Morphometric analysis of Non-Obstructive Azoospermic Testicular Biopsies

Thesis

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By

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Abstract

Aim: To assess the morphometric analysis of non-obstructive azoospermic testicular biopsies.

Patients and Methods: One hundred and sixty two azoospermic men were included in this study divided into; obstructive azoospermia (OA, n=31) and non-obstructive azoospermia (NOA, n=161). NOA group was subdivided according to testicular sperm extraction (TESE) results into; unsuccessful TESE (n=72) and successful TESE (n=89). The patients were subjected to the following: history taking, general and genital examination, semen analysis, testicular biopsies for TESE & histopathology and computerized morphometry for testicular biopsies.

Results: The mean surface area of seminiferous tubules was significantly increased in OA compared with all other NOA histopathological patterns. In NOA testes, the more advanced histological pattern demonstrated significant difference compared with the lower one respectively. The mean interstitial tissue area percentage was significantly decreased in OA compared with all other NOA histopathological patterns except tubular hyalinization. In NOA testes the interstitial tissue area percentage was increased in SCO > severe hypospermatogenesis > germ cell arrest > hypospermatogenesis > tubular hyalinization. Successful TESE of NOA cases compared with unsuccessful TESE had a significant increase in the mean seminiferous tubular surface area and nonsignificant difference in the interstitial tissue percent.

Conclusion: The seminiferous tubular area correlates with advanced spermatogenic activity and successful TESE in NOA cases.

Key words: male infertility; testis, azoospermia, morphometry, spermatogenesis, TESE.

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Contents

Aim of the Work

This study aimed to assess the morphometric analysis of non-obstructive azoospermic testicular biopsies.

Introduction

Starting from a self-renewing stem cell pool, male germ cells develop in the seminiferous tubules of the testes throughout life from puberty to old age. Spermatogenesis occurs in the seminiferous epithelium, which consists of Sertoli cells and several types of germ cells ***(Roosen-Runge, 1952; Clermont, 1972;de Kretser and Kerr, 1994)***. It is subdivided into; spermatogoniogenesis, meiosis, spermiogenesis, spermiation with its end products; spermatozoa (***Bergmann, 2005)***.

Rigid spermatogonial synchronization and coordination along the length of the seminiferous tubules are responsible for the histological appearance of single stages in tubular cross sections ***(Steinberger and Tjioe 1968)***. ***Schulze (1982)*** evidence for the wave of spermatogenic stages along the length of the seminiferous tubules in a helical pattern had been reported. Due to this irregular pattern of spermatogenesis in humans, tubular cross sections are composed of more than one stage and some mixing of germ cells occurs in typical stages ***(Clermont 1963; Bustos-Obregon et al. 1975)***.

To avoid this difficulty, spermatogenesis has been quantified, histologically, in terms of differential germ cell counts expressed per unit length of seminiferous tubular circumferences ***(Steinberger and Tjioe 1968;***

Zukerman et al. 1978), per Sertoli cell ***(Rowley and Heller, 1971; Skakkebaek and Heller 1973)***, or per tubular cross section ***(Silber and Rodriguez-Rigau, 1981; Cortes, 1990)***. Others have evaluated ratios of germ cells ***(Barr et al. 1971)*** and expressed counts per g parenchyma as a measure of efficiency of spermatogenesis in humans ***(Amann and Howards 1980; Johnson et al., 1992; Chaturvedi and Johnson, 1993)***.

Image analysis is a technology that has undergone rapid development since 1960, at first mainly stimulated by the needs of the aerospace and defense industries. Imaging techniques had found wide spread applications in industry, primarily in the field of robotic applications, including radiological imaging and microscopy ***(Sheldon, 1981; Brugal, 1987)***. Computerized testicular morphometry was documented in diverse number of scientific papers being mostly on animals where human studies were rather relatively few.

Spermatogenesis

Starting from a self-renewing stem cell pool, male germ cells develop in the seminiferous tubules of the testes throughout life from puberty to old age. Spermatogenesis is subdivided into; spermatogoniogenesis, meiosis, spermiogenesis, spermiation with its end product; the mature male gametes, the spermatozoa. It depends on intratesticular and extratesticular hormonal regulatory processes, functions of intertubular microvasculature, Leydig cells and other cellular components of the intertubular space ***(Bergmann, 2005)***.

