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INTRODUCTION

<i>V. Gentile</i>	5
Epidemiology of infertility	5
Male infertility	6
Outlines of anatomy of the male genital apparatus	6
Physiology of the testicle.....	7
Sertoli cells.....	9
Interstitial or Leydig cells	9
Peritubular cells	10
Spermatogenesis	10

HYPOTHALAMUS-HYPOPHYSIS-TESTICLE AXIS

<i>R. Rago, P. Salacone</i>	13
Hypothalamus	13
Anterior hypophysis	13
Hypothalamus-hypophysis-Leydig cells axis	13
Testosterone	14
Hypothalamus-hypophysis-Sertoli cells axis.....	15

SEMINAL DIAGNOSTIC

<i>A. Sebastianelli, L. Caponecchia</i>	18
Seminal fluid.....	18
Semen analysis.....	18
Macroscopic evaluation.....	19
Microscopic evaluation.....	20
Computerized study of nemaspermic kinesis	21

FSH HORMONE THERAPY IN MALE INFERTILITY

<i>R. Rago</i>	24
Correlation between plasmatic FSH and seminal parameters	24
Hormone therapy and functional hypogonadotropic hypogonadism	25

Introduction

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Epidemiology of infertility

Infertility is generally defined as the inability to achieve a pregnancy within a determined period of time in couples of reproducing age having regular sexual intercourse who do not use any contraceptive method. There is no general agreement on the length of such a period: the European Society for Human Reproduction and Embryology (ESHRE) consider a normal fertility to achieve a pregnancy within two years of regular sexual activity¹, although the most common tendency in our increasingly fast-lived society that defers the first pregnancy more and more often, at least in industrialized countries, is to not wait longer than twelve months before seeking assistance.

The infertile population includes couples made up of one or two partners for whom there is no possibility of conception, i.e. sterile, and of those for whom there remains a possibility, so-called *subfertile*. This is the problem of ever larger proportions, that also in Italy, involving tens of thousands, also in Italy.

The human species is *per sé* not very fertile: in fact only 20% of couples who have regular unprotected intercourse two or three times a week achieve pregnancy within the first month, while is necessary about a year in the remaining 80% of cases about a year is necessary. The World Health Organization (WHO) estimates around 15-20% of the couples with fertility problems in advanced industrialized countries². This percentage is destined to increase for various reasons ranging from environmental problems to the excessive sophistication of food and lifestyle. In order to resolve the fertility problem, researchers have developed several therapeutic strategies and techniques of artificial reproduction ranging from the simple and non-invasive IUI (Intra-uterine Insemination) to the more complex assisted reproductive techniques (FIVET, ICSI)^{3,4}.

It is important to assess throughout the diagnostic and therapeutic course undertaken by the couple the real possibilities of success of the techniques so as to avoid useless persistence of the therapy at all costs.

With regard to infertility in the couple, it is possible to identify a series of conditions generically defined with the term male factor, in which, associated with an andrological pathology that may be clinically diagnosed, there is an alteration of the characteristics of the seminal fluid,

indicated with the name of *dyspermia* or *oligozoospermia*. This term refers not only to quantitative but also qualitative alterations of the seminal fluid, both of which are capable of reducing or annulling the fertilizing capacity of the male gamete.

Over the last fifteen years, a branch of medicine has developed considerably and affirmed itself in the specialization of the study of the male gonad and its relations with the rest of the organism: *andrology*, and within the field of andrology, a branch of study dedicated specifically to research on seminal fluid – *seminology*. For a man to be fertile, an adequate quantity of vital spermatozoa must be produced in the testicles possessing qualitative characteristics such as motility and morphology, and quantitative characteristics such to consent reaching and fertilizing the oocyte. The mechanism of ejaculation consists not only of emission of the spermatozoa but represents also a series of events during which secretions from accessory glands join the spermatozoa and other secretions from the epididymis. The seminal fluid must be appropriately deposited in the vagina, and must then migrate through the cervical mucus up to the uterus and Fallopian tubes where fertilization will take place. The male factors are the cause for infertility in 30% of couples and represent a concomitant cause in 20%. The remaining 50% of cases of infertility may be attributed to female causes ⁵, according to data reported in the Italian Register of Medically Assisted Procreation.

Male infertility

Male infertility may be defined as the inability to fertilize after at least 12 months of unprotected intercourse with a female partner in perfect conditions of fertility (WHO, 2000) ⁶.

In defining male infertility, it is necessary to consider also the conditions of the partner; a concept that underlines how fertility or hypofertility almost never exclusively involves the single individual but is rather a state that concerns the couple (infertility of the couple). This means that the fertilizing capacity derives from the integration of the potential of the two components of the couple, intended singularly, as well as from their interaction.

In order to assess the male factor of infertility, it is fundamental to consider that, along with the pathological causes of sterility, it is conditioned also by significant socio-cultural and environmental factors. The main factors that can affect the reproductive capacity of the male are multiple, having determined whether the infertility is transitory or not: primitive testicular patholo-

gies, disorders of transport of sperm, pathologies with secondary hypogonadism and even temperature, diabetes, urinary infections, orchitis, medical therapies, surgical treatment. As well as these factors, lifestyle and therefore environmental and occupational factors can also play an important role: the drop in male fertility in the twenty-first century has been widely recognized, also in view of these parameters that must be considered “reproductive risk factors” (radiation, pollution of foodstuffs, noise pollution, not to mention cigarette smoke which leads to direct and progressive damage on the motility of the spermatozoa, coffee, drugs and alcohol). As such, only with an accurate study of the semen of the subject, together with integration of all his seminal parameters with the data deriving from the study of the fertility potential of the partner, are we in a position to define in correct terms the time to wait and relative fertilizing capacity of an individual. In cases where, after identifying and treating the problem appropriately, incapacity to fertilize persists, the male partner may be helped by means of medically-assisted reproductive procedures.

Outlines of anatomy of the male genital apparatus

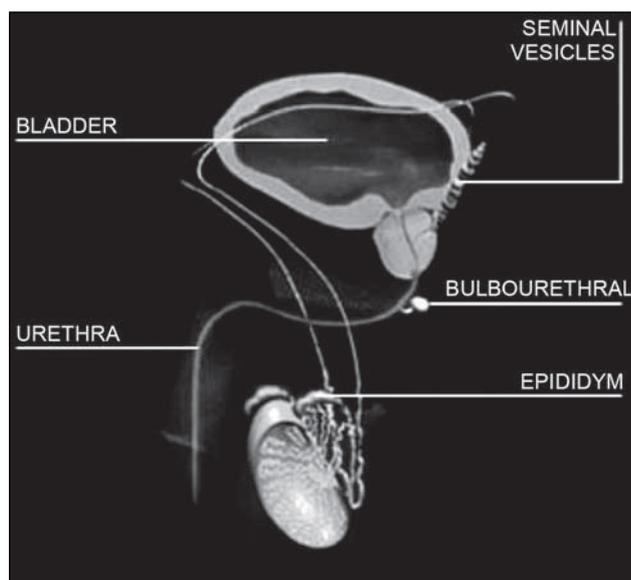
The male genital apparatus consists of the gonads, (testicles), the spermatic canals, glands and external genital organs (Fig. 1) and is constituted as follows:

- gonads or testicles
- spermatic canals
 - tubuli recti and rete testis
 - epididymis
 - deferens ducts
 - (funiulus or spermatic cord)
 - ejaculatory ducts
 - urethra
- glands attached to the spermatic cords:
 - seminal vesicles
 - prostate
 - bulbourethral glands
- external genitals:
 - penis
 - scrotal sac
- rudimentary organs (vestigial structures):
 - appendix of the testicle
 - appendix of the epididymis
 - paradidymis
 - efferent ducts.

The testicles situated in the scrotal sac outside the abdominal cavity produce the spermatogenic cells and male sex hormones.

The spermatic canals begin in the testicle with the

Figure 1. Male genital apparatus.

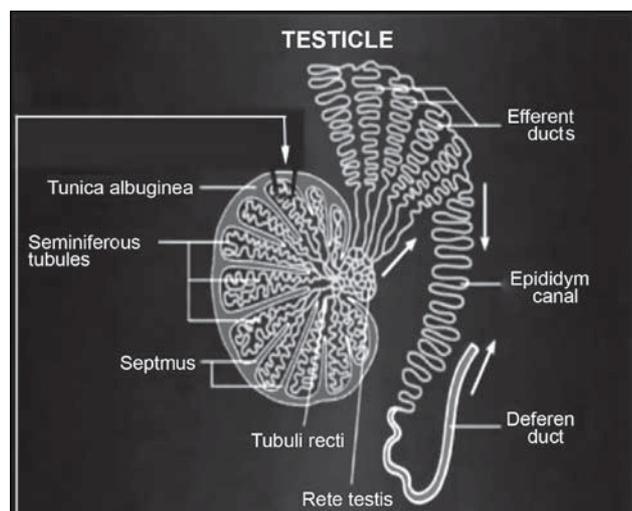


tubuli recti and the *rete testis*, proceed with the epididymis, the deferens duct, the ejaculatory duct and lastly the urethra which represents as such, excluding the initial portion, a common duct for the sperms and urine (Fig. 2).

