöntemlerle elde edilen bulgular doku morfolojisindeki değişikliklerin yorumlanmasını sağlamaktadır. Bu değerlendirmeler araştırmacıların doku örneklerini tanımasını ve morfolojisindeki fizyolojik varyasyonların karşılaştırılmasını sağlamaktadır. Histolojide yapılan laboratuvar çalışmalarında rutin boyamalarda elde edilen sonuçlar kalitatif olduğundan, sonuçların yorumlanması aşamasında görece farklılıklar ortaya çıkabilir. Bu riski ortadan kaldırmak için çeşitli kantitatif ölçüm yöntemleri kullanılmaktadır. Günümüzde, doğru değerlendirme yapabilmek için testis, karaciğer, akciğer, böbrek gibi organlarda kullanılan histometrik ve stereolojik ölçüm yöntemleri önem arz etmektedir. Bu işlemler sonucu elde edilen kantitatif veriler, istatistiksel analizler için yeterlidir. Ayrıca istatiksel analizler yapılırken elde edilen kalitatif ya da semikantitatif verilerin tekrarlanmasında belirli bir standardizasyonun yakalamasında önem arz etmektedir. Bu çalışmanın amacı testis dokusunun histopatolojik değerlendirilmesinde kullanılan yöntemleri özetlemektir.

Anahtar kelimeler: Testis, Histopatoloji, Kantitatif, Seminifer Tübül

1. Introduction

The quantitative measurements are numerical data as the outputs of various processes. Statistical analysis is employed to the collected data. Results obtained from the statistical data are considered today as more reliable. Therefore, measurement processes are the basis of research sciences today. Histology is a discipline that examines the microscopic structure of cells and tissues (Eşrefoğlu 2016). Morphometric measurements can be performed on cell and tissue samples through electron and light microscope. The findings obtained through these qualitative methods provide interpretation of

the changes in tissue morphology. These assessments allow researchers to identify the tissue samples and to compare the physiological variations in its morphology (Cruz-Orive, 1990). The interpretations based on the results of the measurements demonstrate the consequences that are aimed through measurement. In order to employ certain statistical analyses based on the results of the measurement, it is necessary to express the obtained qualitative or semi-quantitative data in numerical terms through quantification. This can be performed by the researcher after the measurement, or it can be performed by quantification of the measurement tool so as to obtain the data during the measurement. The quantitative data obtained from these transactions is sufficient for the statistical analyses. Moreover, the statistical analyses are significant for reaching a certain standardization level in the repetition of qualitative or semi-quantitative data.

Various reasons or diseases cause serious damages to the testicle tissues. Quantitative or semi-quantitative methods have been developed to determine the type, degree, level, and number of these damages. The semi-quantitative scoring usually includes multiple parameters, which are measured individually on an ordered scale and eventually combined as a total score. The data of different experimental groups can be compared statistically. The selection of parameters should be based on the current information, scientific hypotheses or questions concerning the morphological results of the model of the investigated disease (Klopfleisch, 2013).

2. Methods used for morphometric measurements;

2.1. Testicular biopsy score through the Johnsen method

It is a semi-quantitative method used for determining the degree of the damage on the tubules. In the implementation of this method, for approximately each group, 10 different preparations are prepared with 5µm thickness. 100 tubules are evaluated for each group under the 20x objective lens according to the criteria specified in Table I; thus, this method is based on the statistical comparison of the inter-group differences (Özgüner et al., 2004, Jafari et al., 2018).

Table I: Johnsen testicular biopsy score (Johnsen, 1970).

