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Some Techniques of Human Andrology

男性学的实验方法

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Some Techniques of Human Andrology

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前　言

男性学（Andrology），广义地说应称为雄性学，在临床上为了与妇科学相对应，亦以男科学名之。自六十年代末开始，它在男性避孕法研究的推动下，已逐渐发展成为一门新兴的独立学科，而它的理论与实验方法反过来又成为男性计划生育研究不可或缺的基础。

我国男性学研究的起步虽较晚，但随着棉酚抗生育工作的广泛开展，对这门新学科的探索已渐向纵深推进。目前，各有关计划生育科研的院、所、大专院校和医院正纷纷筹建从事男性生育研究的实验室。为此，广大科研人员和临床医生急待参考反映国外先进实验技术的文献，以建立一套我国男性学实验方法的常规。然而，世界上各实验室所采用的方法，种类繁多、标准不一，文献量大，检索费时。有鉴于此，我们特围绕形态学、免疫男性学、男性内分泌学、细胞遗传学和精液分析等五个专题，检索、筛选了国外文献，从中择取比较先进和成熟的方法，编选了这卷专集，供我国从事男性学和男子计划生育研究的科研、教学人员及泌尿科医师参阅。

本卷的编选工作承上海第二医学院王一飞同志大力协作，谨表谢意。

编　者

序

几十年来，世界各国的科学家对生殖生物学这个古老的学科一直兴趣不衰。在人口控制越益受到重视的今天，这门渊深源长的学科更是日新月异，飞速发展。在生殖生物学领域中，对男性的研究大大落后于对女性的研究，这就造成目前男性节育的方法也远远少于女性节育措施。近十年来，世界上不少研究所及实验室开始在男性生殖生物学及男性节育研究方面投注较多的人力与物力，一门新兴学科——“男性学”（Andrology）正在逐步发展壮大。国际男性学会议已开过多次，并已出版多种男性学专刊和专著，世界卫生组织也成立了关于男用避孕方法研究的专题小组。目前世界上几个著名的男性学研究中心，如美国、北欧及南美等均建立了一批配备齐全，水平很高的实验室。我国随着棉酚抗生育科研的不断深入，也开始形成一支男性学的专业研究队伍。

男性学是一门年青的边缘学科，它涉及的范围极广，诸如细胞学、组织学、胚胎学、生理学、生物化学、药理学、免疫学、内分泌学及临床科学。这一学科还处于雏形阶段，并在不断出现新的生长点；它所采用的实验技术种类繁多，各个实验室的技术标准各自迥异，参考文献也分散在各种杂志和书刊中，这就给男性学工作者带来很大的困难。为此，我们感到应尽快编纂一本男性学实验方法手册。经比较与筛选，我们终于从美国 Wayne 州立大学男性学实验室 E. S. E. Hafez 教授近年来编著的几本男性学专著中挑选了一部份比较成熟并且适合我国目前情况的实验技术，汇编成册。全书分成五个专题。其中形态学观察是研究男性生殖活动的结构基础，尤其是细胞化学的研究有助于了解生殖活动的动态过程；免疫男性学的重点是研究精子抗原的免疫现象，我们选编了一些测定精子抗原细胞免疫与体液免疫的技术，这不但对男性不育症的临床诊断有所裨益，而且可在免疫避孕研究中采用；在男性学的实验研究与临床研究中，男性激素测定及染色体核型分析是常用的技术，而精液分析更是衡量男性生育力最简单而实用的指标。这五个方面的实验技术不但适用于男性学基础理论的研究工作，也会受到男性学临床工作者的重视与欢迎。

爱因斯坦有一句名言：“对于真理的探索比对真理的占有更为宝贵”。借鉴是创新的前奏，殷切期望我国男性学工作者在掌握这些技术的基础上，尽快建立我国男性学的研究方法常规，并测出我国男子各项男性学指标的正常值。只要勇于探索，勤于探索，善于探索，我们就一定能为世界男性学的发展作出更大的贡献。