The spermatic canals are responsible both for the passage of spermatozoa from their place of origin to the exterior, and for the maturation of the same, rendering them capable of moving and fertilizing the oocyte. Moreover, they are able to modify the composition of the lumen contents with both secretory and absorbing mechanisms.

The morphofunctional characteristics of the sper-

Figure 2. Representation of the testicle and epididymis. "Andrologia" Fabbrini.



matic canals, especially those of the epithelium, depend closely on the presence of male sex hormones.

The glands attached to the spermatic canals, the seminal vesicles, the prostate and the bulbourethral glands (or Cowper's glands) are those principally responsible for the production of fluid.

The external genitals are represented by the penis, on the apex of which (the glans) the urethra emerges, and by the scrotal sac or scrotum which contains the testicles and part of the sperm ducts. Small formations (vestigial structures) are, inconstantly, attached to the testicles and the first portion of the sperm ducts which represent embryonal residue; these are the appendix testis and appendix of epididymis, paradidymis and the efferent ducts ⁸.

Physiology of the testicle

The human testicles are a pair of organs localized in the scrotum, separated one from the other by a septum and have an ovoid shape with one axis directed obliquely downwards of 3.5-4.5 cm, an anterior axis of 2.3-3.5 cm and a transverse axis of 2-2.5 cm; the weight of each testicle in the male adult is between 10-45 grams. The testicle is surrounded by a capsule of connective tissue called *tunica albuginea* which merges into the testicular parenchyma, subdividing it into septa. Each septum contains many tubules, the *seminiferous tubules* which are minutely convoluted so as to take up the minimum space possible inside the septa (Figs. 3.1, 3.2) ⁹.

The seminiferous tubules are composed of a wall of *peritubular cells* that rest on a basal membrane surrounding both the *Sertoli cells* and the spermatogenic cells. These latter go through six stages of development before becoming mature spermatozoa (Fig. 4). These changes include two phases: 1) *spermatogenesis*, or proliferation of the spermatogonia and reductional division until the spermatid stage (meiosis) is reached; 2) *spermiogenesis*, the maturing of the spermatids into spermatozoa.

The spaces between the seminiferous tubules are occupied by the Leydig cells, the lymphatic vessels and various connective elements.

The intertubular space is rich in interstitial fluid into which testosterone produced by the Leydig cells is released; this hormone may spread from the interstitial fluid in the seminiferous tubules, inducing and regulating the production of spermatozoa, as well as passing through the blood vessels into the circulatory system to produce systemic effects.

The seminiferous tubules continue in the *rete testis*

Figure 3.1. (Left): transverse section of seminiferous tubules delimited by germinal epithelium. In the peritubular stroma small groups of interstitial cells are present. (Balboni-Motta).

Figure 3.2. (Right): note the pluristratified germinal epithelium surrounded by a thin membrane (lamina). The interstitial cells are between the seminiferous tubules. (Balboni-Motta).

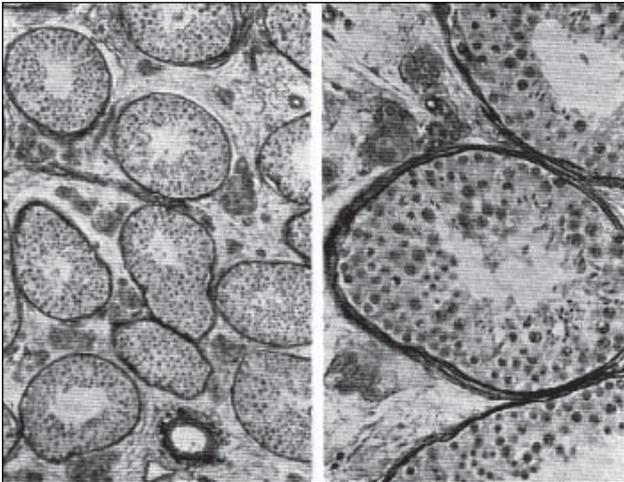
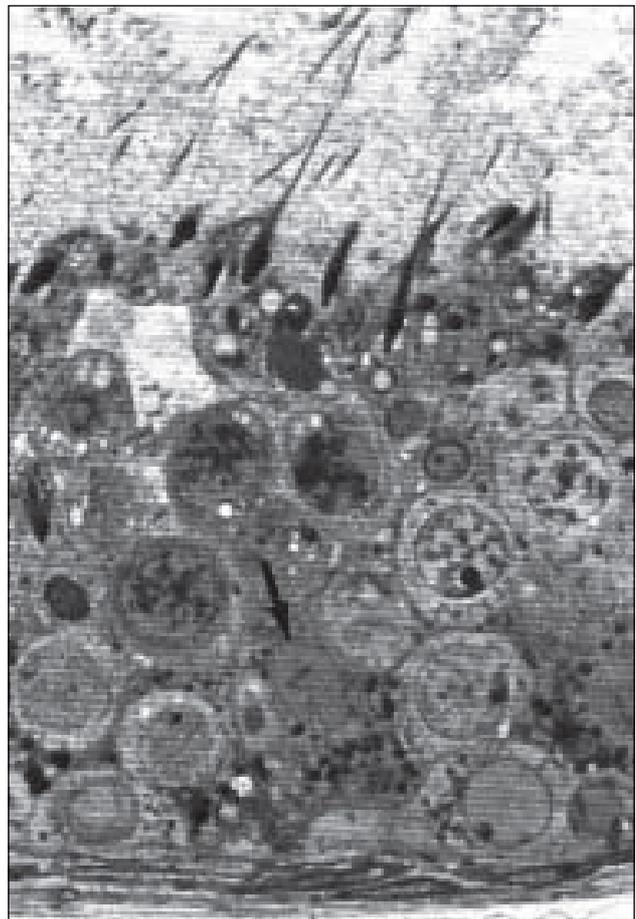


Figure 4. Detail of the wall of a seminiferous tubule. In the germinal epithelium, as well as the sustentacular cells of which a characteristic nucleus can be seen (arrow), the seminal cells are present, in various phases of differentiation. The spermatozoa are visible towards the lumen. Underneath the epithelium there is the lamina. (Balboni-Motta).



which joins to form the head of the epididymis where the spermatozoa begin to acquire the capacity to move ¹⁰.

In the *caput* or head of the epididymis, the smooth muscle surrounding the epididymis duct undergoes rhythmic peristaltic contractions that propel the sperm along the *corpus* and subsequently into the *cauda*. The cauda has less contraction compared to the rest of the epididymis and provides a storage area in which 90% of the fluid containing spermatozoa is re-absorbed.

Part of the maturation process occurs in this area of the epididymis while the final process, including acquiring the capacity to fertilize the oocyte (capacitation), takes place in the female reproductive tract. The *vas deferens* originates in the cauda of the epididymis, passing round the back of the scrotum. The deferent duct or vas deferens is surrounded by a network of interconnected veins, known as the *plexus pampiniformis* which constitutes the necessary mechanism for maintaining temperature lower inside the scrotal sac by means of an exchange of heat with hot blood from the artery and colder blood from the vein.

The deferens ducts contain a thin wall of three-layered smooth muscle cells highly innervated by the sympathetic nerves which, together with the smooth muscle cells of the epididymis, furnish the greater part of the propelling force that is necessary for ejaculation.

Distally speaking, the deferens duct widens, enters

and passes through the inguinal canal to arrive in the peritoneum before penetrating the pelvic region.

Just before coming into contact with the prostate, it is joined by the excretory duct of the seminal vesicles. Secretions of the seminal vesicles mix with spermatozoa coming from the testis and pour into the ejaculatory duct which passes through the prostate to join the urethra in the prostatic utricle. The *prostate* is surrounded by a fibro-elastic capsule and is composed of 40 tubulo-alveolar glands which are immersed in stromal connective tissue, made up of elastic tissue and smooth muscle, and which open up separately into the prostatic urethra that passes through the prostate ¹¹.

The prostate produces the prostatic secretion that constitutes about 15-30% of the seminal fluid; it is composed of a slightly acid (pH 6.4), milky-look-

ing liquid and contains numerous enzymes (acid phosphatase, β -glucuronidase, amylase, fibrinolysin, protease), prostaglandins, spermine and spermidine, immunoglobulin, zinc and citric acid.

The last male accessory glands along the reproductive tract are the bulbo-urethral glands, or *Cowper's* glands, situated posterior and lateral to the membranous portion of the urethra at the base of the penis, and they secrete a clear lubricating fluid that facilitates sexual intercourse.

The human testicle therefore serves two purposes: one is endocrinological, distinguished by the secretion of androgens while the other is exocrinological, characterized by the production of spermatozoa. These two tasks are interconnected, since it has been demonstrated that spermatogenesis depends on the androgens.

