

Aspects biologiques de la fertilité masculine I



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Introduction

- L 'analyse biologique des spermatozoïdes et du liquide séminal est une étape clé de l 'évaluation de la fertilité masculine
- Ces analyses donnent des indications sur la fonction testiculaire et sur l 'intégrité du tractus génital masculin

Type d'essais

1. **Descriptif** : - spermogramme

2. **Fonctionnel** : - pénétration du mucus cervical (test post-coïtal et test de pénétration in vitro)
- liaison des spermatozoïdes à la zone pellucide
- fusion des spermatozoïdes avec des ovocytes dépel-lucidés de hamster
- gonflement hypo-osmotique du flagelle

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

L'analyse de sperme consiste en:

- analyse macroscopique
 - volume, pH, temps de liquéfaction
- analyse microscopique
 - concentration, mobilité et viabilité
- analyse immunologique
 - recherche d'anticorps anti-spermatozoïdes
- analyse bactériologique
 - détection d'infection, en plus de la colonisation toujours présente
- évaluation de la morphologie des spermatozoïdes
 - examen détaillé de la morphologie de 100 à 200 spermatozoïdes
- analyse biochimique du plasma séminal
 - marqueurs des glandes annexes

WHO laboratory manual for the examination of human semen and sperm–cervical mucus interaction

FOURTH EDITION



WORLD HEALTH ORGANIZATION

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 pregnancy 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.
- ---

^aWHO manual, 4th edition, 1999.

Valeurs de références des variables du sperme

- Chaque laboratoire devrait déterminer ses propres valeurs de références pour chaque variable,
- Les valeurs de références devraient être basées sur les caractéristiques du sperme d'hommes ayant engendré un enfant moins de 12 mois avant l'analyse,
- Il faut environ 1000 échantillons, de telles références n'ont pas encore été établies,
- Les références utilisées sont basées sur des hommes fertiles et en bonne santé,
- Ces valeurs ne sont pas les valeurs minimales du sperme nécessaires à la conception,
- Des hommes avec des valeurs de sperme plus basses que celles indiquées peuvent être fertiles.

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	

^aWHO manual, 4th 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 μ mole per ejaculate

Prostate markers

Zinc (total) 2.4 μ mole or more per ejaculate

Citric acid (total) 52 μ mole or more per ejaculate

Acid phosphatase (total) 200 U or more per ejaculate

Seminal vesicle marker

Fructose (total) 13 μ mole or more per ejaculate

—

^bWHO manual, 3rd edition, 1992

- Certains des paramètres mesurés dans le spermogramme sont bien établis avec des valeurs de référence stables au cours du temps, comme :
 - le volume
 - le pH
 - la concentration minimale et le nombre absolu minimal de spermatozoïdes
 - la mobilité
 - la tolérance maximale en leucocytes
- Par contre, le pourcentage minimum de spermatozoïdes de morphologie normale a varié considérablement au cours du temps, de même que la définition de la morphologie normale.

CASA

Computer-Assisted Semen Analysis

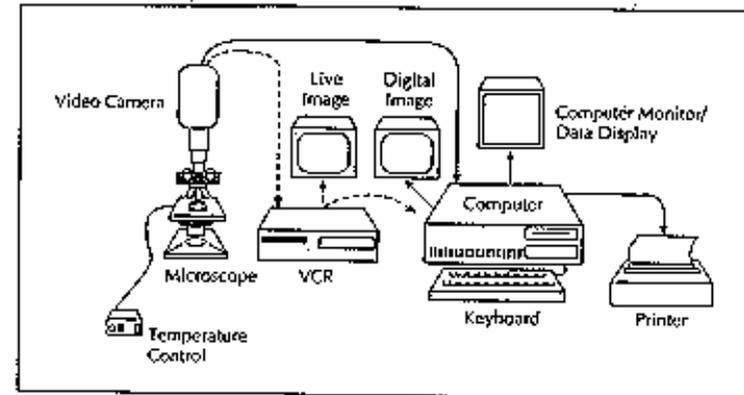


Fig 2.—Schematic diagram of a typical computer-aided sperm analysis system. VCR indicates videocassette recorder (from Boyers et al²⁵).

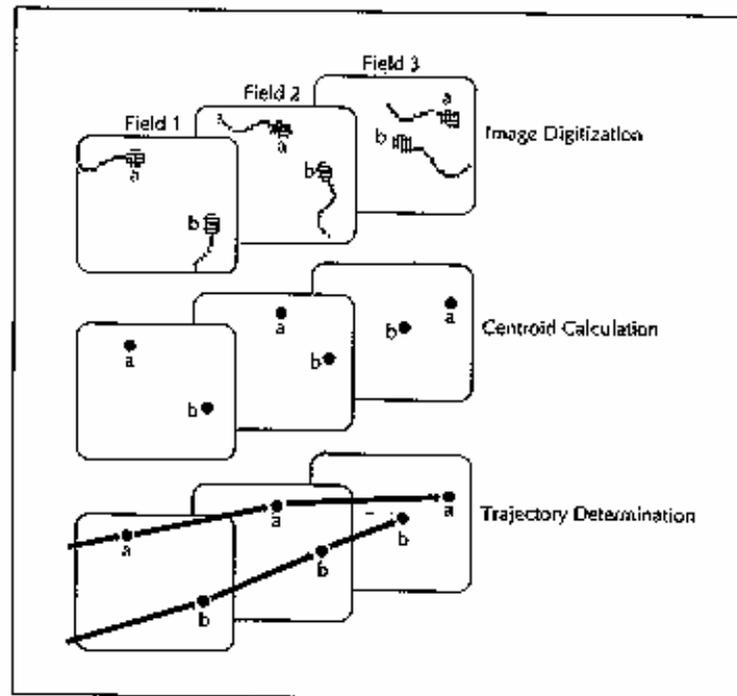
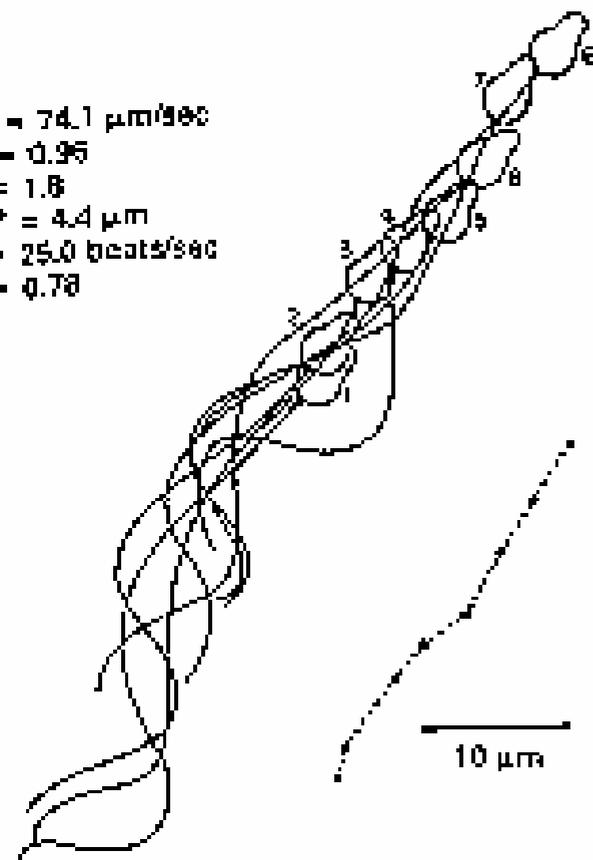