王 一 飞

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Techniques of Human Andrology, edited by E. S. E. Hafez

Evaluation of testicular biopsy

S. M. Girgis and E. S. E. Hafez

Testicular aspiration was first advocated as a diagnostic procedure for men with azoospermia by Huhner (1913). Testicular biopsy was then used extensively for the diagnosis, prognosis and selection of appropriate treatment of various syndromes of male infertility such as azoospermia and unexplained oligospermia (Charney, 1940, 1968; Ragab et al., 1961; Dubin & Hotchkiss, 1969; Amelar & Dubin, 1973; Wong et al., 1973; Shirren, 1974; de Kretser & Holstein, 1976).

It was theorized that bilateral testicular biopsy causes long-term sequelae: an antigen–antibody reaction with subsequent destruction of spermatozoa and more immature cells in the germinal epithelium (Gordon et al., 1965). A transient sperm antibody response was suggested following testicular biopsy. Sera collected within 5 weeks after testicular biopsy revealed evidence of antigenic stimulation in 11 of 35 men (Hjort et al., 1974). However, Ansbacher & Gangai (1975) could not demonstrate sperm-agglutinating or sperm-immobilizing antibodies in the sera of men up to 14 days following bilateral testicular biopsies. Reasons for the lack of sperm antibody response to biopsy of the testis may include: (a) an insufficient insult, i.e. lack of spillage of spermatozoa at the time of the testis biopsy sufficient to initiate the antigen–antibody response; (b) the need for normal spermatogenesis through all its stages to induce the antigen–antibody response after the blood–testis barrier has been disrupted; (c) an immature or incomplete response due to incomplete antigens or the lack of an antigenic stimulus from spermatids; (d) the necessity for more sensitive techniques to identify an antigen–antibody response; and (e) the time interval when sera were examined after the testicular biopsy might have been too short to detect an antibody if the antigenic stimulus was minimal (Ansbacher & Gangai, 1975).

Testicular biopsy is one of the most important diagnostic methods of male infertility. Qualitative and quantitative studies on testicular biopsy, associated with endocrine profiles, provide excellent research tools to investigate the hormonal regulatory mechanism of spermatogenesis.

I Surgical procedure

The patient is shaved and given an appropriate sedative. The operation is done under local anesthesia, using 5–10 ml xylocaine. The spermatic cord is infiltrated, followed by the skin and subcutaneous tissue at the chosen site of operation. The testis is held firmly by the left hand of the surgeon with the skin stretched over its anterior surface, making sure that the epididymis is lying posteriorly and away from the line of incision. Using the scalpel, a 2-cm incision is made into the scrotal skin either transversely or longitudinally choosing an area with no apparent skin vessels. The incision is deepened until the tunica vaginalis sac is opened, with the escape of a few drops of fluid and exposure of the glistening bluish-white tunica albuginea. A small incision is made into the tunica albuginea and protruding testicular matter, the size of a wheat grain is removed with a pair of sharp-pointed scissors and immediately transferred to the fixative.

The tunica albuginea is then closed, followed by the tunica vaginalis, the dartos muscle layer and finally the skin, using fine catgut all through (Fig. 1). After wound dressing, patient leaves the hospital to report again after a few days for wound inspection and the biopsy report. Occasionally there is testicular pain or referred renal pain at the time of puncturing the tunica albuginea so that in overanxious patients general anesthesia is preferable.

Care should be taken to avoid missing the testes for the epididymis during operation, by proper palpation, positioning and maintenance of a firm hold on the testis till biopsy is taken.

Biopsy is usually taken on one side only as the histological picture is similar on both testes, unless there is a difference in the size or consistency of the testes, when bilateral specimens are taken.

Side reactions and complications of biopsy are few and mostly avoidable. These include: hematoma, wound infection, and adherence of the testes to the scrotal skin, which can be prevented by proper hemostasis, antisepsis, and closure of the wound in layers. Also, transient and low titer of sperm antibodies following biopsy has been reported (Hjort et al., 1974).

