Semen analysis

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Introduction

- The availability of semen renders possible direct examination of male germ cells, giving precious data that are not accessible for female germ cells.

- Semen analysis includes the examination of :
  - Spermatozoa
  - Other cells present in semen
  - Seminal fluid

- These data give indications on the testicular function and of the integrity of the male genital tract.

Type of assays

- 1. **descriptive assay**: - spermogram

- 2. **functional assays**: -penetration of cervical mucus (postcoital test,
  in vitro penetration assay)
- - binding of spz to the zona pellucida
- - fusion of spz with zona-free hamster oocyte.
- - hypo-osmotic swelling of the flagella.

- 3. **immunological assay**: - mixed agglutination test (MAR-test).
- - immunobead test.
- - sperm immobilization in cervical mucus

Spermatozoa analysis

- Parameters available from:
- Semen: - Spermogram, analysis of spermatozoa and seminal plasma
  - MAR-test
  - Bacteriolgical analysis
- Cervical mucus - Postcoital test
  - In vitro penetration test
  - Sperm - cervical mucus contact test

Semen analysis includes :

- Spermatozoa analysis

  number, vitality, motility, morphology

- Immunological analysis

  anti-spermatozoa antibodies detection

- Seminal fluid analysis

  biochemical markers of accessory glands secretions

Semen analysis is subdivided in :

- Macroscopic analysis
  volume, pH, liquéfaction time
- Microscopic analysis
  concentration, motility and vitality
- Immunological analysis
  IgG and IgA anti-sperm antibodies detection
- Bacteriological analysis
  Detection of infection, in addition to colonisation
- Evaluation of spermatozoa morphology
  detailed exam of the morphology of 100 to 200 spermatozoa
- Biochimical analysis of seminal plasma
  markers of accessory glands of the reproductive tract

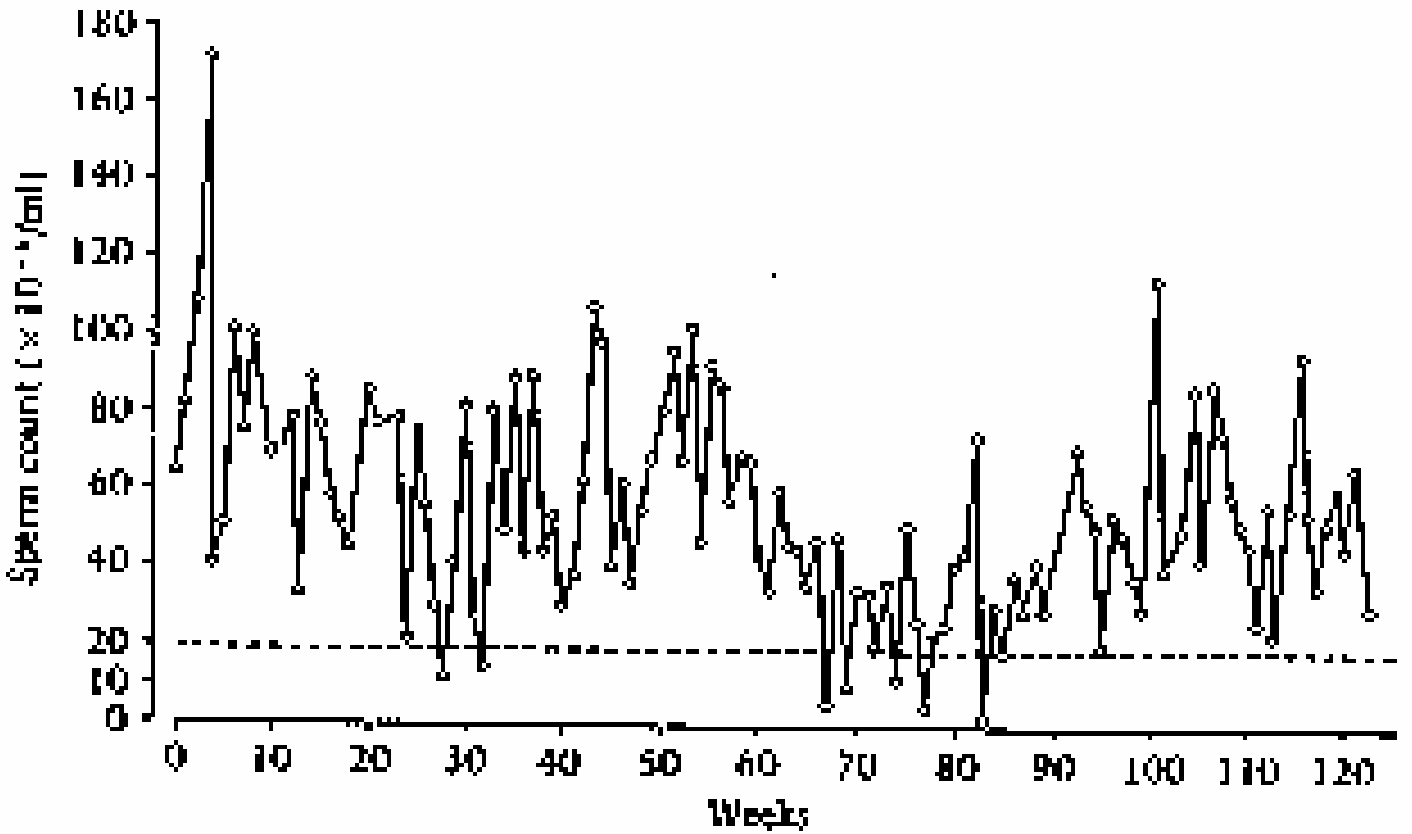
Reference values of semen variables[a]