Fig 3.—Process of sperm digitization and calculation of sperm-swimming trajectories. The analog image of sperm cells is digitized in each video frame (top), the complete image of each cell is reduced to a centroid in each video frame (center), and sperm trajectories are computed from the centroids (bottom). a and b indicate two different sperm cells (from Boyers et al²⁵).

Hyperactivation of human spermatozoa

(a)

VSL = 74.1 $\mu\text{m}/\text{sec}$
LIN = 0.96
Ah = 1.8
AMP = 4.4 μm
 ∂F = 25.0 beats/sec
CR = 0.78



(b)

VSL = 75.3 $\mu\text{m}/\text{sec}$
LIN = 0.42
Ah = 12.3 μm
AMP = 11.3 μm
 ∂F = 17.8 beats/sec
CR = 0.49

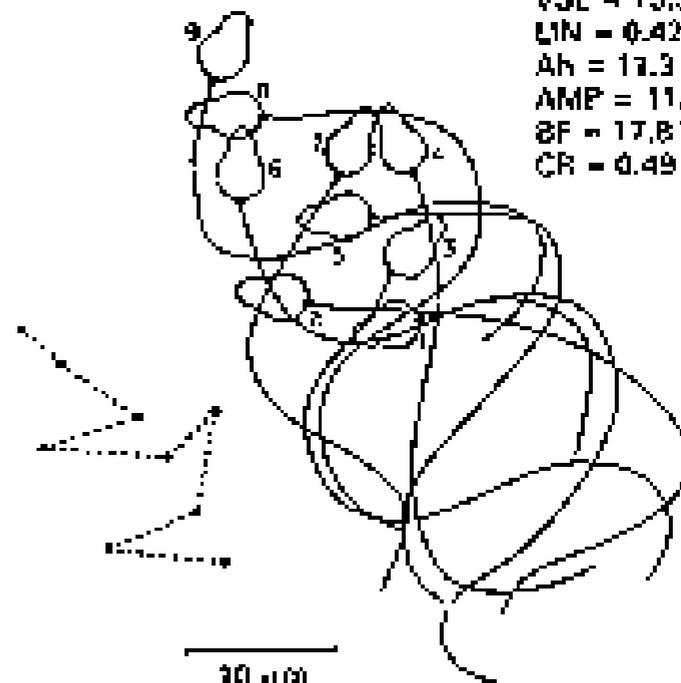


Fig. 3. Movement characteristics (see text for abbreviations) and swimming trajectories of a morphologically normal spermatozoon from a fertile donor at (a) T0 and (b) T6. Consecutive tracings are (a) 0-032 sec or (b) 0-049 sec apart. Points connected by broken lines represent the sequential position of the sperm head.

Morales et al. *J. Reprod. Fert.* 33:119, 1988.

Kobe earthquake and reduced sperm motility

Misao Fukuda^{1,3}, Kiyomi Fukuda¹, Takashi Shimizu², Wakako Yomura² and Seiitsu Shimizu²

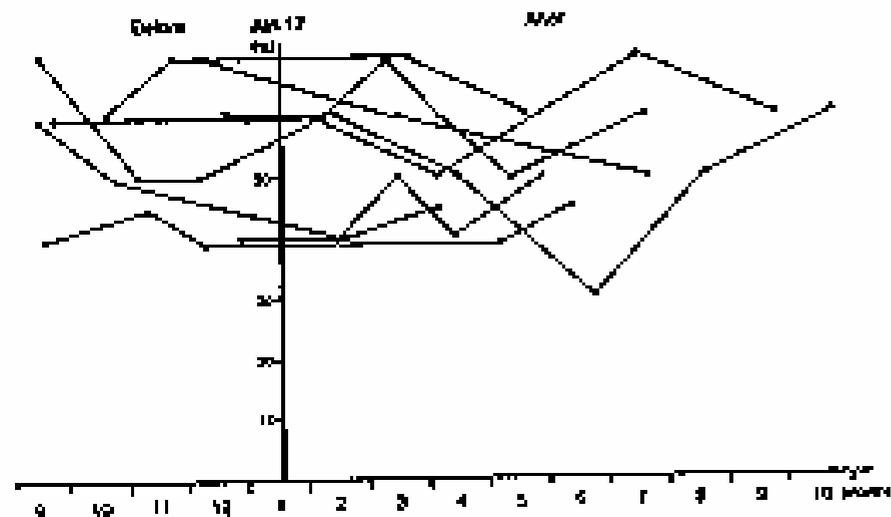


Figure 1. Time course of sperm motility (%) before and after Kobe earthquake (January 17) in eight cases in the area with a magnitude of ≤ 4 on the Richter scale.

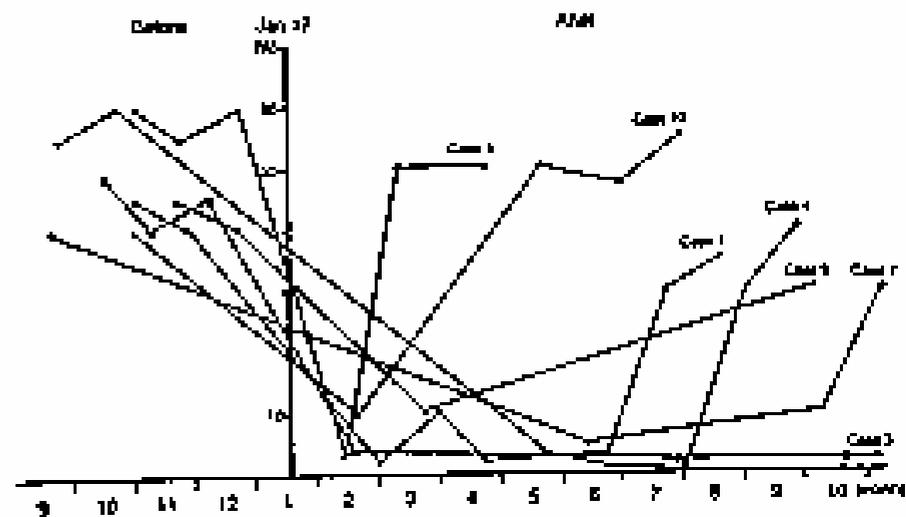


Figure 2. Time course of sperm motility (%) before and after Kobe earthquake (January 17) in seven cases suffering house destruction in the area with a magnitude of ≥ 6 on the Richter scale.