The best fixatives for light microscopy are Bouin's fluid, Cleland's solution, Stieve's solution of Zenker-formol. Formalin causes shrinking of the specimen and disorganization of the tubular contents. Following fixation for 6–12 hours and embedding in paraffin, 5-μm sections are stained with hematoxylin–eosin and with the periodic acid–Schiff–hematoxylin techniques. The topographical arrangement of spermatogonia can be also investigated in dissected tubules, fixed in Bouin's fluid, stained with hematoxylin and mounted into toto between glass slide and coverslip (Clermont, 1970).

II Histology of normal testes

Proper interpretation requires first the knowledge of the normal histological appearance before the pathological lesions can be recognized and evaluated.

From birth until shortly before puberty the testis is rather static, showing tubules of

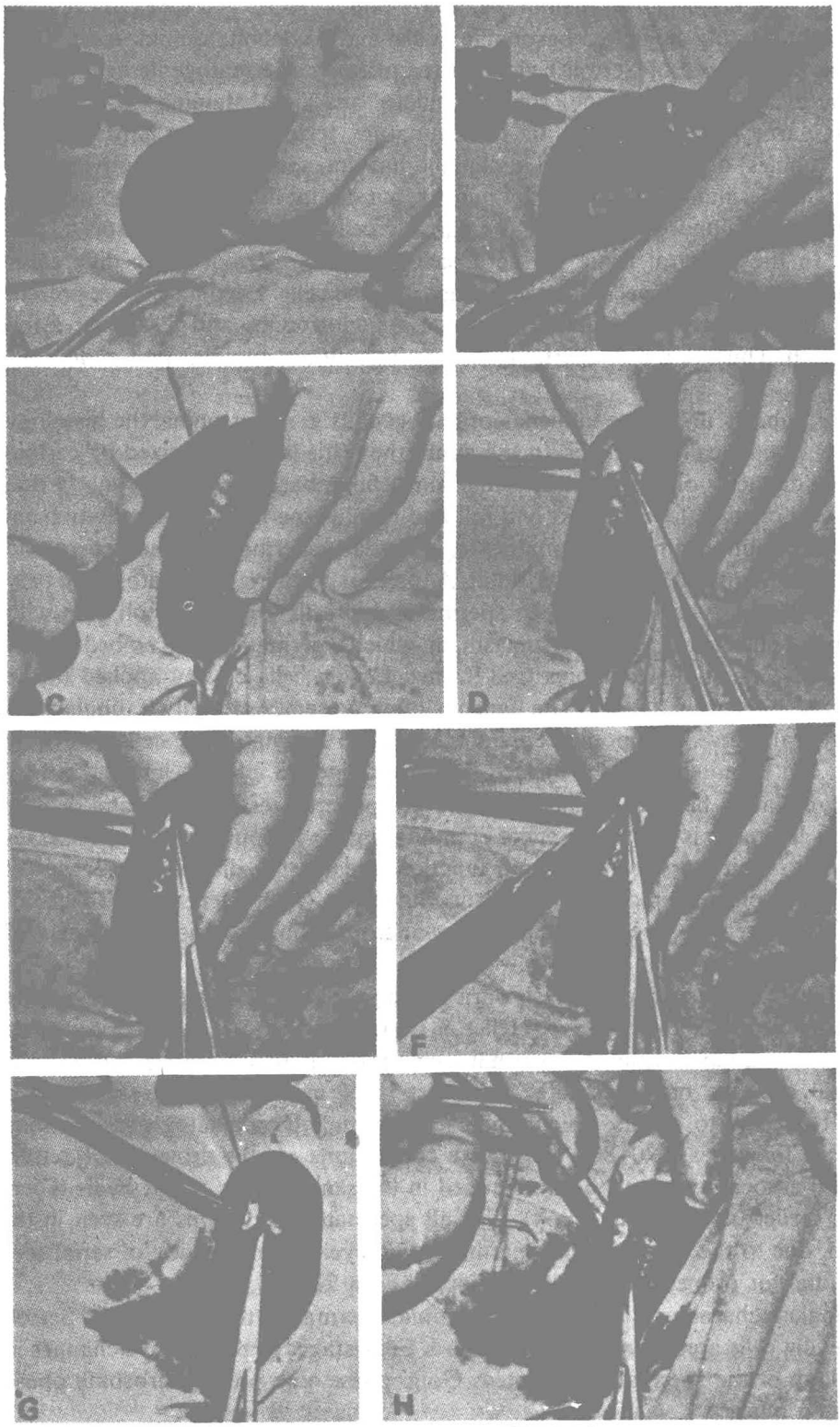
small diameter, with no basement membrane, and is populated by two types of cells: (a) cells with deeply staining elongated basophilic nuclei and no definite cellular membrane which are the progenitors of Sertoli cells, and (b) primary spermatogenic cells which have a definite cellular membrane, rounded nuclei and light staining eosinophylic cytoplasm (Charney et al., 1952). In the immature testes the sex cords (future seminiferous tubules) do not yet have a lumen and have no spermatogenic activity. The gonocytes (stem spermatogonia) divide by mitosis, but do not differentiate into primary spermatocytes. The supporting cells are the somatic cells which are precursors of the Sertoli cells. In the pubertal testis the germ cells undergo spermatogenic differentiation, whereas the supporting cells differentiate into Sertoli cells. Leydig cells are present at birth due to the effect of circulating maternal gonadotropins, and disappear a few weeks after birth. Thus, in a prepubertal testis, the interstitium contains no Leydig cells (Fig. 2A).