Organization of the testis

The human testes are two organs with a shape of rotation ellipsoids with diameters of 2.5 × 4 cm engulfed by a capsule (tunica albuginea) of strong connective tissue ***(Middendorff et al., 2002)***. Thin septula divide the parenchyma of the testis in about 370 conical lobules. The lobules consist of the seminiferous tubules and intertubular tissue, containing groups of Leydig cells and additional cellular elements. The seminiferous tubules are coiled loops that both ends open into the spaces of the rete testis. The fluid secreted by the seminiferous tubules is collected in the rete testis and delivered to the

excurrent ductal system of the epididymis ***(Holstein et al., 2003; Bassas Arnau, 2009)***.

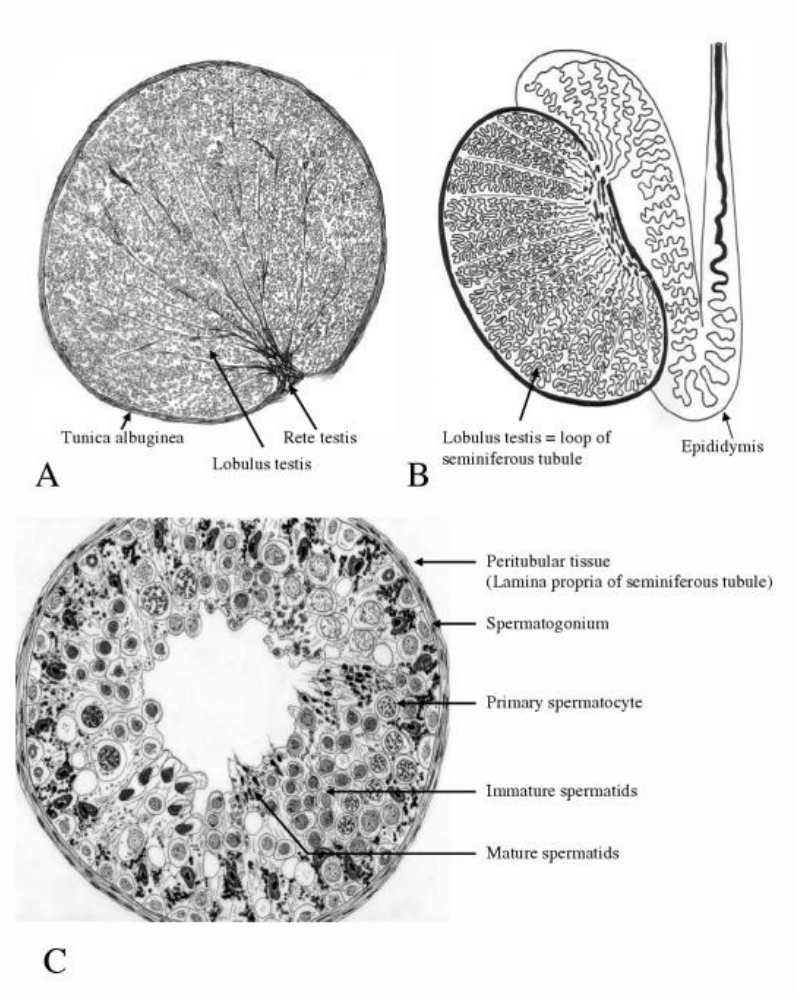

Plate 1

A) Cross-section of the human testis. B) Arrangement of the seminiferous tubules and the epididymis. C) Semi-schematic drawing.