BACTERIOLOGIE DU SPERME

La bactériologie générale du sperme est toujours positive mais on trouve rarement des germes pathogènes.

Culture et antibiogramme

- Culture Aerobe
 - Gram + germs
 - Gram - germs
 - Mixt germs
 - Anaerobe

Cultures sur milieux enrichis

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

PCR dans l'urine

Chlamydia trachomatis: traitement par la tétracycline, pas de résistance connue.

Micro-organismes capable de causer une infection du tractus génital

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

Marqueurs biochimiques du plasma séminal

Le volume du sperme est constitué par la sécrétion séquentielle des glandes de Cowper (petit volume), de la prostate (40%), des testicules and epididymes (10%) and des vésicules seminales (50%).

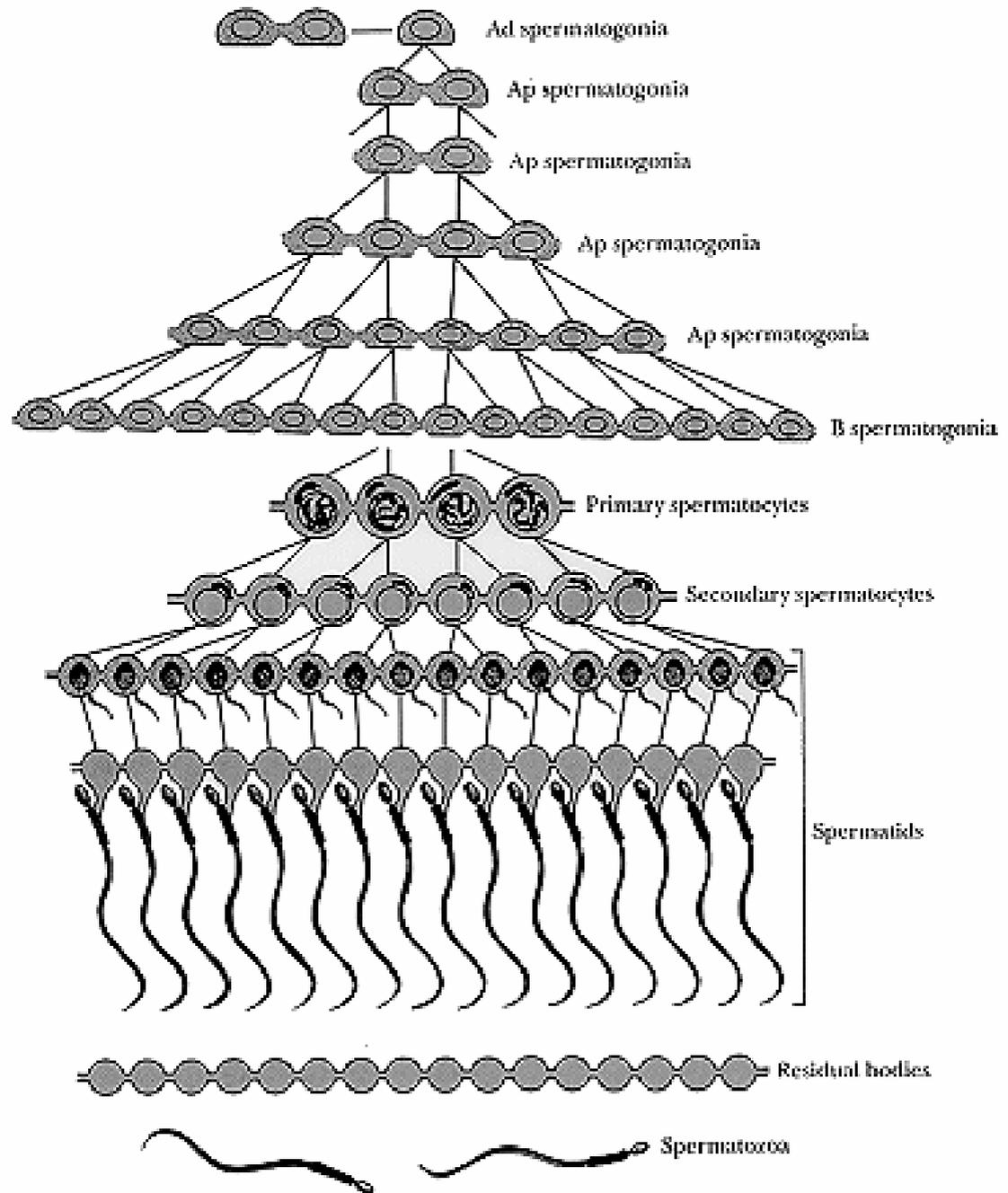
Des marqueurs biochimiques spécifiques permettent de tester les sécrétions de ces différents compartiments.