Score	***Histological Symptoms***
1	Tubules with no cells
2	Tubules containing Sertoli cell but no germinal cell
3	Tubules containing only spermatogonium as the germinal cell
4	Tubules without spermatozoa and spermatid, and containing less than 5 spermatocytes
5	Tubules containing spermatocytes but no spermatozoa and spermatid
6	Tubules without spermatozoa, and containing less than 10 spermatids
7	Tubules with a large number of spermatids, but containing no spermatozoa
8	Tubules containing multi-layered germinal epithelium but containing less than 10 spermatozoa in their lumens
9	Tubules containing spermatozoa, but the order of the germinal epithelium is impaired, and packed towards the lumen
10	Tubules with multi-layered and smooth germinal epithelium and containing numerous spermatozoa

2.2. Cosentino classification

Table II: Cosentino classification (Cosentino, 1986, Aydıner et al., 2012)

Stage	*Symptom*
1	Normal testicular architecture with an orderly arrangement of germinal cells
2	Less orderly germ cells, and close packed seminiferous tubules
3	Disordered germinal cells with shrunken pyknotic nuclei and less distinct seminiferous tubule orders
4	Seminiferous tubules that are closely packed with coagulative necrosis of the germinal cells

2.3 Other parameters that can be scored in interstitium

The parameters for edema, bleeding, and inflammatory infiltration reactions in the interstitium can be assessed based on their intensity and according to the criteria given in Table III.

Table III: Interstitial Scoring (Bozkurt, 2018).

Lesion Density	*Symptom*
-	No Lesion
+	Slight Lesion
++	Medium-Level Lesion
+++	Severe Lesion

3. Measurement method in the seminiferous tubules

3.1. Measuring the diameter of the seminiferous tubules

Another symptom indicating testis damage can be diagnosed through measurement of the diameter of the seminiferous tubules. The measurement is employed under 10X, 20X, and 40X magnification, at least over 10 seminiferous tubules in randomly selected different areas of each animal, and at least over totally 100 tubules for each group. The number of the seminiferous tubules that will be exposed to measurement can be decreased or increased based on the number of the groups and the type of the study. As is seen in the Figure 1 A, for each tubule, it is calculated through the averages of the lengths of the short edge and the long edge (two diameters perpendicular to each other) measured in μm (Kazemi et al. 2016, Songur 2016).

In another interpretation, the measurement is employed under 10X magnification, over randomly selected 50 tubules from each animal. The measurement is conducted with totally 350 tubule diameters from each group, as is seen in the Figure 1 B, by drawing lines perpendicular to each other touching 8 dots in a computer program and the averaged results are analyzed through the appropriate statistical analysis method (Şahin, 2016).

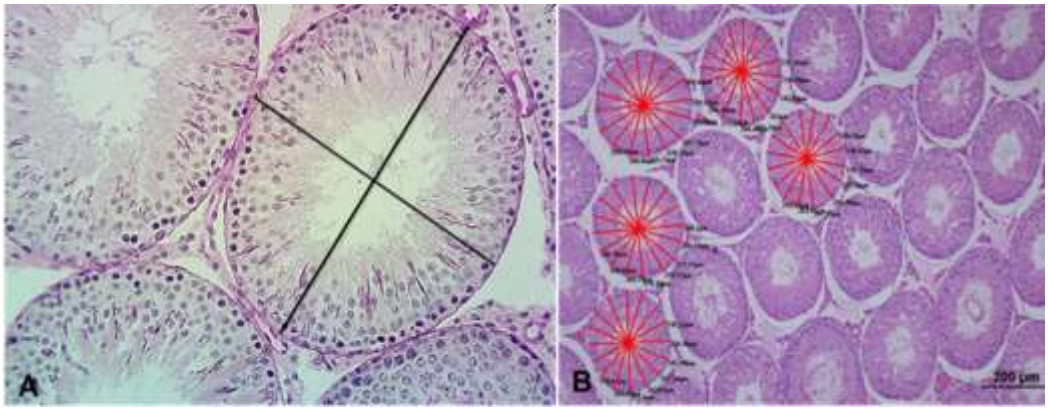

Figure 1. Measurements of the diameters of seminiferous A (Yalçın 2018), B (Şahin 2016)

3.2. Measurement of the epithelium thickness of the seminiferous tubules

The thickness measurement of the epithelium of the tubule is conducted by measuring the thicknesses of the epitheliums of at least totally 100 tubules from the randomly selected different areas of each preparate with 10X, 20X, and 40X magnification, measuring from the four sides and from the four angles in µm, as is seen in the Figure 2, and the calculation is completed by taking their averages (Kazemi et al., 2016). Additionally, the epithelium thicknesses of the tubules can also be measured by measuring the distances between the sperm closest to the lumen and the basal membrane over at least 20 tubules randomly selected from different areas of each preparation (Kianifard et al., 2012).