Structure of the seminiferous tubule

It consists of the germinal epithelium and the peritubular tissue (lamina propria). Its mean diameter is about 180 μm where the height of the germinal epithelium is 80 μm and the thickness of the peritubular tissue is about 8 μm. The germinal epithelium consists of different developmental stages of germ cells, namely spermatogonia, primary and secondary spermatocytes and spermatids. These are located within invaginations of Sertoli cells ***(Maekawa et al., 1996; Amann, 2008).***

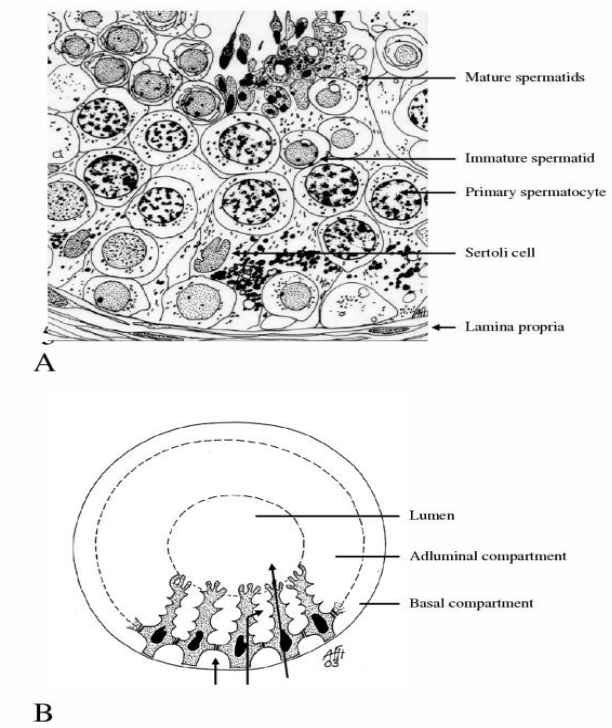

Plate 2

A) Germinal epithelium in the seminiferous tubule. B) Sertoli cells divide the germinal epithelium in a basal and adluminal compartment. Arrows indicate transport of substances only to the basal compartment, via Sertoli cell into the adluminal compartment, via Sertoli cell into the lumen.

The prismatic Sertoli cells are connected by specialized zones of tight junctions of cellular membranes separating the germinal epithelium in a basal and an adluminal compartment. These specialised zones, the so-called "tight junctions" form the blood-testis barrier of the testis. During maturation the germ cells pass this barrier entering the adluminal compartment where they find protection from diffusion of extraneous substances ***(Mruk and Cheng 2004; Lee et al., 2007)***.

Sertoli cells exhibit increased amounts of lipid droplets correlated with advanced age being an indicator of the "biological clock" of the testis. Further functions attributed to Sertoli cells are; 1. Sustentacular and nutritive functions for the germ cells. 2. Organization of the delivery of mature spermatids into the tubular lumen (spermiation). 3. Production of endocrine and paracrine substances for the regulation of spermatogenesis. 4. Secretion of androgen binding protein (ABP) for the maintenance of epithelia of the excurrent duct system. 5. Interaction with the intertubular endocrine Leydig cells ***(Russell and Griswold, 1993; Mruk and Cheng, 2004)***.

The peritubular tissue consists of about 5 layers of myofibroblasts with intermingled connective tissue ground substance. The myofibroblasts cause peristaltic contractions of the seminiferous tubule giving rise to transport of the immotile spermatozoa to the rete testis. The thickness of the

peritubular tissue normally is about 8 μm that in cases of disturbed spermatogenesis it may be thickened by connective tissue ground substance up to 12 μm or more ***(Davidoff et al., 1990; Holstein et al., 1996)***.