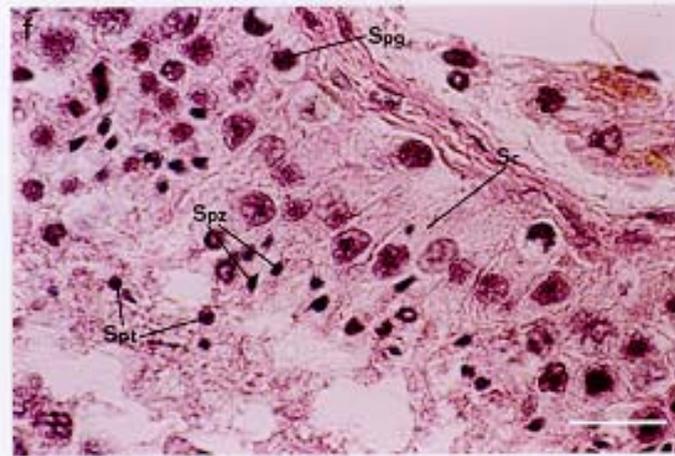
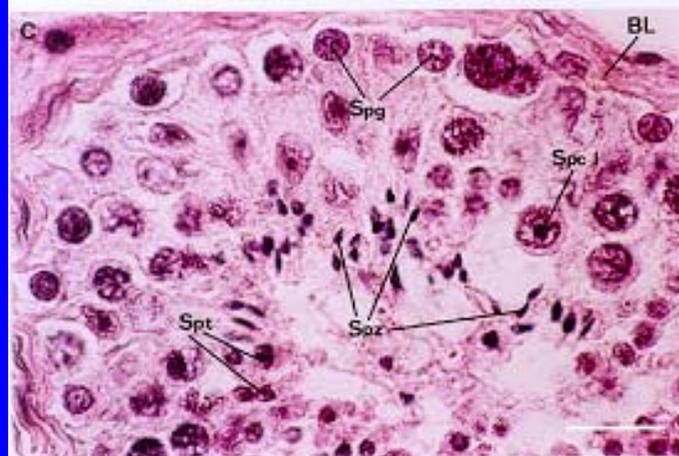
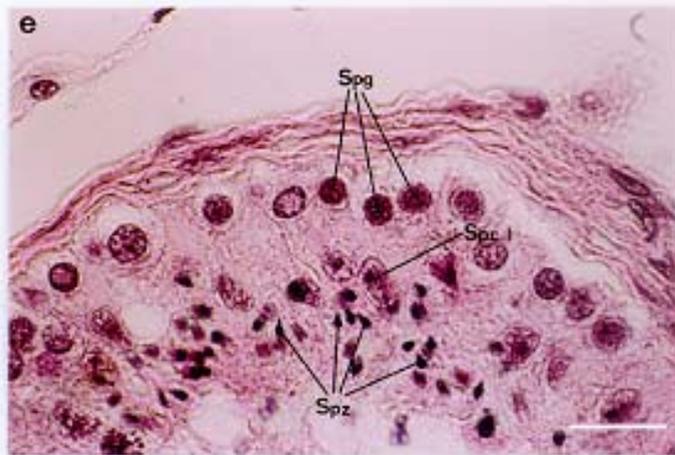
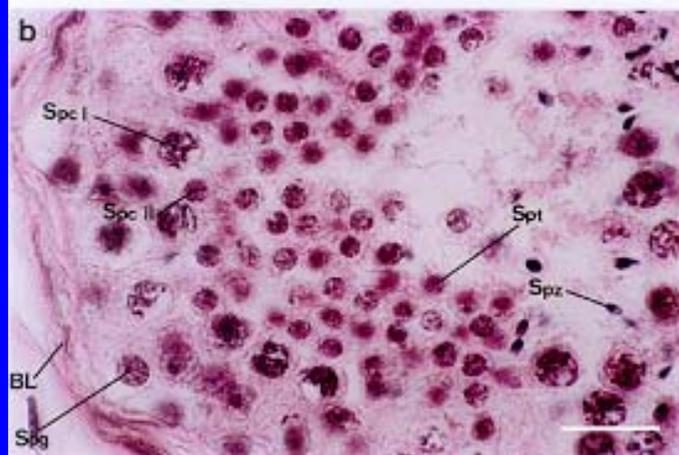
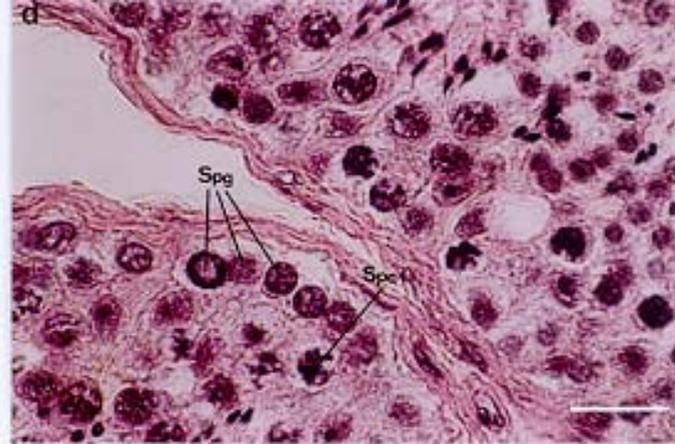
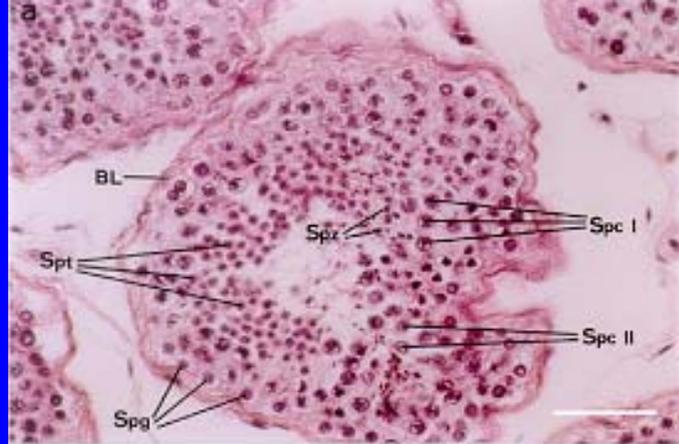
Nous utilisons couramment les marqueurs suivants :