- Each laboratory should determine its own reference range for each variable.
- Reference semen from men who have achieved a preganancy within 12 months
- About 1000 reference samples needed.
- These references ranges have not been established
- The reference ranges given are based on data from healthy fertile men
- These values are not the minimum semen values needed for conception
- Men with semen variables lower than those indicated may be fertile.

- [a]WHO manual, 4rth edition, 1999.

Variability in sperm count

Fig. 2.1. Sperm concentrations in the semen of one man collected biweekly over 120 weeks. During this period the man received no medication and experienced no febrile illness. The dotted line indicates 20×10^6/ml (see Appendix 1A). The data illustrate the marked variations in sperm concentration that can occur in the semen of some men. (Unpublished data from C.A. Paulsen.)



- Some parameters measured in semen analysis are well established and their reference values have remained stable over time such as :
    - volume
    - pH
    - minimal concentration of spermatozoa and minimum absolute number of spermatozoa per ejaculate
    - minimal motility
    - maximal leucocyte concentration tolerance

- In contrast, the minimum percentage of spermatozoa with normal morphology has considerably varied over time, and the definition of normal morphology as well.

Reference values of semen variables[a]

- **Volume** 2.0 ml or more
- **pH** 7.2 or more
- **Sperm concentration** $20x10^6$ spermatozoa/ml or more
- **Total sperm count** $40x10^6$ spermatozoa or more
- **Motility** 50% or more motile (grade a+b)or 25% or more with progressive motility (grade a) within 60 min after collection.
- **Morphology** *
- **Vitality** 75% or more live
- **White blood cells** Fewer than $1x10^6$/ml
- **Immunobead test** Fewer than 50% spermatozoa with adherent particles
- **MAR test** Fewer than 50% spermatozoa with adherent particles

[a]WHO manual, 4rth edition, 1999.