Sertoli cells

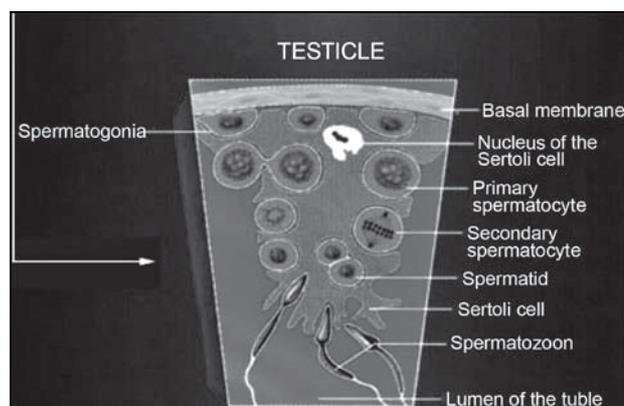
About 120 years ago Sertoli described a type of cell inside the seminiferous tubules placed along the peritubular membrane. These extend towards the lumen of the tubule¹² and numerous cytoplasmic offshoots are everted that surround and make contact with the germinal cells. These cells, called Sertoli cells, have the important role of nourishing and supporting the germinal cells in the stage of development. Each Sertoli cell has close morphological ties with the surrounding cells through specialized structures called tight junctions which distinctly separate the adluminal compartment from the basal one. The basal compartment contains spermatogonia and spermatocytes and is the only one that may come directly into contact with the substances present in the blood, through the interstitial fluid. This compartmentalization excludes the mature germinal cells from direct contact with the blood and peritubular fluid, acting as a proper barrier, called the *blood-testis barrier* (Fig. 5).

Furthermore, the Sertoli cells contribute to the nurturing and transport of the spermatozoa within the genital pathways. The surface of the cell has specific FSH-receptors and is sensitive to this hypophysis hormone¹³.

Interstitial or Leydig cells

Leydig cells (LC) or the interstitial cells of the testicle, described for the first by Leydig in 1850, are highly specialized cells whose primary function is steroidogenic, under the strict control of the luteinizing hormone. This hormone is tied to specific cellular

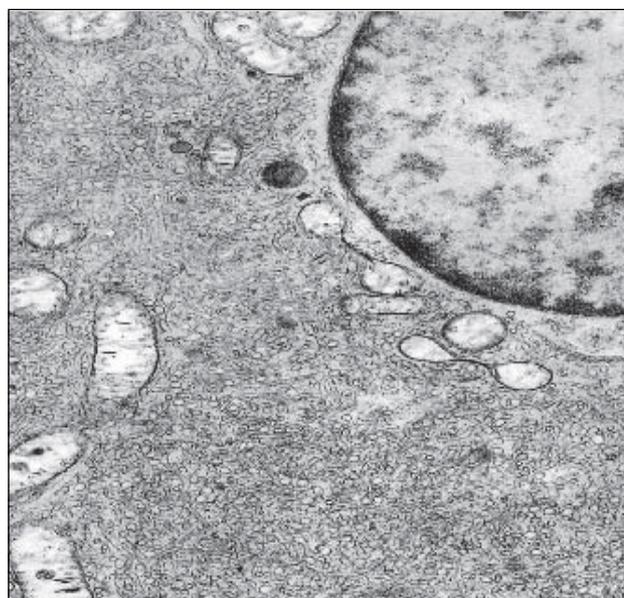
Figure 5. Schematic representation of the seminiferous epithelium. "Andrologia" Fabbrini.



receptors and induces the conversion of cholesterol into androgens. In fact, the LCs secrete testosterone and also oxytocin, thought to be responsible for contraction of the myoid cells that surround the seminiferous tubules¹⁴.

The results of an ultrastructural examination of the Leydig cells show the characteristics of the steroidogenic elements: the agranular endoplasmatic reticule is particularly developed, the mitochondria are numerous and equipped with tubular crests, the Golgi apparatus is evident and furthermore lipid droplets, lysosomes and granules of lipofuscin can be seen (Fig. 6)¹⁵.

Figure 6. Electronic microphotograph of an interstitial Leydig cell. (Fawcett DW. An atlas of fine structure. "The Cell. Philadelphia", W.B. Saunders Company 1966).



Peritubular cells

The peritubular cells are contractile myoid cells that surround the seminiferous tubules. They contain receptors for androgens and oxytocin and as such are susceptible to the control of the LCs. There is a complicated system of paracrine interactions between the peritubular cells and the LCs: both produce different raw material for the formation of the tubular wall ¹⁶.

Spermatogenesis

Spermatogenesis is the process of differentiation and maturation of the *spermatogonia* (indifferentiated germinal cells that cover the basal and external surface of the seminiferous tubules) into *spermatozoa* ¹⁷ (Fig. 7).

The formation of the spermatozoa, defined by the term spermatogenesis, occurs in the seminiferous tubules in the testicle and is subdivided into three phases: the *mitotic* phase, the *meiotic* phase and *spermiogenesis*.

The mitotic phase includes the division of the spermatogonia until formation of the primary spermatocytes occurs, a process defined spermatocytogenesis.

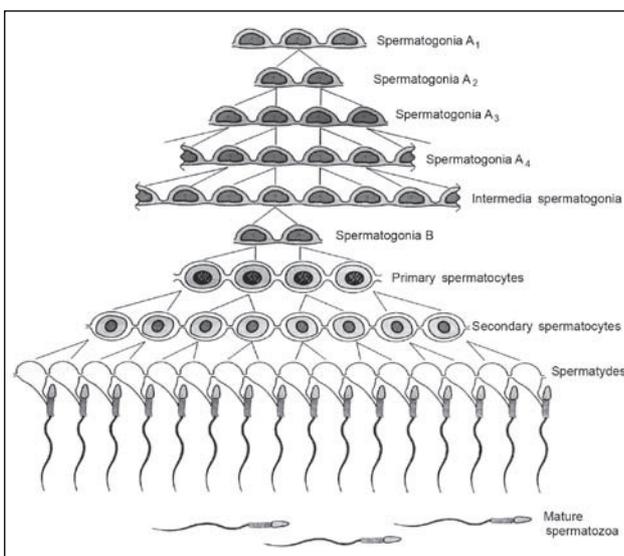
The spermatogonia, small diploid germinal cells located in the basal compartment of the seminiferous tubules (Fig. 5), receive a stimulus from the testosterone after puberty to begin the cycle of cellular division and replication.

According to the staining of the chromatin, in the human testicle three different types of spermatogonia can be distinguished, denominated type A dark, (A-d, "dark" – with dispersed chromatin, intensely coloured), type A pale (A-p, "pale" – with finely dispersed chromatin, faintly coloured) and type B. Maintenance of the spermatogonial reserves is assured by the A-d type spermatogonia which, following mitotic division, produce both further stem cells (A-d), indifferentiated, that will make up the reserves of stem cells necessary to guarantee subsequent cycles of multiplication, and more differentiated (A-p) spermatogonia. From mitotic division of the type A-p spermatogonia derive the spermatogonia type B which in turn give rise, again through mitotic division, to the primary spermatocytes that after having duplicated their DNA contents enter in the first meiotic division.

The meiotic phase consists of a double cellular division with one single duplication of the number of chromosomes: in this way the daughter cells will have a haploid chromosome complement. The two haploid daughter cells deriving from the first meiotic division (reductional meiosis) are called secondary spermatocytes (with haploid structure) of which one has 23 y and the other has 23 x. Between the first and second meiotic division there is a very short interphase, since there is no synthesis of DNA. Almost immediately the process of the second meiotic division begins (equational meiosis), and from each secondary spermatocyte two haploid spermatids advance and form. Thus, at the end of the meiotic process, one primary spermatocyte has divided itself twice to give rise to four spermatids.

The subsequent phase consists of the process of *spermiogenesis*, by means of which each spermatid, without further cellular division, is transformed into a mature spermatozoon, taking on its definitive form: a nucleus with a compact and homogeneous structure, a thin tail and a little cytoplasm. During spermiogenesis, also special structures such as the *flagellum* and the *acrosome* are differentiated. As the cells gradually advance through the spermatogenetic process, they move, propelled by the tubular fluid, towards the lumen of the seminiferous tubules and at the end of spermiogenesis the mature spermatozoa are released into the lumen of the tubules. From the seminiferous tubules, through the *tubuli recti*, the *rete testis* and the efferent ducts the spermatozoa reach the epididymis. The last residues of cytoplasm, called "*cytoplasmic droplet*" are eliminated here. The process of release of mature germinal cells is defined *spermiation*. When the sper-

Figure 7. Schematic diagram of spermatogenesis in which intracellular connections can be seen that permit the persistence of the syncytium during differentiation and maturation. (Modified by: Ren and Russell).



matozoa arrive in the head of the epididymis, they are unable to move and have no fertilizing capacity, two conditions which derive from a complex process that begins during transit through the epididymis and which is completed in the female reproductive tract. The duration of the spermatogenesis, that is, the time necessary for a stem cell to differentiate into spermatozoon, is in man about 70 days and daily production in the testicles is approximately 120 million spermatozoa in a normal subject¹⁸. This period may not be modified, either with hormone therapy or extrinsic factors such as temperature, or toxic agents such as radiation.