Vésicules séminales : Fructose

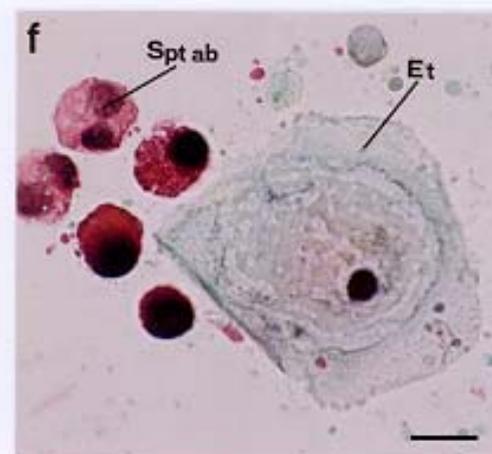
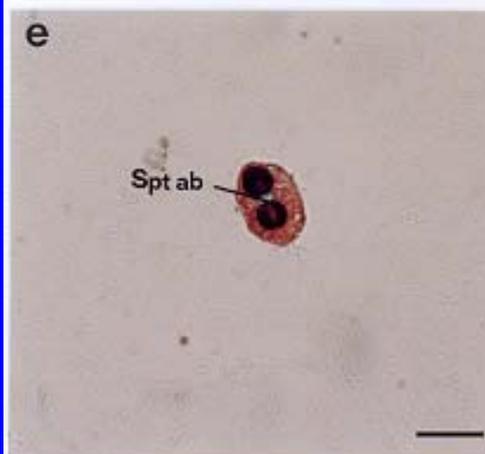
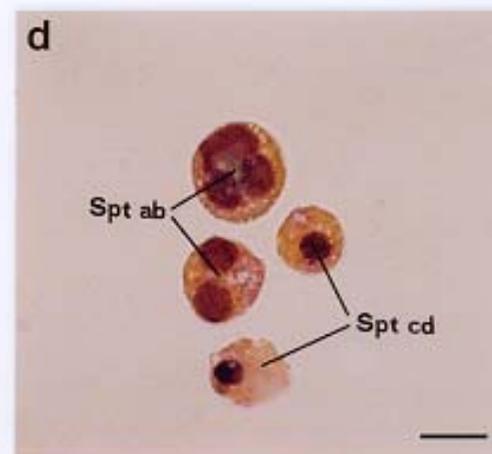
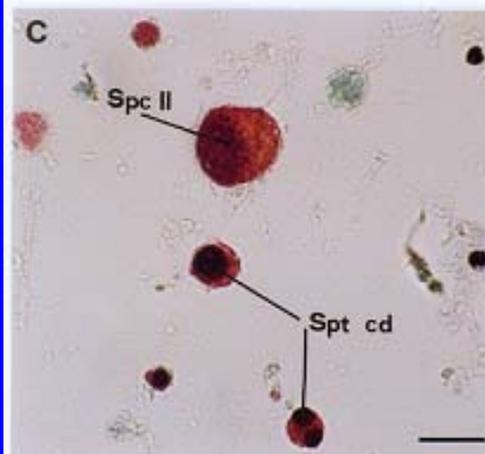
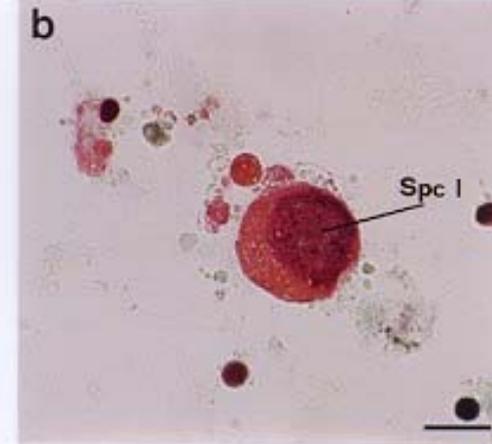
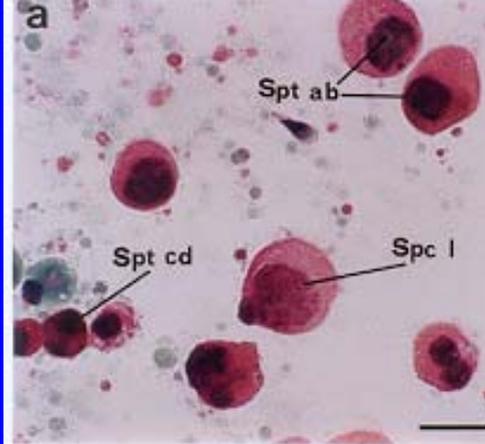
Prostate : Zinc

Epididyme : Carnitine

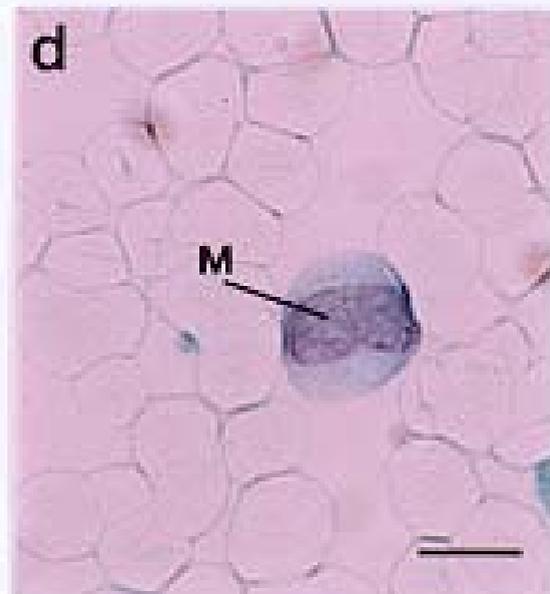
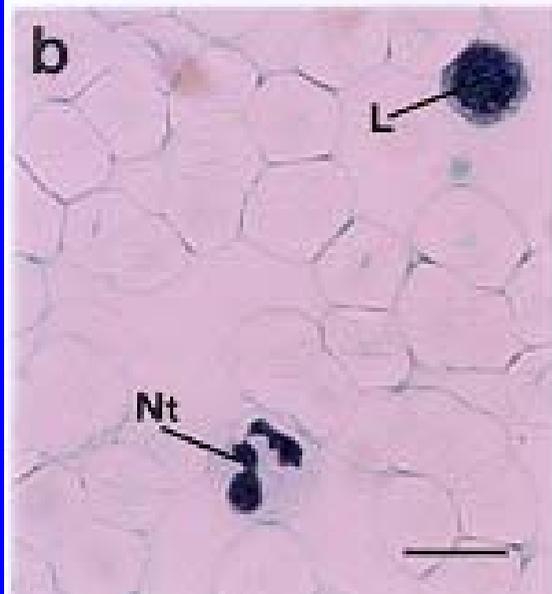
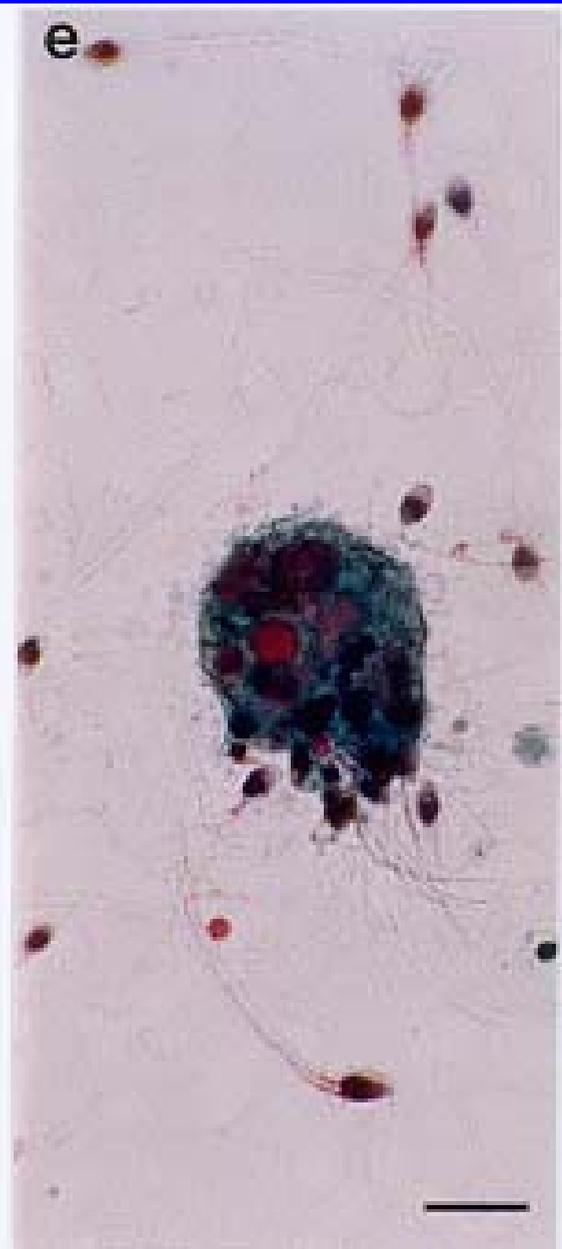
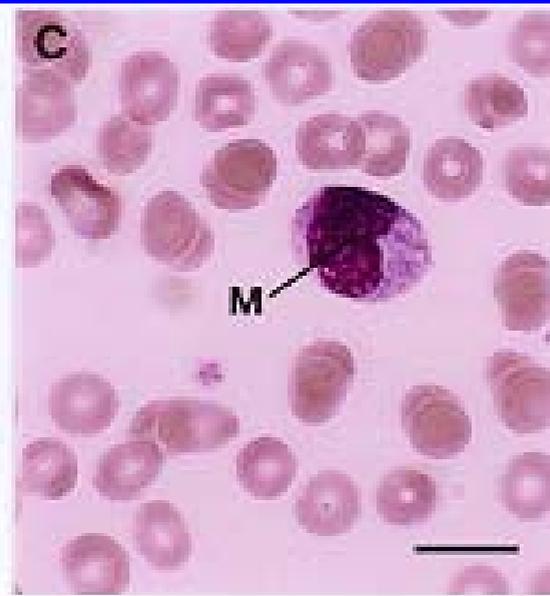
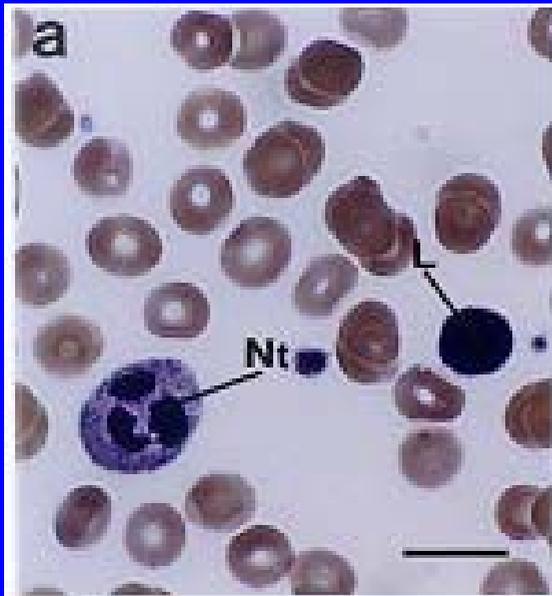