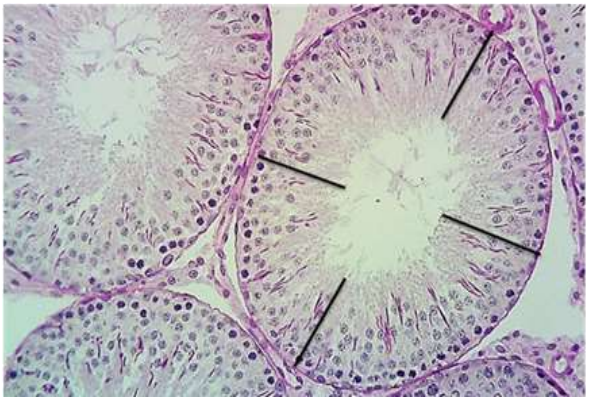

Figure 2. Measuring the epithelium thickness of the seminiferous tubule (Yalçın 2018)

3.3. Measurement of the cross section surface area of the seminiferous tubules

The entire area of the seminiferous tubule (STA) is determined through a calculation (Figure 3). There are certain types of software used to calculate the area (i.e.: Motic Software) (Kazemi et al., 2016).

3.4. Calculating the Cross Section Area of the Epithelium of Seminiferous Tubule

The cross sectional surface area of the epithelium of the tubule (AET) can be calculated (Figure 3) by extracting the area of the seminiferous lumen surface area (SLA) from the seminiferous

tubule surface area (STA), and there are certain types of software to make these calculations (Kazemi et al., 2016).

AET = STA – SLA

Figure 3. Measurement of the cross section surface area of the seminiferous tubule (Yalçın, 2018)

3.5. Quantification of the Seminiferous Tubules

In order to quantify the number of the seminiferous tubules, a counting is employed at least in 40 different areas randomly selected for each group, with 10 X objective lens. For a better and more accurate result, a 6,5 x 6,5 cm square is drawn using the "+" symbol, the tubules both on the lines of the square and in the central part are counted (Kazemi et al., 2016). Subsequently, in order to get the number of seminiferous tubules per 1 cm^2, the total number of the counted seminiferous tubules are divided by the total area of the square, which is 42.25 cm^2 (Kazemi et al., 2016).

4. Measurement methods applied at cellular level

4.1. Germinal Cell Counting

All of the germinal cells in all experimental groups were manually counted through a computerized image processing and analysis program (Bab Bs200Pro) as is seen in Figure 4, by means of 20 X magnification over 10 tubular areas randomly selected from each animal, and totally on 70 tubular areas for each group (Şahin 2016).

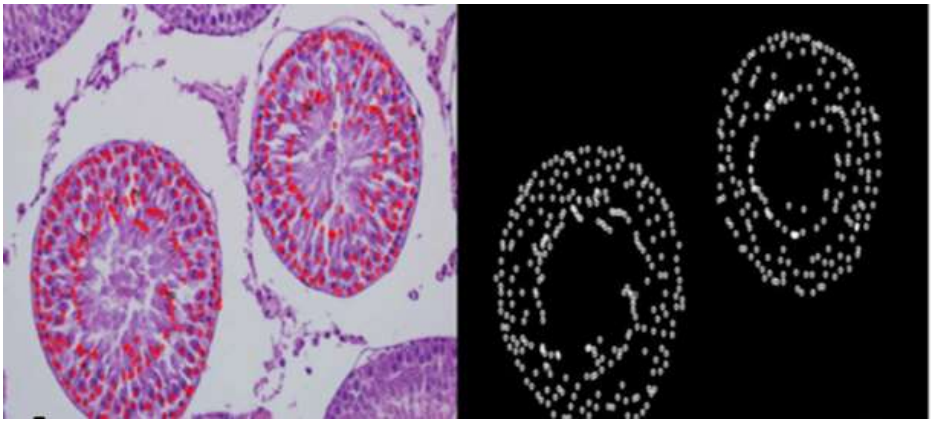

Figure 4. Germinal cell counting (Şahin 2016)