At onset of puberty under the influence of endogenous gonadotropins, the histology of the testis is dramatically changed. In the adult, the testis is characterized by certain features: large diameter of the tubules; a thin but definite basement membrane; a thin tubular wall two or three layers thick, and full and regular spermatogenic activity from basal spermatogonium, primary spermatocyte, secondary spermatocyte and spermatid to terminal spermatozoa. The typical adult A pale or A dark spermatogonia only appear during puberty. Lodged between spermatogenic cells are the Sertoli cells with their prominent nuclei and wavy cytoplasm in which spermatids are embedded. A narrow compact interstitium contains well-formed Leydig cells closely applied to the outer walls of seminiferous tubules. The lumen is often present, especially if tubular section is exactly transverse, commonly containing sloughed spermatogenic cells. Only few spermatozoa can be noted, as once formed they are dislodged into the lumen.

Thus, differentiation between prepubertal and postpubertal testis is easy and definite and, since the difference is due to the effect of pituitary gonadotropins, the biopsy serves as a parameter of pituitary gonadotropic function and for the diagnosis of cases of prepubertal hypogonadotropic hypogonadism. Since the testis exerts a feedback influence on FSH secretion, severely damaged seminiferous tubules are usually associated with high FSH (de Kretser et al., 1972).

The time required for spermatogenesis varies among mammalian species and in man spermatogenesis requires 74 $\pm$ 4 days (Heller & Clermont, 1964). It would appear that once spermatogonia have begun the spermatogenic process, they progress through the developmental changes or degenerate. This fact is of importance in assessing the response of the testis to agents which supposedly stimulate spermatogenesis as they should be used for at least 70–80 days before conclusions can be drawn (de Kretser, 1974). Six cell associations have been identified in the seminiferous tubule cycle (Clermont, 1963; Heller & Clermont, 1964). Not all spermatogenic stages are seen in the same section, due to the nature of the spermatogenic cycle, so that study of serial sections is essential for proper reading of the biopsy (Figs. 2B and 2C).