Immature germ cells in semen
Papanicolaou staining

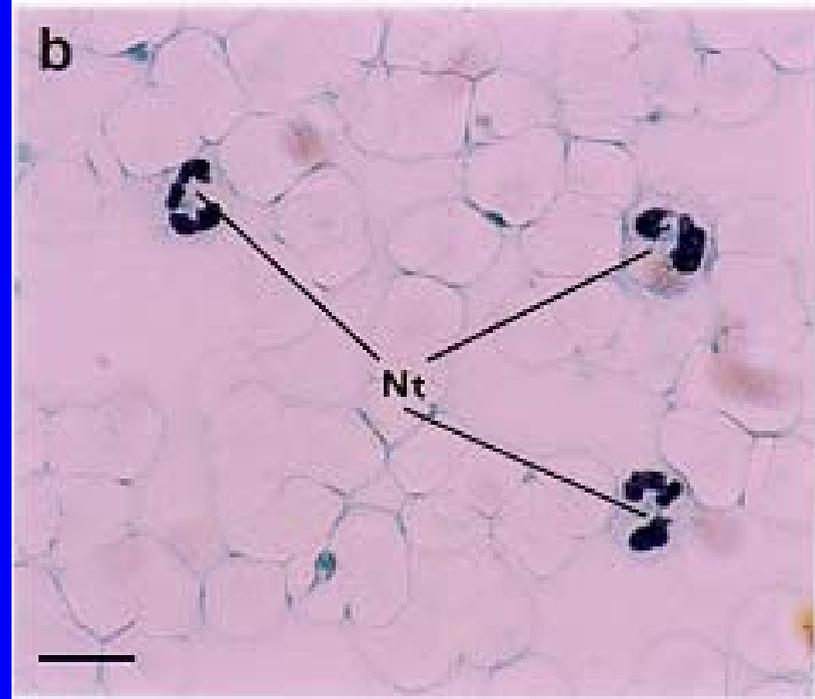
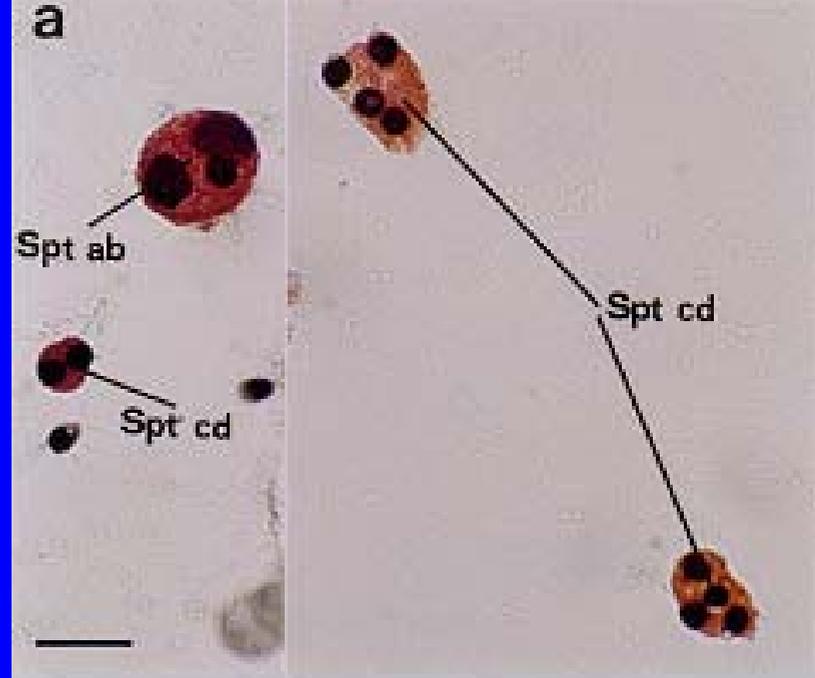