4.2. Leydig cell counting

All of the Leydig cells in all experimental groups were manually counted through a computerized image processing and analysis program (Bab Bs200Pro) as is seen in Figure 5, by means of 20 X magnification over 10 tubular areas randomly selected from each animal, and totally on 70 tubular areas for each group (Şahin 2016).

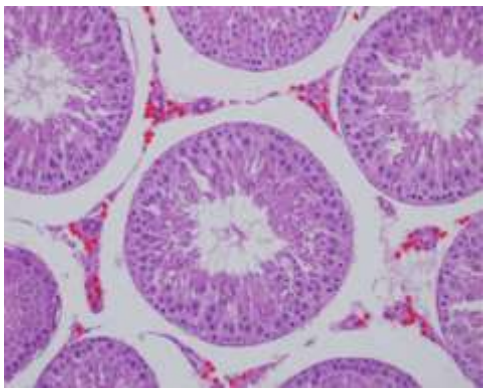

Figure 5. Leydig cell counting (red spots; counted Leydig cells) (Şahin 2016)

4.3. Counting the Spermatogonia and Spermatocytes in 1 cm^2

In order to count the spermatocytes and spermatogonia in the seminiferous tubules, initially, the area of the seminiferous tubule is calculated. Subsequently, the spermatogonia are counted that are observed as dark circles at the outer layer of the epithelium, and in the next step, the spermatocytes are counted. Lastly, in order to get the numbers of the spermatogonia and the spermatocytes in each $1cm^2$,

the numbers of the spermatogonia and the spermatocytes are divided by the surface area of the seminiferous tubule (STA) (Kazemi et al., 2016).

4.4. Measurement of the spermatogenic yield cycle

Evaluations are being conducted concerning the light and electron microscopy images obtained from the histologic and immune-histochemical applications. Counting of the Germinal and Sertoli cells is carried out according to the methodology developed by Abercrombie and revised by Amann and Almquist. Additionally, the histo-morphometric properties of the testicular tissue and the estimated duration of the spermatogenic process cycle can be estimated according to the spermatogenic production indices (meiotic index, mitotic index, Sertoli cell index, testicular sperm reserves, daily sperm production) as indicated in Morais's studies (Abercrombie, 1946; Amann & Almquist 1962; Morais & ark. 2017).

Histopathology is often used in the diagnosis of infectious, degenerative and neoplastic diseases in humans and animals. These qualitative diagnoses are based on observable changes in the morphology of the analyzed tissue. These changes allow for the comparison with the known variation (histology) in tissue morphology. However, absolute measurement of lesion size and severity is difficult (Renshaw & Gould 2007). In order to overcome these difficulties, various imaging methods and software have been developed to automatically calculate the tissue surface area or existing cells per area. These approaches allow for appropriate statistical processes, and to prevent individual errors, they aim at a reliable and reproducible histopathology conducted on a rational scale (Riber-Hansen and Ark 2012). In our article, we attempted to summarize the methods used in the evaluation of the testicular tissues on which there are intensive studies concerning histopathology. In the coming years, with the development of advanced versions of measurement methods, imaging methods, and software in line with the improvements in technology and science, a certain standardization will be achieved in evaluation of testicular tissues, on which there are frequent studies concerning histopathology.