The maturation of the spermatozoa is therefore the result of processes that are intrinsic to the spermatozoa themselves, as well as to interaction with the epithelium of the epididymis duct. This phase includes among other things the depositing on the surface of the spermatozoa of some epididymo-specific glycoproteins (*molecule coat*) that represent a system of immunological protection of the gamete and at the same time seem to be elements of stabilisation of the plasmatic membrane (decapacitating substances) that are lost in the female genital pathways during the phase of capacitation preceding the acrosomal reaction (Fig. 8).

In the testicle the spermatozoon is deprived of any progressive motility, even if the flagellum is capable of some movement, but of little amplitude (6-7 μm)¹⁹. In the head of the epididymis the flagellum

begins to make more movement (15-16 μm) and the spermatozoa start to show propulsive action, tracing circulatory or completely irregular trajectories. During transit through the epididymis, the frequency and amplitude of the movement of the flagellum increase significantly (18-19 μm), thus the spermatic motility becomes progressive.

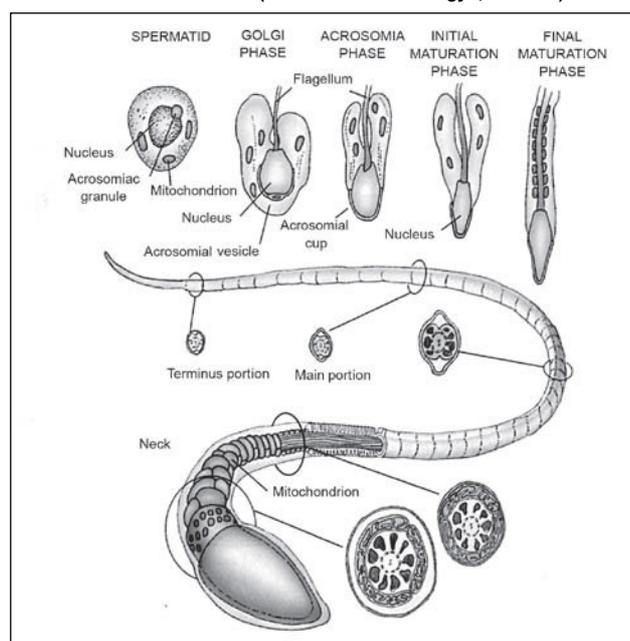
As well as these factors, there are others which contribute to the development of motility: the increase of osmolarity and diminishing of the pH, the stabilisation of numerous structures of the spermatozoon, through the formation of disulfide bonds or bridges such as the flagellum, the mitochondrial sheath of the median, the external mitochondrial membranes and the dense accessory fibres of the axoneme, the intraspermatic increase in calcium ions and cAMP.

In the epididymis the spermatozoa are stored as in a reservoir and only at the moment of ejaculation mix with the seminal plasma which is the product of the combined secretory action of the accessory glands of the male genital tract (seminal vesicles, prostate, bulbourethral glands, deferens ducts and deferential ampullae).

It is important to underline that the various secretions are not emitted externally in a casual and irregular way but rather following a precise sequence. First there is a prostatic quota, that permits an improvement of the motility of the spermatozoa for the contribution to the seminal plasma of some substances such as albumin, that are capable of stimulating the motility of the spermatozoa in the epididymis, and of those present in the ejaculate. There then follows a quota from the epididymis and testicle (rich in spermatozoa) and lastly a quota from the vesicle, characterized by its yellowish colour and alkaline properties with large quantities of fructose, the principal specific biochemical marker of the vesicle fluid, and other constituents including ascorbic acid, inorganic phosphorus and potassium that is the principal cation since there is practically no sodium present^{20 21}.

This explains the necessity to analyse the whole amount of ejaculate, after its complete homogenisation, in order to make a correct diagnosis and to also make use of the sample *in vitro*.

Figure 8. Diagram figuring the six stages of spermatogenesis in human seminiferous tubules (taken from "Histology", Gartner).



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Hypothalamus-hypophysis-testicle axis

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Hypothalamus

The hypothalamus is the cerebral region that supervises the regulation of the glands involved in the maintenance of hormonal homeostasis by means of the action exerted on the pituitary gland. This control is mediated by two mechanisms: 1) the secretion of neuropeptides synthesized in the hypothalamic neurons and transported to the posterior hypophysis along the hypophyseal peduncle; 2) the neuroendocrine control of the secretion of the adenohypophysis or anterior hypophysis by means of the secretion of peptics that mediate adenohypophyseal response (hypophysiotropic hormones) ¹. The GnRH-secreting neurons are in the preoptic-medial area and in the arcuate nucleus of the medial hypothalamus and their axons end in the median eminence where a portal vein running along the hypophyseal peduncle transports the GnRH to the gonadotropic cells. GnRH is a decapeptide, codified by a gene localised on chromosome 8 which acts through a seven transmembrane domains receptor binding to the G_q protein.

Anterior hypophysis

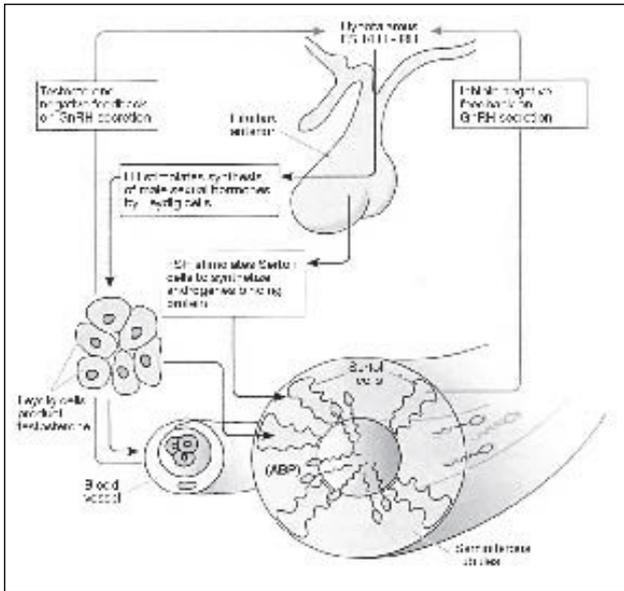
Gonadotropins (FSH and LH)

The LH and FSH gonadotropins are synthesized and secreted by the gonadotropic cells (65-70% of the hypophyseal cells) in response to the gonadotropin-releasing hormone, GnRH. Most of the gonadotropic cells produce both LH and FSH (60%), the remaining part of LH (18%) or FSH (22%). The GnRH is synthesized and secreted by the hypothalamus in pulsating waves and binds to receptors coupled to the G_{q/11} protein which determines activation of the phospholipase C, leading to an increase in turnover of inositols and of mobilization and influx of intracellular calcium. The activation of this path of transduction determines the increase of the transcription of genes codifying subunits α and subunits β of the LH and the FSH and therefore of the circulating hormones ² (Fig. 1).

Hypothalamus-hypophysis-Leydig cells axis

The production of androgens is regulated by the GnRH which induces hypophyseal secretion of LH (luteinizing hormone) that in turn stimu-

Figure 1. Schematic representation of hormone control on spermatogenesis (from: Fawcett, "A textbook of Histology").



lates the production of testosterone by the Leydig cells.

The pulsatility of the GnRH seems to be a fundamental requisite for a normal functioning of the gonadotropic cells, given that constant levels of GnRH determine a desensitization of the hypophysis with reduction in LH. Peaks of LH which may reach values even three times greater than basal levels follow one another irregularly with an average interval at around 90 min.

LH is a glycoprotein composed of two polypeptide chains, one α and one β . The subunit α is common also to FSH, hCG and TSH; the subunit β is specific and its gene, located on chromosome 19, codifies for a protein of 121 aminoacids.

Daily production of LH in males is from 500-1.000 UI, metabolic clearance of 25 ml/min and the complete turnover of hypophyseal contents is 12-24 hours. LH Secretion is controlled by a negative feedback mechanism of the testosterone. It is debated whether the inhibitor effect of androgen is exerted directly at a hypothalamic and hypophyseal level or indirectly through their conversion into estrogens. Studies carried out with non-aromatizable androgens such as dehydrotestosterone (DHT), taking account of the pulsatility of the secretion of LH, have demonstrated a differential effect: androgens would appear to reduce the frequency of the peaks of LH, while estradiol would seem to determine a reduction in amplitude. The LH receptor, situated on the wall of the Leydig cells is a seven transmembrane domain receptor binding to the G_s proteins with cAMP as

second messenger, the activation of which gives rise to the cascade of events of steroidogenesis.