* Data from ART programmes suggest that, as sperm morphology falls

Reference values of semen variables[b] (2)

- **Seminal plasma biochemical analysis**
- ***Epididymal markers***
- α-glucosidase (neutral) 20 mU or more per ejaculate
- Carnitine 0.8-2.9 μmole per ejaculate
- ***Prostate markers***
- Zinc (total) 2.4 μmole or more per ejaculate
- Citric acid (total) 52 μmole or more per ejaculate
- Acid phosphatase (total) 200 U or more per ejaculate
- ***Seminal vesicle marker***
- Fructose (total) 13 μmole or more per ejaculate
- —

- [b]WHO manual, 3rd edition, 1992

Definitions of semen classifications

- Normozoospermia	When all the spermatozoal parameters are normal together with normal seminal plasma and WBCs, and there is no agglutination.
- Oligozoospermia	When sperm concentration is < 20 million/ml.
- Asthenozoospermia	Fewer than 50% spermatozoa with forward progression (categories (a) and (b) or fewer than 25% spermatozoa with category (a) movement).
- Teratozoospermia	spermatozoa with decreased % of normal morphology.
- Oligoasthenoterato-zoospermia	signifies disturbance of all the three variables zoospermia (combination of only two prefixes may also be used)
- Azoospermia	No spermatozoa in the ejaculate
- Aspermia	no ejaculate

Definition of specificity and sensitivity of an assay

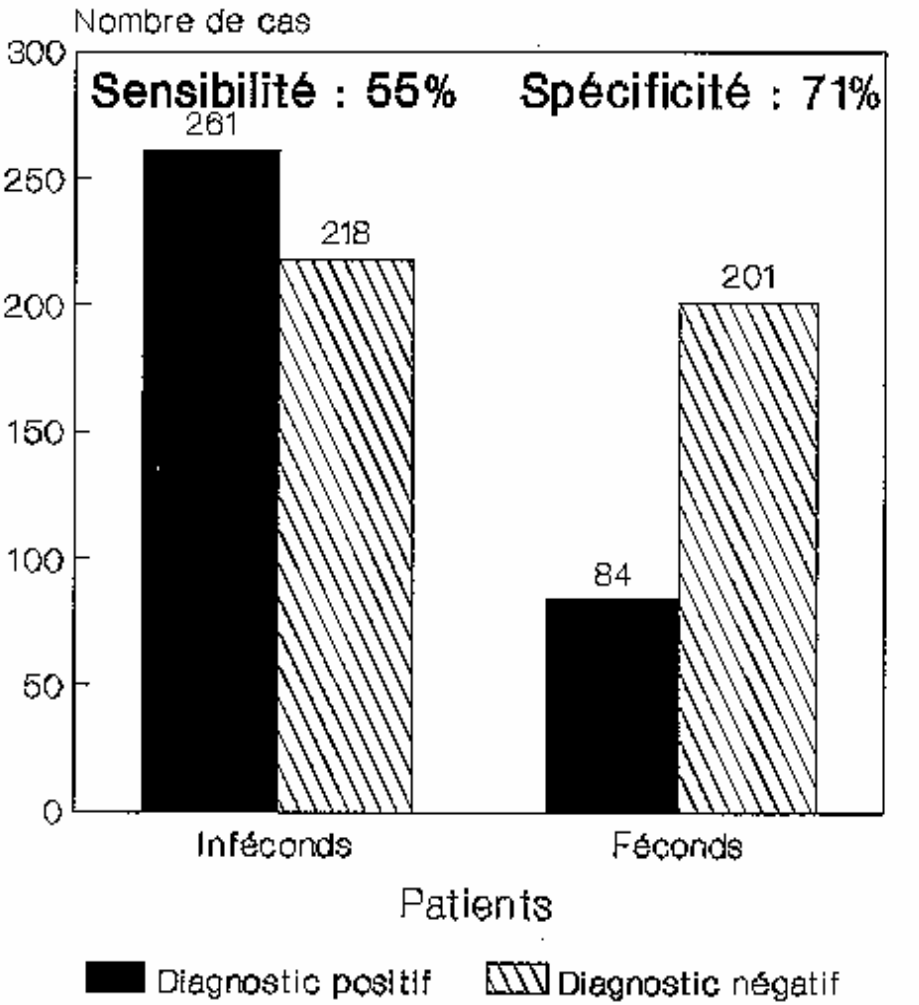
Sensitivity :
- Probability of of positive test in the positive events
- Proportion of positive results within the positive cases
- For semen analysis : proportion of abnormal semen analysis in infertile men

Specificity :
- Probability of a negative test in the negative events
- Proportion of negative results within the - negative cases
- For semen analysis : proportion of normal semen analysis in fertile men

55% sensitivity means that in 55% of the infertile men have abnormal semen analysis results

71% specificity means that 71% of the fertile men have a normal semen analysis resutls

SENSIBILITE ET SPECIFICITE DU SPERMOGRAMME



Feneux D et al. Recherches récentes sur l'épidémiologie de la fértilité. 1986

SEMEN BACTERIOLOGY

The general semen bacteriology is always positive but pathogen germs are rarely present.