The cytological characteristics of spermatogonia, spermatocytes, spermatids, Sertoli cells, and Leydig cells are summarized in Table I. Four stages of progressive changes in the morphology of the spermatid are noted: Golgi phase, cap phase, acrosome phase and maturation phase.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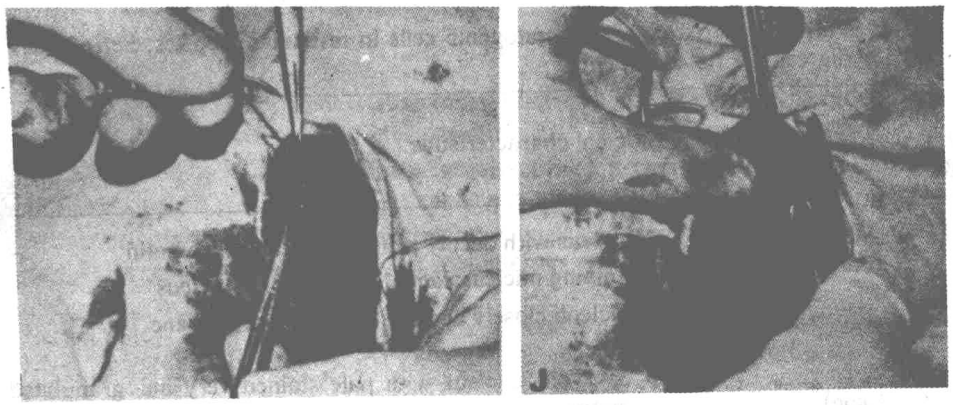

Fig. 1. Surgical procedures of testicular biopsy in man.
A: Infiltration of the cord. B: Infiltration of skin and subcutaneous tissue at the chosen site of biopsy. C: Skin incision 2–3 cm long. D: Skin incision is deepened to expose tunica albuginea. E: Tunica albuginea is incised and testicular matter bulges out. F: A small piece of testicular matter is excised using a sharp pointed scissors.
Wound is closed in layers: first: the tunica albuginea is closed (G), second: the tunica vaginalis is closed (H), third: the dartos muscle layer is sutured (I), finally the skin incision is closed (J).

Leydig cells are usually arranged around blood capillaries or in clusters in the interstitial spaces. As in most steroid-secreting cells, the cytoplasm of these cells is characterized by abundant smooth endoplasmic reticulum composed of interconnecting tubules extending throughout the available cytoplasmic space (Christensen, 1970). The rare occurrence of interstitial cell tumors in childhood is often associated with spermatogenesis in the seminiferous tubules nearest to the adenoma. The 'crystalloids of Reinke' are found in the interstitial cells of the testis and the hilar cells of the ovary (both types of cells secrete testosterone). The abundance of crystals varies greatly from person to person, and it had not been possible to correlate this variation with any functional condition. Crystalloids seem to be absent in some men of normal fertility, and evident in patients with Klinefelter's syndrome, and in hypogonadotropic patients before and after gonadotropic therapy (cf. Christensen, 1970).

III Assessment and indications of testicular biopsy

Assessment of testicular biopsy is better done by the andrologist who conducts the clinical examination and takes the biopsy. In reading the biopsy, several criteria are noticed: size of the tubules, thickness of tubular wall and of basement membrane, integrity of basal line of spermatogonia and Sertoli cells, mitotic index, regularity and uniformity of various stages of spermatogenesis and their completeness, extent of sloughing and disorganization, relative frequency of abnormal cells, the condition of the intertubular space and its contents of Leydig cells and blood capillaries, average number of germ cells per seminiferous tubules (spermatogonic, spermatocyte, spermatid), and the number of germinal epithelium: number of Sertoli cells ratio.