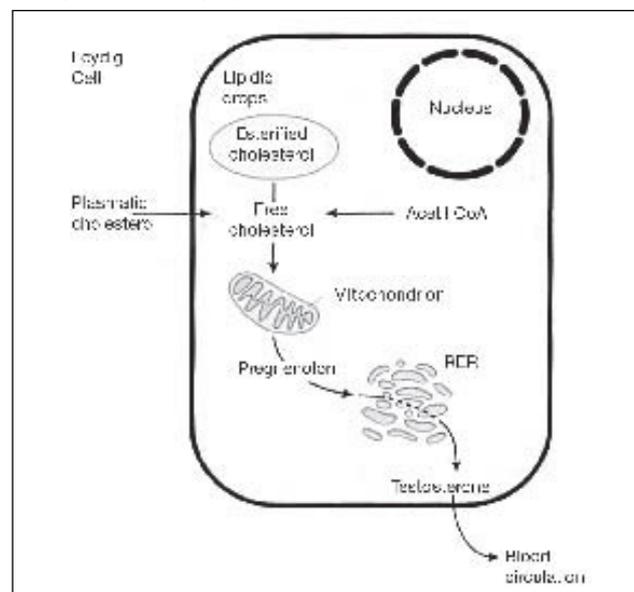
The secretion of testosterone has a less accentuated pulsatile character compared to that of LH, and follows circadian rhythms, more accentuated at puberty, with maximum values on waking and minimum values in the evening. The testicle also secretes estradiol and estrone, but respectively only 20% and 5% of the circulating hormones are of testicular origin while the majority come from the peripheral conversion of testosterone and androstenedion³.

Testosterone

Testosterone stimulates the development of the accessory glands and assumes a fundamental role in the development of secondary male sexual characteristics, yielding metabolic and psychic effects. Intratesticular testosterone, moreover, is diffused as far as the Sertoli cells and stimulates spermatogenesis. In fact, subjects who present a post-puberal androgenic deficit have lesser bone mineral density, lesser muscular mass and diminishing of libido and erectile capacity, compared to normo-testosterone subjects.

The luteinizing hormone (LH) binds to the LH receptors present on the Leydig cells, activating the adenylate cyclase which forms cyclic adenosine-monophosphate (cAMP). The increase in cAMP induces activation of the cholesterol esterase that is able to extract free cholesterol from the intracellular lipid droplets (Fig. 2).

Figure 2. Schematic representation of the synthesis of testosterone by interstitial Leydig cells.

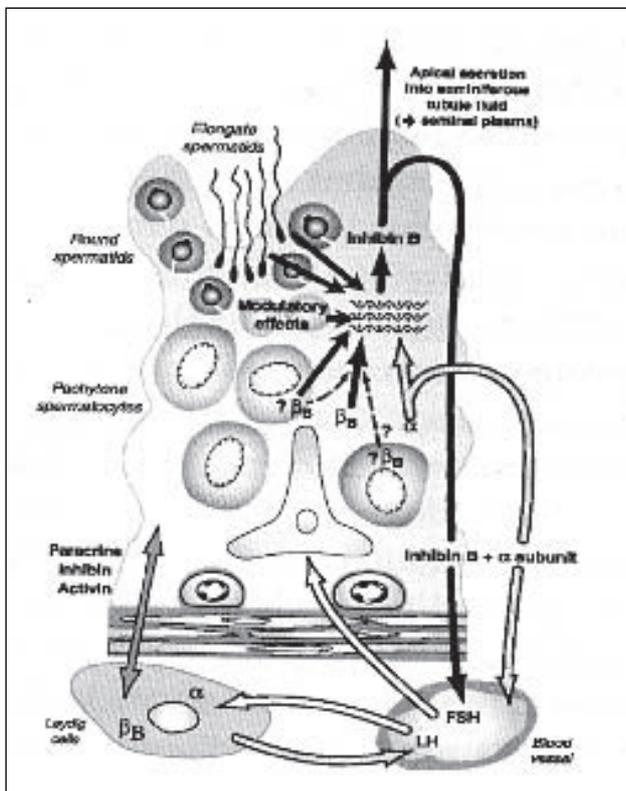


This cholesterol is then transported from the smooth endoplasmic reticulum to the mitochondria where the precursor of the male hormone is constructed, that is finally secreted out of the cell. Secretion of LH is inhibited through negative feedback of the testosterone produced.

Control over spermatogenesis takes place above all by means of the follicle-stimulating hormone (FSH) that induces synthesis and release on behalf of the Sertoli cells of the *androgen-binding protein* (ABP) (Fig. 3). The ABP binds the testosterone, preventing leakage from the seminiferous tubules, thus increasing the intratesticular concentration that is necessary for spermatogenesis. FSH release is inhibited by the increase of inhibin-B produced by the Sertoli cells (Fig. 3).

Testosterone circulates, binding to specific transport proteins, the *sex hormone binding globulin* (SHBG) (30%) and to *albumina* (68%). The free testosterone, about 2-3% of the total, is responsible for the action on organs and tissues. This fraction may vary, however, depending on the SHBG rates that increase for example in conditions of obesity, hyperprolactinemia, hypothyroidism, hyperthyroidism, cyrrhosis and in elderly subjects. An increase in SHBG determines a diminishing in free testosterone levels.

Figure 3. Control of secretion of inhibin-B by testicle (from Anderson RA. Mol Cell Endocrinol 2001;180:190-216.



Hypothalamus-hypophysis-Sertoli cells axis

The GnRH stimulates secretion of FSH, albeit in reduced amounts. Recent studies have demonstrated that a reduction of pulsatile frequency of the GnRH determines a preferential secretion of FSH compared to LH. FSH is composed of two subunits, α and β . The gene of specific subunit β is situated on chromosome 11 and codifies for a protein of 110 aminoacids. In the male, metabolic clearance of FSH is of 4-12 ml/min and daily production is from 140-280 UI³.

The FSH is composed of heterogeneous molecules that differ for their diverse composition from the glucidic groups. The glycosylation of the hormone determines also the half-life and therefore the biological activity *in vivo*⁴; FSH isoforms, characterised by few residues of sialic acid (basic forms), in fact have a short half-life, while those characterised by a greater number of residues of sialic acid (acid forms) have a half-life 10 times greater^{5,6}.

The basic forms circulate in reduced numbers but present greater receptor-binding affinity (from 2-5 times greater than the acid forms)^{4,7,8}.

The FSH plays out its testicular action on the spermatogenesis almost entirely through action on the Sertoli cells, in which a specific seven transmembrane domain receptor correlated with the G_s proteins is present.

Therefore, these cells, as well as constituting the blood-testis barrier, regulate meiosis and spermiogenesis, secretion of specific and non-specific proteins (ABP, plasminogen activator, transferrin, ceruloplasmine, antiMüllerian hormone, H-Y antigen, inhibin-B), the conversion of testosterone into DHT (5 α -reductase activity) and estradiol (aromatase activity), as well as many other functions including functional modulation of the Leydig cells and peritubulars.

Inhibin-B

Inhibin-B has a central role in regulating the secretion of FSH, a proteic hormone produced by the gonads, hypothesized as far back as the 1930s, which exerts a negative feedback on the FSH. Inhibin-B is composed of a subunit α and a subunit β connected to each other by means of a disulfide pons; the subunit α (134 aminoacids) is common in all forms of inhibin while subunit β , of which two isoforms are known (β_A and β_B , respectively of 116 and 115 aminoacids), participates in the constitution of inhibin-B through the isoform β_B ⁹.

Both forms of inhibin, therefore, are able to exert an FSH-suppressing action¹⁰. Also heterodimers and

homodimers can be formed of the subunits beta, β A- β B, β A- β A, β B- β B, to which the name of FSH-releasing protein (FRP) has been given, or activin (AB, A and B respectively)^{11 12}; these peptides stimulate the exclusive secretion of FSH with a reduced frequency and in a different manner compared to the GnRH. Furthermore its hypophyseal action seems to be different to that of the GnRH¹³⁻¹⁸.

In recent years, the hypothesis that testicular inhibin-B represents the principal hormone controlling secretion of FSH has been supported by much clinical and experimental evidence. Reduced concentrations of inhibin-B found in some pathologies such as hypogonadotropic hypogonadism and Klinefelter's syndrome, not to mention its absence in agonadic subjects, has led to the belief that the testicle is the only place where this peptide is produced¹⁹. The subunits α and β B have been localised by means of studies of immunohistochemistry prevalently on the Sertoli cells²⁰, while, coinciding with the lack of inhibin-A circulating in man, the search for the subunit β A in the testicle has proved negative.

Secretion of inhibin-B by the Sertoli cells is gonadotropin-dependent; in fact, the administration of levonorgestrel and testosterone is capable of suppressing secretion of endogenous gonadotropins, and secondarily reducing also the concentrations of inhibin-B²¹. Furthermore, in patients suffering from hypogonadotropic hypogonadism due to isolated GnRH deficiency, subsequent treatment with pulsatile GnRH is capable of normalizing levels of FSH, LH, testosterone and inhibin-B^{22 23}. We can therefore conclude that FSH stimulates the secretion of inhibin-B by the testicular Sertoli cells which in turn, via negative feedback regulate the hypophyseal secretion of gonadotropin.

There seems to also be a gonadotropin-independent component in humans in the controlling of inhibin-B secretion by the Sertoli cells, regulated by interaction of the latter with the surrounding testicular germinal cells^{24 25}. In particular, experimental studies on rats have shown that elongate spermatids are capable of influencing the production of inhibin²⁶ and that the concentrations of immunoreactive inhibin vary depending on the different stages of the cycle of the seminiferous epithelium²⁷. It can be inferred that secretion of inhibin is associated with the more advanced phases of the spermatogenetic process²⁸, thus indicating the specific interaction between the cells implicated in spermiogenesis and the Sertoli cells. The effect of the spermatids in controlling the secretion of inhibin-B, and therefore FSH, seems to be documented also in man; in fact, subjects with

severe oligozoospermia or azoospermia with arrest in the spermatidic maturation process present normal FSH levels²⁹⁻³¹ (Fig 3).