Culture et antibiogram

Aerobic Culture

Gram + germs

Gram - germs

Mixt germs

Anaerobic Culture

Cultures on enriched media

Mycoplasma hominis, Ureaplasma urealyticum: pathogenicity threshold 10^4 CFU/ml

PCR in urine

Chlamydia trachomatis: treatement with tetracyclin, no known resistance.

Micro-organisms capable of causing infection of seminal tract

Classical germs	**Aerobic, gram -**	**Aerobic, Gram +**	**Anaerobic**
Chlymydia Trachomatis	E. Coli	Gardnerella Vaginalis	Bacteroides
Nesseria Gonorrheae	Enterobacter	Streptococcus Faecalis	Bifidobacterium
Treponema Vaginalis	Klebsiella	Staphyloccocus Aureus	Fusobacterium
Mycoplasma	Proteus	Staphylococcus Epidermidis	Lactobacille
Ureaplasme Urealitique	Pseudomonas	Streptococcus Agalactiae	Peptococcus
Corynebacterium		Streptococcus Saprophyte	Propionibacter
			Peptostreptoccus

Biochemical markers of seminal plasma

Semen volume is constituted by the sequential secretion of Cowper's glands (small volume), prostate (40%), testicle and epididymis (10%) and seminal vesicles (50%).

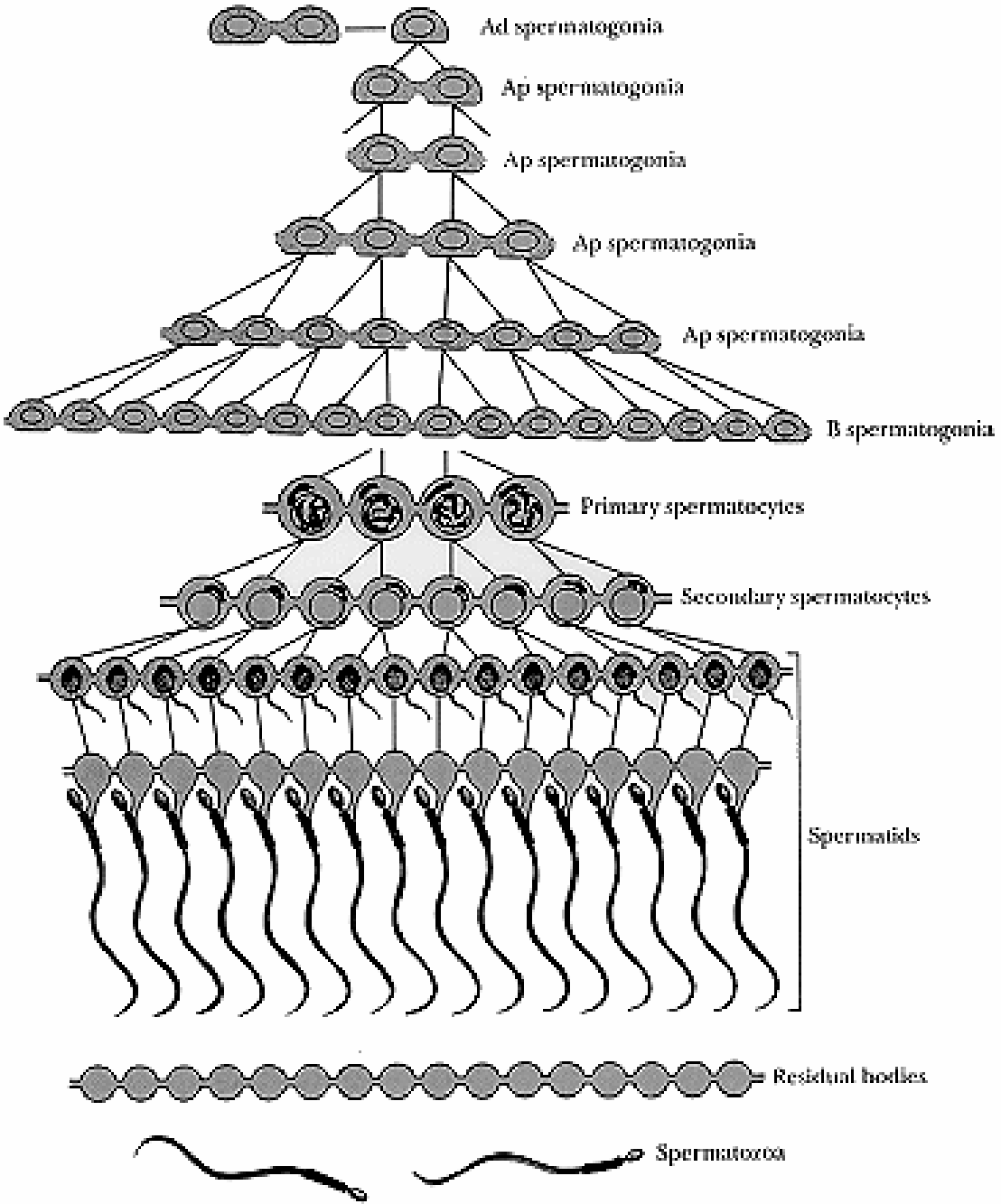
Specific biochemical markers allow to test the secretions of these different compartments.