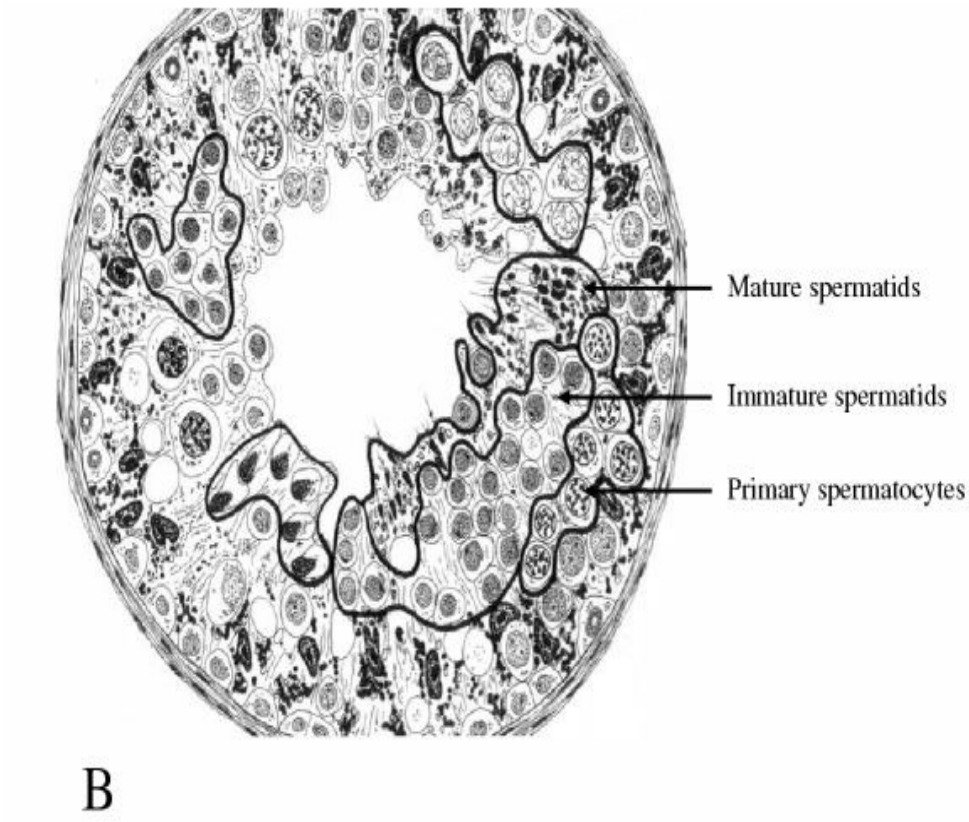

Plate 3

Seminiferous tubule with marked clones of germ cells.

Spermatogenesis Processes

Spermatogenesis begins at puberty after a long preparatory period of "prespermatogenesis" in the fetus and the infant. Three major stages can be distinguished: spermatogoniogenesis, maturation of spermatocytes and spermiogenesis, which is the cytodifferentiation of spermatids.

Spermatogoniogenesis

Several types of spermatogonia are distinguished by their position in the basal part of the germinal epithelium, their morphology and stainability of nuclei: A pale type-, A dark type- and B type-spermatogonia . A-type spermatogonia belong to the stem cell pool of spermatogenesis. B-type-spermatogonia represent the onset of germ cell development up to spermatids ***(Clermont, 1966; Amann , 2008)*** .

Spermatogonia multiplicate continuously in successive mitoses. Spermatogonial cell divisions are usually incomplete. The daughter cells remain interconnected by cytoplasmic bridges so that a clone derived from one stem cell forms a syncytium of cells. Syncytial connections are maintained through spermatogonial and spermatocytic stages and are dissolved only in advanced phases of spermatid development. It is thought

that the formation of these clones is the basis for the synchronuous development of germ cells ***(Amann, 2008)***.

Both A type spermatogonia are necessary for intact spermatogenesis. In reduced spermatogenesis A dark-type spermatogonia are often absent. In the absence of both types of spermatogonia, no spermatogenesis takes place and the germinal epithelium consists of Sertoli cells only. Spermatogonia may be absent from birth (congenital SCO Syndrome) or may be destroyed by different noxes, e.g. X-radiation, (acquired SCO Syndrome). In cases of disability of spermatogonia to develop B-type spermatogonia the number of A pale type spermatogonia increases and bi- or multilayered groups of spermatogonia in the basal compartment are formed without further developed germ cell stages. This aspect represents an arrest of spermatogenesis at the stage of spermatogonia. The barrier of Sertoli cells can not normally be passed by A type-spermatogonia but under special conditions as intratubular tumor cells, the barrier is interrupted and spermatogonia are dislocated into the adluminal compartment where they disintegrate ***(Bergmann et al., 1989; Nistal et al., 1998)***.