Since Inhibin-B is produced by the testicle depending on the grade of spermatogenesis, it may rightfully be considered as the best indicator of testicular function³².

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Seminal diagnostic

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Seminal fluid

The seminal fluid is composed of a *corpuscolate fraction*, represented by solid elements and by a liquid or *soluble fraction* (seminal plasma) in which sperm are suspended. The seminal plasma is the product of secretory combined accessory glands of the male genital tract.

At the moment of ejaculation the spermatozoa are transferred from storage in the *cauda* of the epididymis (which contributes from 5-10% of the total volume of the ejaculate), mixed with the accessory secretions gland in a well-defined sequence (first that of the prostate, then that of the vesicles) to then be emitted through the penile urethra.

The first part of the ejaculate contains most of the spermatozoa suspended in prostatic fluid and represents from 15-30% of the whole volume of the ejaculate (including a small rate of epididymal flux). The colour is whitish with an acid pH value of between 6.4 and 6.8. The prostatic fluid is particularly rich in fibrinolytic enzymes (responsible for the liquefaction of the coagulated vesicular secretion), citric acid, zinc and acid phosphatase.

The epididymal secretion, though scarce in quantity is rich however in carnitine, glycerophosphorylcholine and α -glucosidase. The secretion of the seminal vesicles (alkaline and of a yellowish colour) constitutes the last part of semen ejaculated and contributes about 80% of the entire ejaculate. It contains fructose, ascorbic acid, inorganic phosphorus and potassium.

Semen analysis is the basic analysis for the study of male infertility and must be evaluated according to WHO 2000 criteria ¹. In fact only with a correct recording of the seminal parameters is the andrologist able to utilize and integrate the laboratory data with clinical data in order to then classify the patient as fertile, hypofertile or sterile.

Semen analysis

The standard semen analysis must be performed respecting certain rules for collection of the sample and the detection of a minimum number of seminal and nemaspermic variables that are indispensable for maintaining a diagnostic value and/or optimal prognostic ².

One of the instructions for the collection of semen is related to the period of sexual abstinence that must precede the collection itself. By agreement linked to knowledge relating to the physiology of the nemaspermic intraepididymal maturation, and to the transit and per-

manence of mature sperm in the caudal tract of the epididymis, it has been determined that the period of abstinence from ejaculation preceding that of the semen analysis must be from 3-5 days long. Only adhering scrupulously to this rule permits the possibility of comparing seminal data with normal standard values so as to classify the individual according to his fertilising potential. Too prolonged abstinence will in fact provoke an accumulation of nemaspermic cells that are often endowed with reduced motility, while too short abstinence will cause a reduction in the sperm concentration and frequently a diminished volume of ejaculate.

Another fundamental instruction to follow, in order to perform the semen analysis as far as possible without errors due to other variables, regards specifically the methods used for collection³. The only acceptable method is that of collecting the sample through masturbation and transferring it to a container for urine collection, as this is the only way to guarantee recovery of the whole ejaculate without contamination of any female secretions.

The seminal sample must arrive in the laboratory within 60 minutes of collection in order to assess times of liquefaction, or more correctly fluidification, and to carry out the first evaluation of the motility. Moreover, the patient must be carefully instructed as to how to avoid excessive thermal changes to the seminal fluid, as both excess heat and low temperatures can interfere with the sperm motility.

For the anamnesis, it must be remembered that upon delivery of the sample to be examined, certain questions must be put to the subject in order to collect data regarding any psychical pathological event (particularly conditions of acute stress) or physical, relating to the preceding three months, as well as any use of drugs or medicine in the same period. Febrile conditions of viral or bacterial origin, antibiotic therapies, local or general anaesthetics, anabolizers for sports and many other pathological or therapeutical questions may interfere with the characteristics of the semen and should be referred to the clinician so any incidence on the results of the seminal sample may be assessed.

Macroscopic evaluation

Volume

The volume of the ejaculate is generally between 1.5-5 ml, with marked oscillations also in the individual. Below 0.5 ml constitutes a condition of hypoposia while quantities greater than 6 ml are described as hyperposia.

Hypoposia may indicate a pathological obstruction

of the ejaculatory ducts or a secretory deficit of the seminal vesicles due to functional or anatomical causes; in very rare cases it may indicate a reduced hormonal secretion of primitive or secondary testosterone. Hyperposia is frequently associated with flogistico-irritative pathologies of the seminal vesicles and the prostate.

pH findings

The pH of the seminal fluid is alkaline (normal values 7.2-7.8) and is the result of the mingling of the acid secretion of the prostate with the alkaline secretion of the seminal vesicles.

Aspect

The physiological aspect of semen is ivory-white coloured and opalescent. A milky aspect, especially if accompanied by hypoposia and azoospermia may indicate an ejaculate consisting exclusively of prostatic secretion. A yellowish aspect (pyoid) indicates the presence of white globules⁵: a number of white globules ≤ 1 million/ml (counted with Thoma-Zeiss or Neubauer counting chamber) is considered physiological. A pinkish or intense red colour indicates instead the presence of red globules (haemospermia). Research of red globules may be particularly investigative, given the frequent deformation of the erythrocyte as a consequence of the seminal osmolarity. From a clinical point of view, pyospermia is associated with acute or subacute infections of the male genital tract, while haemospermia may be caused by slight haemorrhages due to the capillary fragility of the penial or prostatic urethra, or alternatively may be a symptom of intense flogosis⁶.

Viscosity and fluidification

Immediately after ejaculation, the human seminal fluid undergoes a process of coagulation allowing the semen to cling to the cervix and form an interface with cervical mucous in the posterior fornix of the vagina. After coagulation, the process of fluidification of the semen begins and terminates in a period ranging from 10-60 minutes. The analysis of this process is macroscopical and targeted at evaluating whether the homogeneization following fluidification is complete or incomplete (presence of smaller or larger coagulates) and whether it occurs in a normal physiological period of time or is delayed. Alteration of the fluidification may be observed where there is inflammation of the accessory glands, and may justify, though only in part, the consequent hypofertility. Assessment of the seminal viscosity, in contrast with the fluidification, is not a parameter with which to

study a characteristic physiological process of the semen, but it evaluates a characteristic relating to its biochemical and cytological constitution. In the case of increased or decrease viscosity, the terms “hyper-viscosity” or “low viscosity” respectively are used. In the first case, this often indicates local flogosis regarding the accessory glands; the second refers to cases in which the cellular component of the seminal fluid is very scarce.

Microscopic evaluation

Sperm concentration

The sperm concentration must be evaluated on the optic microscope, setting up a minimum of two preparations per sample (observation with 10x, 20x, 40x lens) with a special Makler counting chamber (Fig. 1); dilution of the sample is not necessary.

A drop of 10 μ l of seminal fluid is deposited directly in the specific area of a slide containing four quartz pins on which is placed a cover glass equipped with a metallic ring and a counting grid. The chamber consents the monolayering of 10 microns and the possibility to multiply by a million the number of cells evidenced. This technical support does, however, present limitations in terms of precision that can be observed in cases of low sperm concentration and in those of strongly dishomogeneous seminal samples. For this reason samples must always be tested fresh, immediately after collection.

In fertile subjects aged between 20-40 years and considered normal also from a clinical point of view, a marked oscillation in numbers of spermatozoa may be seen in the same individual between one and ejaculate and another. Maximum values vary between 100-200 million/ml (higher values may be defined as *polyzoospermia*). In contrast, the minimum concentration may be established around 20 million/ml. Anything under this constitutes *oligozoospermia* while absence of spermatozoa in the ejaculate is defined as *azoospermia*. In the case of the number of spermatozoa being lower than 1 million/ml, the term *criptozoospermia* is used. A diagnosis of azoospermia may only be declared with relative certainty after being confirmed on at least three differentiated samples and verified accurately also on sediment after centrifugation (3000 rpm per 10 min).

Sperm motility

As with all mammals, also in human movement of the spermatozoa is flagellating and consists of a series of waves which originate at the base of the flagellum and propagate along it, causing the head

Figure 1. Makler's counting chamber.



to be propelled along passively with regular rhythmic oscillations⁷. Motility undoubtedly represents a fundamental property of the spermatozoon⁸. This parameter must not be confused with that of sperm vitality; in fact a spermatozoon may well be vital from a biochemical viewpoint while at the same time being immobile. Sperm motility is assessed at the end of fluidification, setting up a preparation and observing it on the optic microscope with a 20x lens.

At least twenty microscopical fields per preparation must be assessed, and in any case not less than 100 nemaspermic elements.