Markers currently used in our laboratory :

Seminal vesicles : Fructose

Prostate : Zinc

Epididymis : Carnitine


Ad spermatogonia
Ap spermatogonia
Ap spermatogonia
Ap spermatogonia
Ap spermatogonia
B spermatogonia
Primary spermatocytes
Secondary spermatocytes
Spermatids
Residual bodies
Spermatozoa

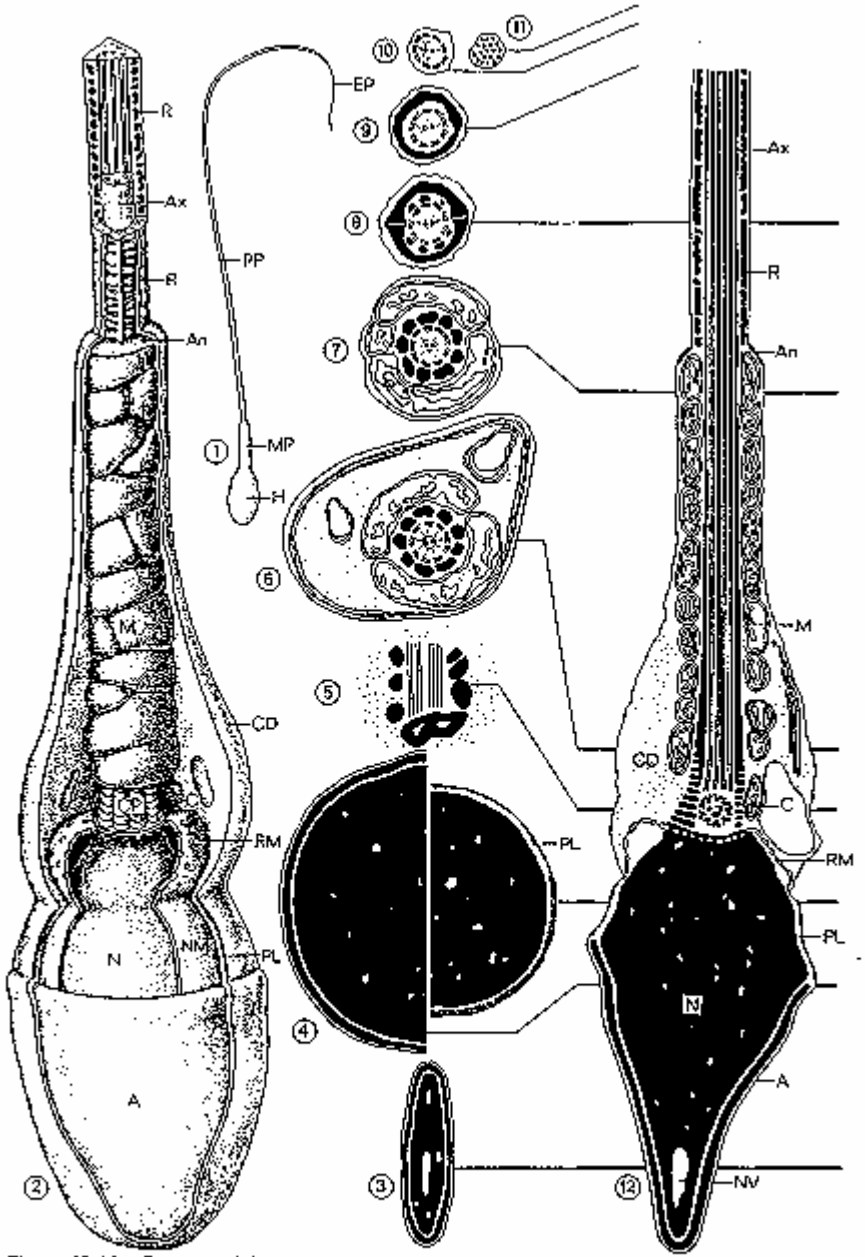


Figure 19.11. Structure of the mature spermatozoon. *1*, the relative size of the head (*H*), midpiece (*MP*), principal piece (*PP*) and end piece (*EP*) is shown. *2*, a three-dimensional cutaway drawing showing the acrosome (*A*), nuclear envelope and nucleus in the head, the connecting piece (*CP*) of the neck, the mitochondria (*M*) in the midpiece, fibrous sheath (*R*), and axoneme (*Ax*) of the principal piece. *3* to *11*, cross sections at the level indicated on diagram *12*. (Courtesy of Dr. E. C. Roosen-Runge.)