Inflammatory cells in blood and semen

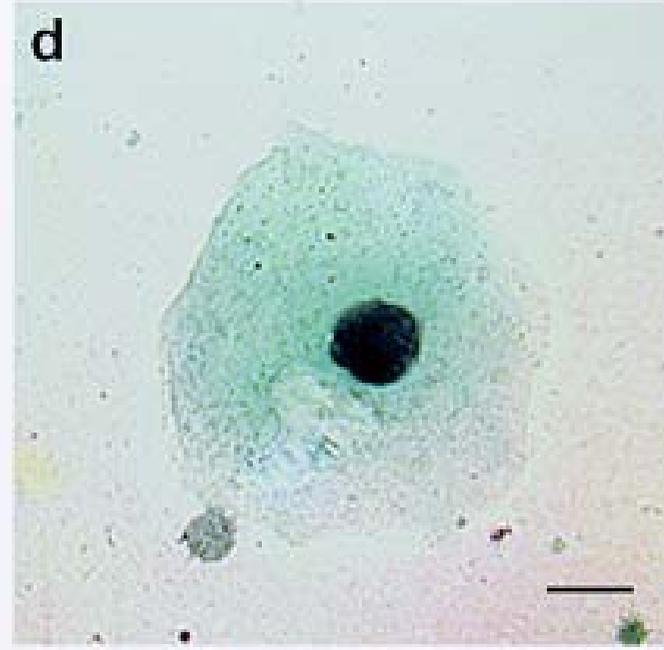
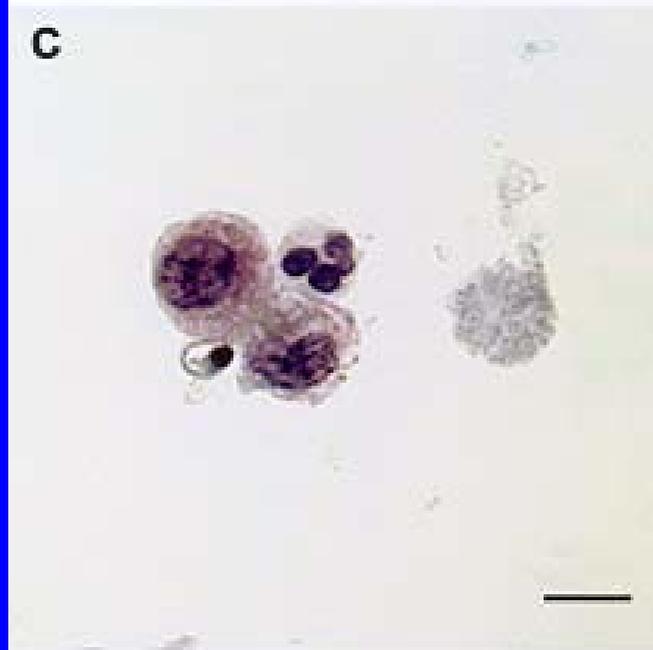
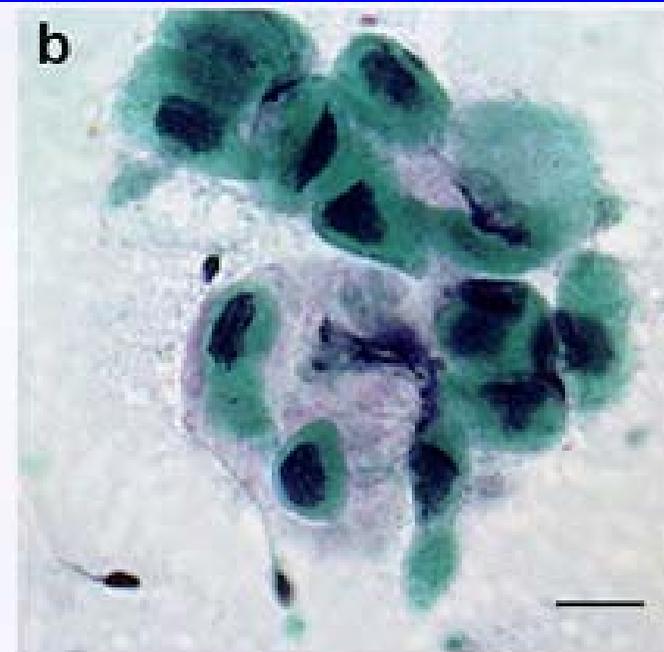
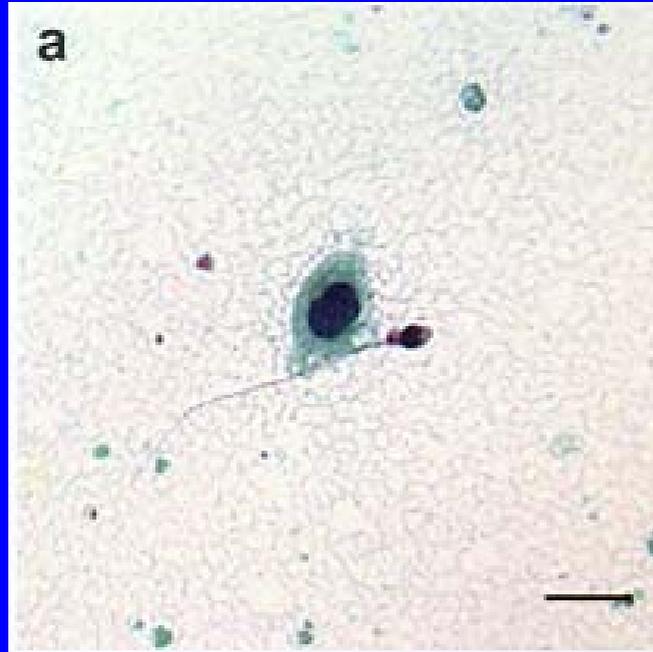