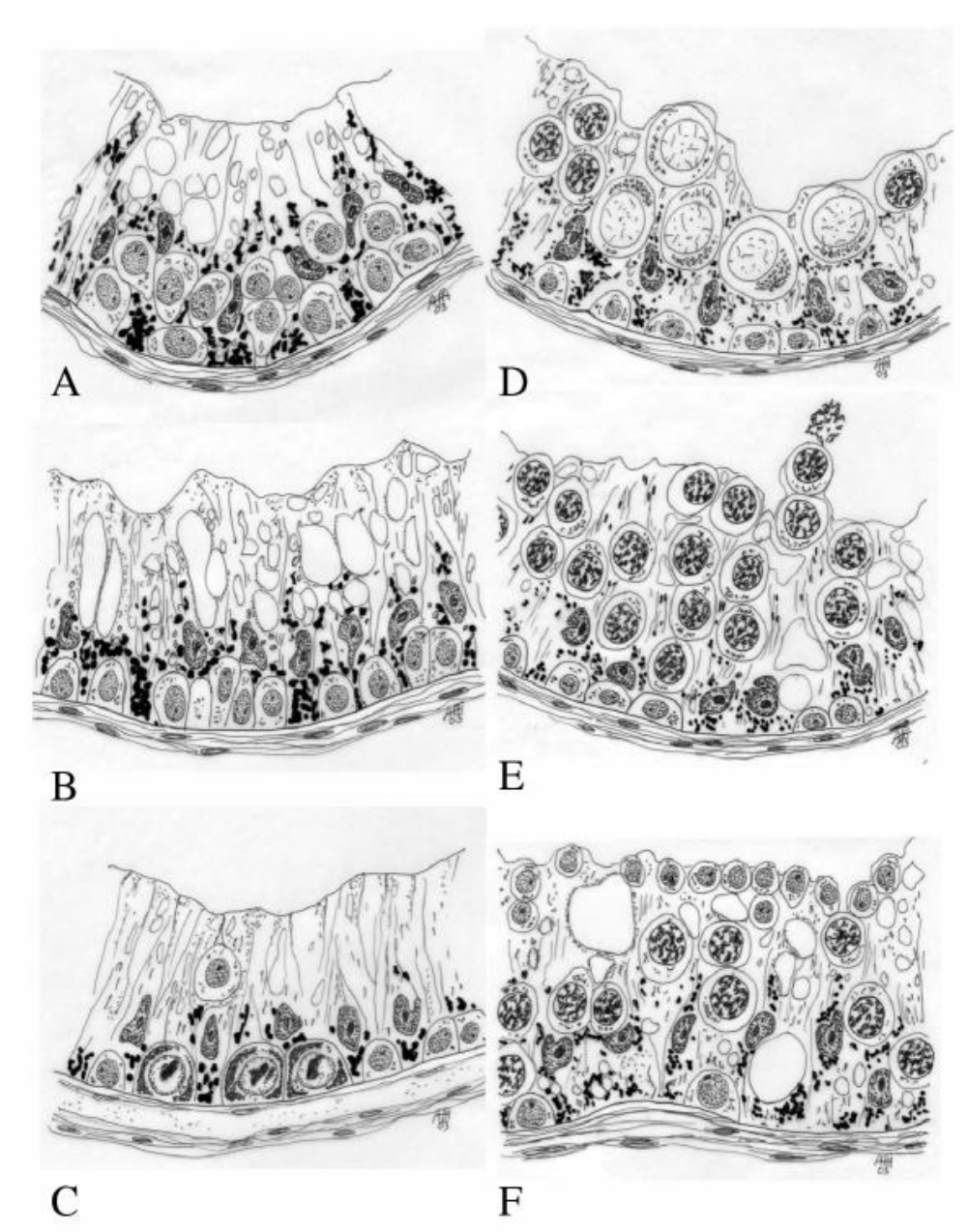

Figure 4

A) Germinal epithelium with multilayered spermatogonia. B) Arrest of spermatogenesis at the stage of A pale type-spermatogonia. C) Tumour cells in the basal compartment of the germinal epithelium dislocate a pale type-spermatogonia. D) Megalospermatocytes do not complete meiosis. E) Arrest of spermatogenesis at primary spermatocytes. F) Arrest of spermatogenesis at immature spermatids.