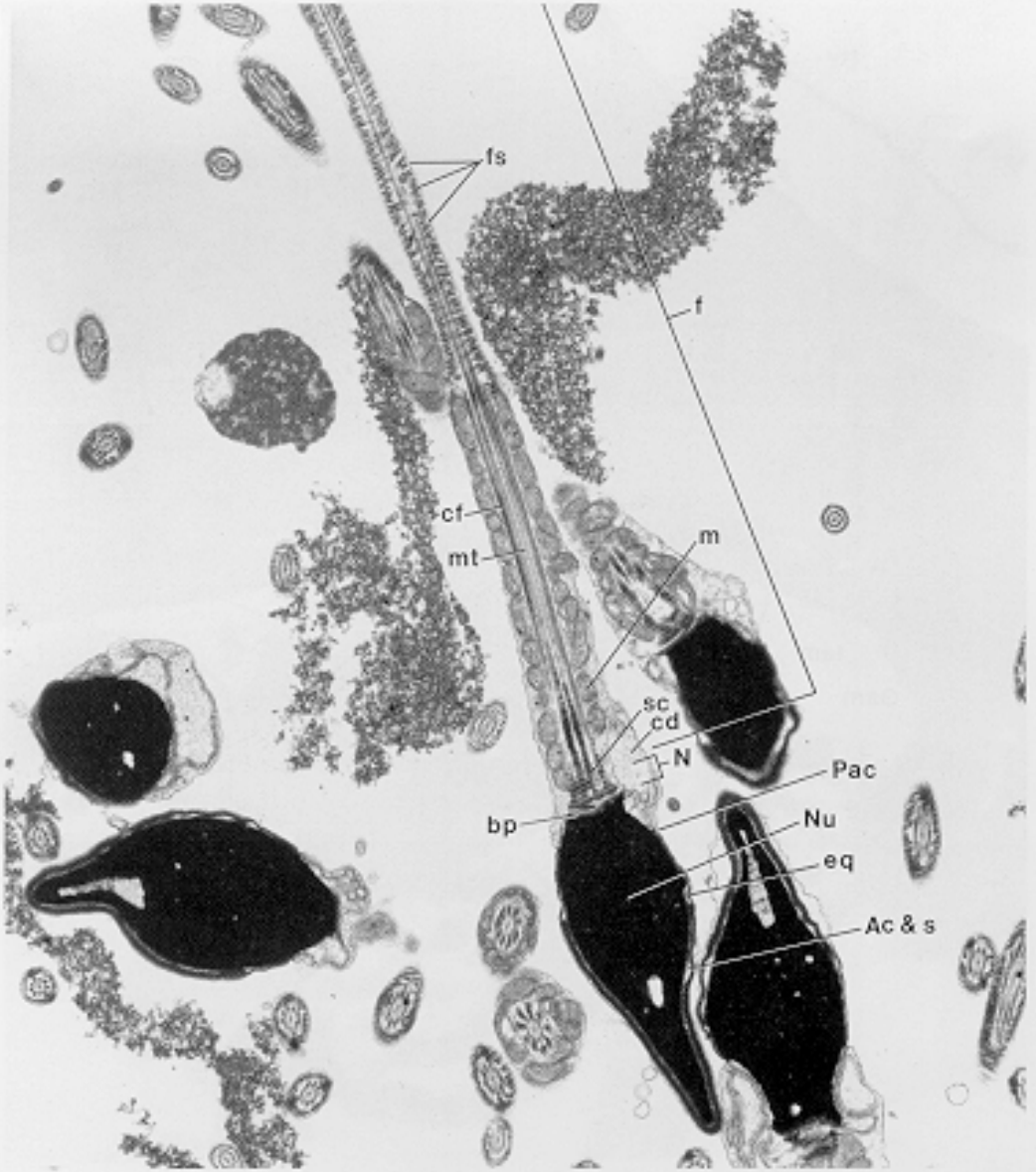


Figure 3.56. Longitudinal section of a normal spermatozoon. Nu = nucleus; Ac = acrosome; eq = equatorial region; Pac = postacrosomal region; cd = cytoplasmic droplet; sc = segmented columns of the proximal centriole; f = flagellum; bp = base plate; s = subacrosomal space; m = mitochondrion; N = neck; mt = microtubules; cf = coarse fibers. (Magnification: 17,400 ×.)

Certain morphological defects can compromise the fertilizing capacity of spermatozoa

- Defects of the midpiece or of the flagella can interfere with motility
- An incomplete or absent acrosome can prevent penetration of the oocyte zona pellucida
- Excessive head size is a sign of incomplete DNA condensation

For many other defects of the head it is impossible to predict if they have an impact on the spermatozoa fertilizing ability

The relationship between sperm morphology and fertility is dependent from evaluation **at two distinct levels** :

1. Each spermatozoa is individually examined and classified as normal or abnormal according to a **definition of the normal morphology of spermatozoa**

2. The percentage of spermatozoa with normal morphology in semen is calculated and used to predict fertility according to **threshold values under which fertility decreases**

Regional differences in semen quality in Europe

Jorgnesen et al. *Hum.Reprod.* 2001 16 :1012.

Region	Rank		Seasonal variation (%) winter / summer	Concentration range (million/ml)	
	Concentration and Total sperm count	Motility	Concentration and Total sperm count	winter	summer
Turku	4#	3#	100 % / 70 %	132	93
Edingurgh	3*	4*		119	84
Paris	2* #	1* #		103	73
Copenhagen	1	2		98	69

CONCLUSION

Semen analysis brings valuable informations about the male reproductive function

It allows :

- to assess spermatogenesis and accessory glands function
- to detect immunological, inflammatory or infectious problems

However, the current lack of adequate standards renders some parameters difficult to evaluate, in particular spermatozoa morphology

The ongoing multicentric studies of large populations of fertile men will hopefully soon allow to set validated threshold values for semen parmeters that will help to distinguish cases with reduced fertility