REFERENCES

Abercrombie M, (1946) Estimation of nuclear population from microtome sections. *Anat Rec,* 94:239–247. PMID: 21015608

Amann RP, Almquist JO, (1962) Reproductive capacity of dairy bulls.VIII. Direct and indirect measurement of testicular sperm production. *J Dairy Sci,* 45:774–781

Aydıner Ç.A, Pul M, İnan M, Bilgi S, Çakır E, (2012) Can N-acetylcysteine play a role in preventing tissue damage in an experimental testicular torsion model? *Cumhuriyet Journal of Medicine,* 34: 462-471. Doi: 10.7197/1305-0028.1498

Bozkurt M, (2018) Protective effect of hydrogen sulfide in experimental testicular ischemia reperfusion injury in rats. Health Sciences University Okmeydanı Health Application and Research Center, Urology Clinic, Thesis.

Cosentino MJ, Nishida M, Rabinowitz R, Cockett A.T, (1986) Histopathology of prepubertal rat testes subjected to various durations of spermatic cord torsion. *J Androl,* 7: 23-31.

Cruz-Orıve L.M & Weibel E.R, (1990) Recent stereological methods for cell biology: a brief survey. *Am J Physiol,* 258(4 Pt 1):L148-56. Doi: 10.1152/ajplung.1990.258.4.L148

Eşrefoğlu M, (2016) General Histology, İstanbul Medicine Bookstore, İstanbul 2; 3p.

Jafari O, Babaei H, Kheirandish R, Samimi A.S, Zahmatkesh A, (2018) Histomorphometric evaluation of mice testicular tissue following short- and long-term effects of lipopolysaccharide-induced endotoxemia. *Iran J Basic Med Sci,* Vol. 21, No. 1.

Johnsen SG, (1970) Testicular biopsy score count- a method for registration of spermatogenesis in human normal values and results in 335 hypogonadal males. *Hormones,* 1:2-25.

Kazemi S, Feizi F, Aghapour F, Joorsaraee G.A, Moghadamnia A.A, (2016) Histopathology and histomorphometric ınvestigation of bisphenol a and nonylphenol on the male rat reproductive system. *North American Journal of Medical Sciences,* 8(5): 215-221. Doi: 10.4103/1947-2714.183012

Kianifard D, Sadrkhanlou R. A, Hasanzadeh S, (2012) The ultrastructural changes of the sertoli and leydig cells following streptozotocin induced diabetes. *Iranian Journal of Basic Medical Sciences,* 15(1), 623.

Klopfleisch R, (2013) Multiparametric and semiquantitative scoring systems for the evaluation of mouse model histopathology - a systematic review. *BMC Veterinary Research,* 9:123. Doi: doi.org/10.1186/1746-6148-9-123

Morais DB, Puga LCHP, Paula TAR., Freitas MBD, Matta SLP, (2017) The spermatogenic process of the common vampire bat Desmodus rotundus under a histomorphometric view. *Plos One,* 1-18. https://doi.org/10.1371/journal.pone.0173856

Özgüner M, Şenol A, Ural M, İşler M, (2004) Histological changes caused by experimental hyperthyroidism in adult rat testicular tissue. *S.D.Ü. Medicine Facultary Journal,* 11(3):1-6

Renshaw A.A, Gould EW, (2007) Measuring errors in surgical pathology in real-life practice - Defining what does and does not matter. *Am J Clin Pathol*, 127(1):144–152.

Riber-Hansen R, Vainer B, Steiniche T (2012) Digital image analysis: a review of reproducibility, stability and basic requirements for optimal results. *APMIS,* 120(4):276–289.

Şahin T.C, (2016) Protective effect of montelukast on torsion and detorsion injury in rats. Eskişehir Osmangazi University Institute of Health Sciences Department of Histology and Embryology, Master Thesis.

Songur A, Karateke H, Tosun M, Gönül Y, Turamanlar O, (2016) Histomorphometric and immunohistochemical evaluation of testis tissue and blood testis barrier development in rats in postnatal period. *Kocatepe Medicine Journal,* 17(2).

Yalçın T, (2018) The effect of oleuropein on apoptotic changes in testicular tissue in diabetic rats. *Inonu University Journal of Health Sciences,* 7 (2): 29-34. ISSN:2146-6696