The evaluation of the total percentage of the mobile forms is fundamental, but also the discrimination of the type of motility must be taken into consideration. This must normally be rapid and forward (type "a" 40 μ /sec). When the average speed is significantly reduced, but the shifting of the cell continues along a straight line, the motility is described as slowly progressive (type "b"). Non-rectilinear motility is defined as dyskinetic (type "c") or "*agitoria in loco*" (moving on the spot), in other words "non-progressive", in cases in which there is no real movement in the visual field.

Two hours after ejaculation, in a normally fertile subject aged between 20-40 years, the percentage of cells having forward motility, both rapid and slow, is generally $\geq 50\%$. Values lower than this determine a more or less accentuated condition of hypokinesis that is defined with the term *asthenozoospermia*. A total absence of motility is termed akinesis.

Another extremely important characteristic is the persistence of total motility in time; in normal conditions if the rheological conditions of the seminal plasma permit it, motility is maintained above 30-35% also after 24 hours.

Sperm morphology

The mature human sperm, observed through an optic microscope, appears as having an apical portion denoting "head", oval-shaped and flattened, and a long, thin flagellate segment called the "tail". This in turn is divided into an *intermediate segment* (length 5-6 μ m) that contains the mitochondrial system which is essential for the production of energy, a *principal segment* (length about 45 μ m) and a very fine *terminal segment* (length 5 μ m). The head (from 4 to 5.5 μ m long and from 2.5 to 3.5 μ m wide) is mainly occupied by the nucleus containing DNA, and in the anterior part the acrosome is situated, consisting of glycoproteins deriving from the Golgi apparatus of the spermatid. The neck of the spermatozoon, a very short tract about 1 μ m long, extends from the posterior of the nuclear membrane up to the joining of the intermediate segment with the head. The total length therefore of the spermatozoon is about 60 μ m. The thread-like, axial unit is constituted of nine voluminous fibres (external cylinder), nine fine fibres (internal cylinder) and lastly two fibres forming the central couple that run the length of the whole flagellum.

The sperm morphology must be assessed immedi-

ately under the optic microscope with a 40x lens. As for the motility, at least 20 microscopical fields per preparation must be considered, and in any case no less than 100 nemaspermic elements. The percentage of the atypical forms must not exceed 70%; anything above this value constitutes a condition of *teratozoospermia*.

Morphological anomalies are indicated according to the classification proposed by the WHO 2000 ¹, regarding the head (microcephaly, amorphous head, acephaly etc.), the neck (angulated neck) or the tail (cropped, swollen, double tails etc.) of the nemaspermic cell.

Non-nemaspermic cell component of seminal fluid

- *elements of the spermatogenetic line*: these are prevalently represented by primary spermatoocytes and spermatids;
- *epithelial cells*: these derive from the ducts and canals of the genito-urinary apparatus;
- *prostatic corpuscles*: their presence is physiological, an increased concentration does not necessarily indicate the presence of prostatic pathologies;
- *spermioagglutination zone*: spermatozoa tend to clump together and condense, (spontaneously, due to bioelectric and chemical phenomena of interaction of the membrane) around leucocytes, epithelial cells, cell detritus and immobile spermatozoa. In this case, areas of aspecific condensing or spermioagglutination may be seen through the microscope. When this agglutination is specific, formed only by nemaspermic cells, it is more clinically significant as it indicates the presence of antispermatozoa antibodies.

In order to perform a correct "screening" of elements in the seminal fluid, it is sufficient to set up a May-Grunwald-Giemsa, Papanicolau or Bryan-Leishman ⁹ staining.

Computerized study of nemaspermic kinesis

In the last twenty years, computerised systems have been developed which use the analysis of digitalized images in order to assess seminal parameters such as concentration, motility and morphology.

This sector of seminology is known today as CASA ¹⁰ (*Computer Assisted Sperm Analyzer*). The first attempts to automatise the process of assessing nemaspermic kinesis were made using the technique of *Laser Light Scattering*, which was based on modifying the frequency of light diffused by a low-energy laser by spermatozoa in movement, by

the technique of *Time-Lapse Photography* and by *Multiple Exposure Photography* in which spermatozoa in movement leave traces or signs on the photographic images. Other techniques involve spectrophotometric methods, based on absorption of ultraviolet light and videocinematography with calculation on monitors of the trajectories described and average speed of the spermatozoa^{11 12}.

Among the methods which use analysis of images, one of the most recent is represented by the systems for study of the human nemaspermic cell by means of electronic programmes of video imaging. These systems effect proper analyses of the semen, in particular of the concentration, the motility and in some cases also of the morphology of the spermatozoa¹³.

The first commercial analyser for sperm motility appeared in 1985, and used a microcomputer to obtain an image that was digitalized from a video signal. Since then, various systems of computerized analysis have become available (Cell Soft, Hamilton Thorn, Cell Track).

The SCA SYSTEM (Sperm Class Analyzer) represents an evolution in computerized sperm analysis. It consists of a personal computer equipped with specific software for data management and uses an phase-contrast optical microscope, a Makler counting chamber, a high-resolution videocamera and PC monitor (Fig. 2).

From the microscope the image is transferred by means of the videocamera, where the PC monitor can be controlled by the operator; from the screen the image is sent to the processing software that is present in the computer which converts the real

image into an electronic trace with a resolution of 512x512 pixel (digitalisation). In order to obtain statistically stable and reliable data, it is considered ideal to analyse at least 500 mobile cells from at least three different fields.

It is important to analyse the fields at random. The system at the end of the reading of each field permits to check how the machine is assessing the sample in question by making a report of the kinetic parameters as well as providing each histograms time relating to velocity and linearity.

Analysis is carried out using a digitalised image in different shades of grey; this threshold of grey is regulated subjectively by the operator up to the point of eliminating the tails of the spermatozoa and the detritus on the digitalised image. Unfortunately, to date, no objective system exists for establishing a correct digitalisation of the threshold which, as such, represents a source of possible error.

The system is capable of distinguishing the spermatozoa from the other cells, cell detritus and aggregates present in the seminal fluid, integrating the data referring to size, brightness and sperm motility (Fig. 3).

The computer transforms the head of the spermatozoon into a digitalised image, on the basis of the coordinates of its initial position and its maximum velocity, and calculates a circular area in which it is possible for the centroid in question to move.

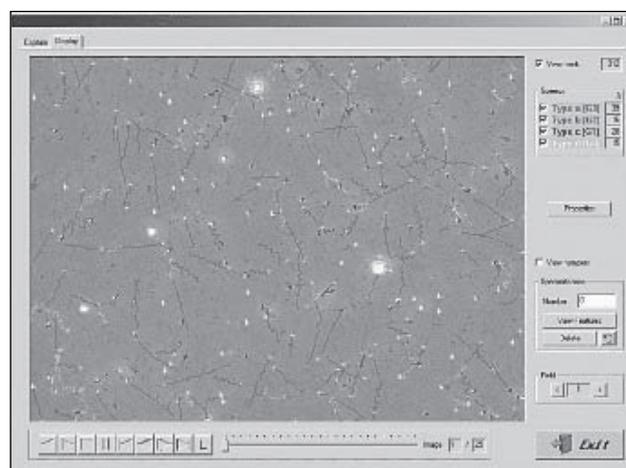
The concentration of this sequence of images is calculated, together with the percentage of mobile spermatozoa, motility, linearity, amplitude and the frequency of lateral beats of the head.

The system is able to assess the curvilinear velocity (VCL), the linearity of the intermediate trajectory

Figure 2. Computer and phase-contrast optical microscope.



Figure 3. A reading of the Sca System.



(straightness STR), the amplitude of lateral movement of head (ALH) and the frequency of beats (BCF)¹⁴.

Once the reading has been completed, all the data collected, both global and relating to the single fields analysed, may be filed. This registration or filing of data consents access via modules for a detailed analysis for each field, thus enabling, for instance, the selection of one single spermatozoon in order to analyse its kinetic parameters of linear velocity, linearity, interval of movement of the various cells during microscopic observation, linear acceleration, direction of nemaspermic course, directional changes, velocity of directional changes and lastly, lateral movements of the nemaspermic head. All these parameters can be extracted directly from the system to file a medical report.

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FSH hormone therapy in male infertility

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Correlation between plasmatic FSH and seminal parameters

The importance of the gonadotropic action on spermatogenesis has been known for some time, although the mechanisms of action and the relative role of the follicle-stimulating hormone (FSH) and of testosterone (T) in the beginning of spermatogenesis are still being debated.

Clinical evidence of hypogonadism suggests that the FSH is necessary, together with T, to start off spermatogenesis, also if there may be some minimal spermatogenetic activity in the absence of FSH as well.

Studies carried out on prepuberal monkeys have in fact demonstrated that treatment with FSH alone is unable to start spermatogenesis, even when protracted for two years and in combination with T¹, while the latter alone has shown to induce a premature presence of primary spermatocytes in 3 months and starting off the spermatogenetic process in the following 12 months². In contrast, again in the prepuberal monkeys, complete spermatogenesis of all four differentiated spermatogonia was observed after treatment with both gonadotropins. Also treatment with only hCG (similar to LH action) in males with hypogonadotropic hypogonadism determines an increase in plasmatic and intratesticular levels of T and leads, in sporadic cases, to the presenting of mature spermatozoa³.