TABLE I Cytological characteristics of spermatogenic cells in testicular biopsies, Sertoli cells and Leydig cells of normal men

Spermatogenic stage	Type	Cytological characteristics
Spermatogonium	Ad (dark)	ovoid nucleus with deeply stained dust-like chromatin
		a pale staining nuclear vacuole in center of nucleus
		1 or 2 nucleoli closely applied to nuclear membrane
	A (pale)	disoid or ovoid nucleus with pale stained very fine granulated chromatin
		1 or 2 nucleoli attached to nuclear envelope
		spherical nucleus with clumps or granules of heavily stained chromatin distributed along nuclear membrane, centrally located nucleolus
	B	spherical nucleus with clumps or granules of heavily stained chromatin distributed along nuclear membrane, centrally located nucleolus
Primary spermatocytes	General characteristics	earliest modification of primary spermatocyte, which characterizes it from spermatogonia is the appearance of very fine single leptotene threads
		3 or 4 nucleoli, one main nucleolus and 2–3 secondary nucleoli; secondary and main nucleoli are similar and all of them are related to acrocentric chromosomes
	(preleptotene)	spherical nucleus with deeply stained granulated chromatin accumulating on nuclear membrane nucleolus
	prophase (1st division)	nucleus undergoes progressive swelling
		chromatin assumes characteristics of leptotene, zygotene and pachytene
Secondary spermatocyte		spherical homogeneous nucleus with finely granulated chromatin and some larger deeply stained globular masses
		nucleolus is very inconspicuous and is absent much of the time
		not frequently observed because of their short life span
Spermatid	A. Golgi phase	newly-formed spermatids have a spherical nucleus with Golgi zone, mitochondria, centrioles, and chromatoid body, well demarcated nucleus, elaborate small granules which stain vividly with PAS
	B. Cap phase	proacrosomic granules, coalesce to form a single layer acrosome granule closely attached to surface of nucleus
		head cap expands around acrosomic granule and grows over surface of nucleus
		acrosomic granule and head cap, stained well by PAS

TABLE I (*continued*)

	C. Acrosome phase	acrosomes nucleus and flagellum undergo remarkable modifications
		nucleus migrates to periphery of cell, nuclear chromatin condenses into coarse dense granules
	D. Maturation phase	spermatid rotates and acrosome becomes directed toward wall of seminiferous tubule
Sertoli cell		closely associated with germinal cells; outlines of germinal cells occupy deep recesses of conforming shape on face of Sertoli cells
		nucleus and cytoplasm undergo changes of shape and activity in relation to seminiferous epithelium cycle
		ovoid nucleus with characteristic infoldings of its surface nucleoplasm homogeneous, and granulofilamentous with clusters of dense particles
Leydig (interstitial) cell		polygonal epithelioid cells scattered or more often irregularly grouped in angular spaces or in stands along the intertubular spaces
		finely granular cytoplasm contains vacuoles representing lipid globules which dissolves during specimen preparation
		cytoplasm stains by many acid dyes with little affinity for basic dyes
		cell contains glycogen, hydrolytic enzymes (lipases, estrases, and phosphatases) and oxidative enzymes

(from Burgos et al., 1970; Clermont, 1970; Courot et al., 1970; Girgis & Hafez, unpublished data; Hooker, 1970; Solari & Tres, 1970; Vilar et al., 1970).

Several methods have been developed to evaluate frequency distribution and volume of interstitial cells, e.g. point counting or the Leydig:Sertoli cell ratio (Ahmad et al., 1969; Heller et al., 1971). For example, Leydig cell numbers do not increase in normal men treated with HCG, whereas the size of the cells becomes consistently larger, an indication of hypertrophy rather than hyperplasia (Heller & Leach, 1971). A scoring technique (Table II) may be used for overall assessment of testicular biopsies (Johnsen, 1970; Franchimont et al., 1972).

Abnormal spermatogonia with large, presumably polyploid nuclei or multinucleate cells may be found in healthy men or in testosterone-treated oligozoospermic patients (Barham & Berlin, 1974). Anomalies of spermatids may involve malformation of the tail or acrosome and/or nuclear condensation (de Kretser & Holstein, 1976). It is possible that abnormal types of germinal cells in the biopsy may correspond to the counter-