In summary, from these studies, it can be seen how FSH is actually not indispensable for the beginning and maintenance of spermatogenesis, however, its presence is fundamental if the process is to be conducted at the highest levels.

Therefore, FSH and T must develop a synergic action in order to obtain a quantitatively normal spermatogenesis. In the foetal and neonatal periods, FSH activates the proliferation of the Sertoli cells; subsequently, in the puberal phase it influences the mitotic activity of the spermatogonia and assists differentiation through meiosis to the spermatogenetic process, up to the stage of the round spermatids⁴. The Leydig cells produce testosterone which, present in elevated concentrations within the testicle, seems to influence spermiogenesis and the following phases of maturation⁵. These observations, together with knowledge of tubular physiology, have enabled FSH to be used as pharmacological therapy in treatment of male infertility, although to date its efficacy remains a debatable issue.

Hormone therapy and functional hypogonadotropic hypogonadism

Up to a few years ago, the only clinical condition for which treatment with gonadotropins was revealed to be positively efficacious was hypogonadotropic hypogonadism⁶⁻⁹. The use of gonadotropins has an unequivocal rationale in this clinical setting; in fact, the deficit of gonadotropins is the cause for azoospermia (aspermia), and thus the administration of gonadotropinic compounds or preparations is capable of activating the process of spermatogenesis. Both the gonadotropins (FSH and LH) are necessary to activate the spermatogenetic process in so much as the maturation of the germinal cells occurs in the seminiferous tubules in the presence of FSH and of an elevated intratesticular level of T, produced by the Leydig cells under the effect of the luteinizing hormone^{10,11}.

The success achieved with the FSH treatment in subjects with hypogonadotropic hypogonadism has led to the use of this type of therapy also in the treatment of normogonadotropic infertile patients with idiopathic oligozoospermia, hypothesizing that increase in the levels of gonadotropins is able to stimulate and improve spermatogenesis.

Several different types of Gonadotropin are available on the market for substitutive therapy of male hypogonadism, some of extractive origin and others, more recently, of the recombinant type (Tab. I). With regard to FSH, in Italy preparations deriving from menopausal urine (hMG or urinary FSH, FSHu) are available, or highly purified urine, or otherwise preparations obtained by means of the recombinant DNA technique (FSHr). Also for LH there are hormones of extractive origin highly purified with an activity similar to the luteinizing hormone, such as the human chorionic gonadotropin (hCG) and recombinant preparations.

The use of gonadotropins in therapy for male hypogonadotropic infertility has a foundation both in the use of extractive and recombinant preparations. The limitations as to the use of one and not the other type is represented in Italy by the AIFA Nomenclature which authorises the prescription of the above-mentioned preparations upon realisation of a therapeutic programme regulated by the so-called "nota 74". This postulate limits the use of recombinant preparations to only male infertile subjects who present a condition of hypogonadotropic hypogonadism. To date, literature offers ample space to discussion as to what is intended by "hypogonadotropic hypogonadism" and there is plenty of material available in support of the theory that this condition is more

associated to the clinical aspect (hypospermatogenesis) rather than to the laboratory aspect (FSH). It is common in endocrinological contexts, in relation to other pathologies such as thyroidism for example, even in the presence of normal hormone values to determine a clinical condition that is secondary to a functional deficit of the hormone. In these cases, we would thus speak in terms of a "subclinical" pathology or "functional deficit". This concept, translationally speaking, may be adopted also in the case of hypogonadism and as such, may justify the use of recombinant gonadotropins also in the presence of a normogonadotropic hypogonadism because in the case of dyspermia, it would take on the meaning of a functional hypogonadotropic hypogonadism.

In literature, nevertheless, there are not many prospective and randomised studies dedicated to assessing the efficacy of the therapy with rFSH in oligoasthenoteratozoospermic (OAT) patients compared with untreated control groups, and the results obtained appear contrasting. In fact, some authors^{12,13} have not found any significant improvement of the seminal parameters, while others have shown not only an improvement in seminal parameters but also an increase in the percentage of pregnancies^{14,15}.

Bartoòv¹⁶ documented after FSH treatment an improvement of the ultrastructural characteristics of the spermatozoa analysed under the electronic microscope, characterized by a reduction in the percentage of acrosomal agenesis and amorphous testes, comparable to that found in studies on the rat¹⁷. Similarly Baccetti¹⁸ found a reduction of the seminal apoptotic process after treatment with FSH and evidenced how some qualitative aspects of the acrosome, of chromatin and of the axoneme are sensitive to the action of this gonadotropin. The improvement of such ultrastructural characteristics may justify finding an increased percentage of pregnancies obtained in the couples undergoing assisted reproductive treatment, in whom the male partners had been previously been treated with FSH¹⁹.

In particular, Foresta's group²⁰ showed a statistically significant increase in the sperm concentrations, according with a more recent study of Paradisi²¹ regarding use of high doses of r-FSH taken for a 4-month period on a small number of patients.

These contrasting results may be in part justified by the different criteria adopted for selection of patients, the differing interpretation of the seminal parameters, the different treatment dose and its lengths.

Even our group²² conducted a research study on idiopathic normo-gonadotropic OAT patients and we found, in line with the studies cited, an increase in

sperm concentration after three months of treatment with r-FSH. In our study, the preliminary results of which were presented during the course of the SIA Congress of Ancona 2007, 154 male patients were selected, who were part of couples with infertility for a period exceeding 2 years. The components of the couples, men and women, presented a mean age respectively of 36.9 and 35.4 years. The subjects selected were randomized blinded enrolled into two groups, utilising a pre-determined numerical sequence in which in turn was associated with a patient that matched the inclusion criteria according to a 1:1 ratio. 77 subjects (group A) were allocated for treatment with r-hFSH at a dose of 100 IU s.c./day for three months, while 65 subjects (group B) were allocated for representing the control group. The female partners of those enrolled did not have specific diseases that could be considered causes for infertility. We shall not go into further detail here so as not to invalidate the originality of the work and its publication, although we can say that the criteria which influenced the selection of the patients was seminological (sperm concentration $\leq 20 \times 10^6/\text{ml}$; forward motility: $\leq 20\%$) and the primary endpoint was to verify improvement of the seminal characteristics after therapy.

The study was conducted through a semen analysis on the Optic Microscope (WHO, 2000) and in parallel by means of a computerized reading. In addition to the preceding studies, in the group treated an improvement of sperm motility was observed after treatment, particularly in the forward component, which continued to be significant also at three months after the end of the therapy. This data represents a novelty that cannot be overlooked in a therapeutic course targeted at improving the seminal characteristics of an individual. Automatized analysis, using software

SCA SYSTEM of the MICROPTIC S.L confirmed the results of optical microscope analysis. The use of computerized analysis has consented to evidence also an increase in percentage of kinetic parameters VCL and STR which are not evaluable by the use of only optical microscopy. These parameters may represent a criterion of selection of the spermatozoa in PMA technic and a guide to the assisted fertilization methods towards less invasive.

The study, in line with that of Foresta ²³, has evidenced also an improvement in the percentage of pregnancies in treated patients, if compared with untreated ones. These data confirm the importance of a correct use of therapy with recombinant gonadotropin in infertile males, in order to increase the percentage of spontaneous pregnancies and to reduce the use of second-level PMA, giving more importance to the benefits of the first level ones.

In summary, in these patients, treatment with r-FSH could exercise a local priming, promoting expression of the r-FSH receptors on the Sertoli cells and on the surface of the germinal cells, thus producing an increased post-receptorial response which would result in an improvement of the spermatogenesis.

The results of the study also suggest that the improvement of the seminal parameters could be attributed to the influence of the r-FSH on the testicular paracrine environment, on the Sertoli cells and on the spermatogenesis. Therefore, in the presence of normal levels of endogenous FSH and in the absence of specific pathological parameters of reference between healthy subjects and OAT subjects, we cannot exclude the hypothesis of a "functional hypogonadotropic hypogonadism" due to receptorial alterations, puntiform mutations or different isoforms of endogenous FSH.

In literature, different isoforms of FSH have been

Table I. Preparations of gonadotropins for male use.

DRUG	NAME	PRODUCER	NOTE
Chorionic Gonadotropin (hCG)	Gonasi HP	Amsa	/
Chorionic Gonadotropin (hCG)	Pregnyl	Organon	/
Menotropine	Menogon	Ferring	74 hypo-hypo-normo
Urofollitropine	Fostimon	Amsa	74 hypo-hypo-normo
Recombinant Follitropine alfa	Gonal F	Serono	74 hypo-hypo
Recombinant Follitropine beta	Puregon	Organon	74 hypo-hypo
Lutropine alfa (Recombinant LH)	Luveris	Serono	74

reported that lead to the expression of different biological properties^{24,25}. In these cases, human recombinant FSH could be the choice treatment, since its biological characteristics are comparable to the endogenous FSH.

This reasoning paves the way to a new concept of hypogonadism that we could define subclinical or functional, and to a new use of therapy with gonadotropin in males.

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