Papanicolaou staining

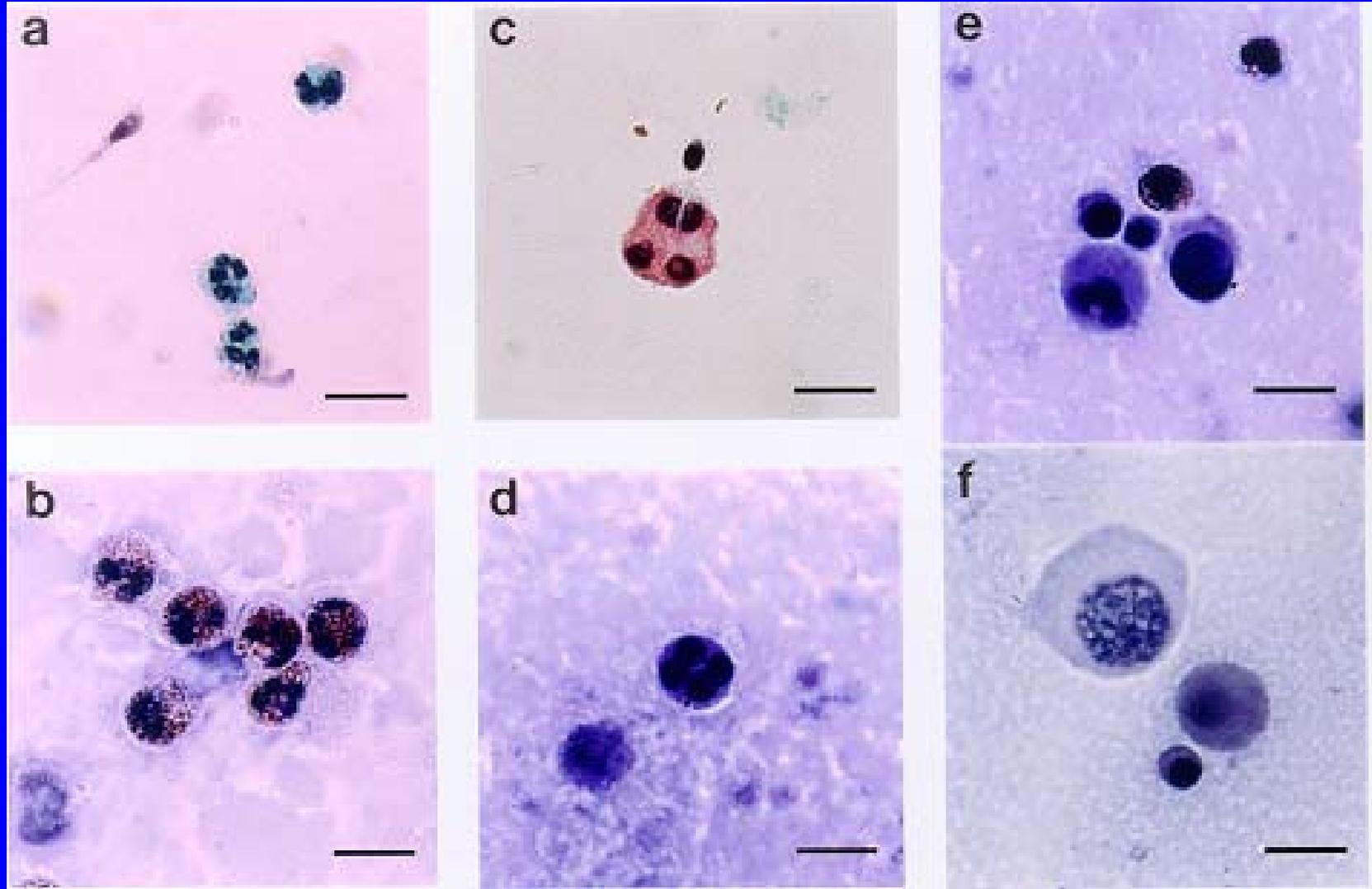
- a) semen
- b) blood



Papanicolaou staining
of epithelial cells
from male genital tract



Peroxidase staining distinguishes neutrophils from spermatids in semen



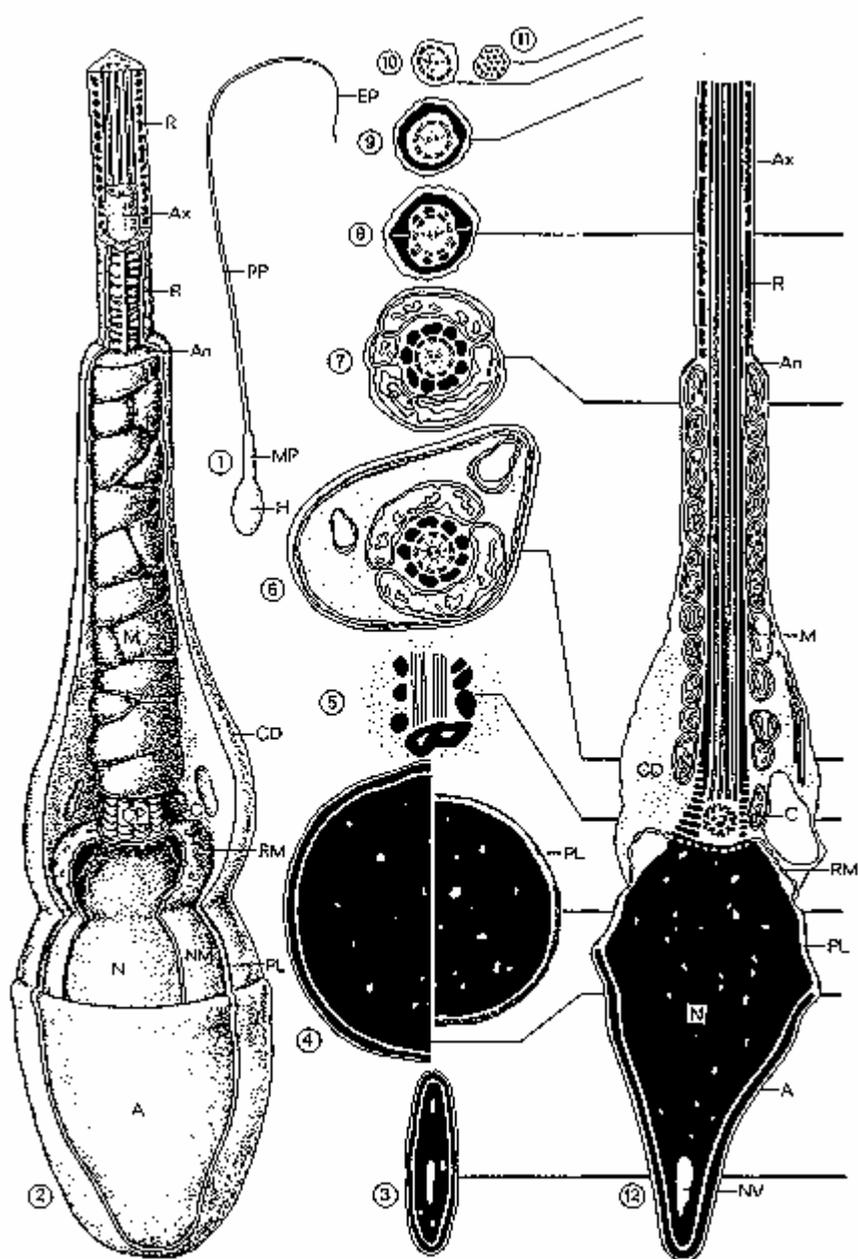


Figure 19.11. Structure of the mature spermatozoon. 1, the relative size of the head (H), midpiece (MP), principal piece (PP) and end piece (EP) is shown. 2, a three-dimensional cutaway drawing showing the acrosome (A), nuclear envelope and nucleus (N) in the head, the connecting piece (CP) of the neck, 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.)

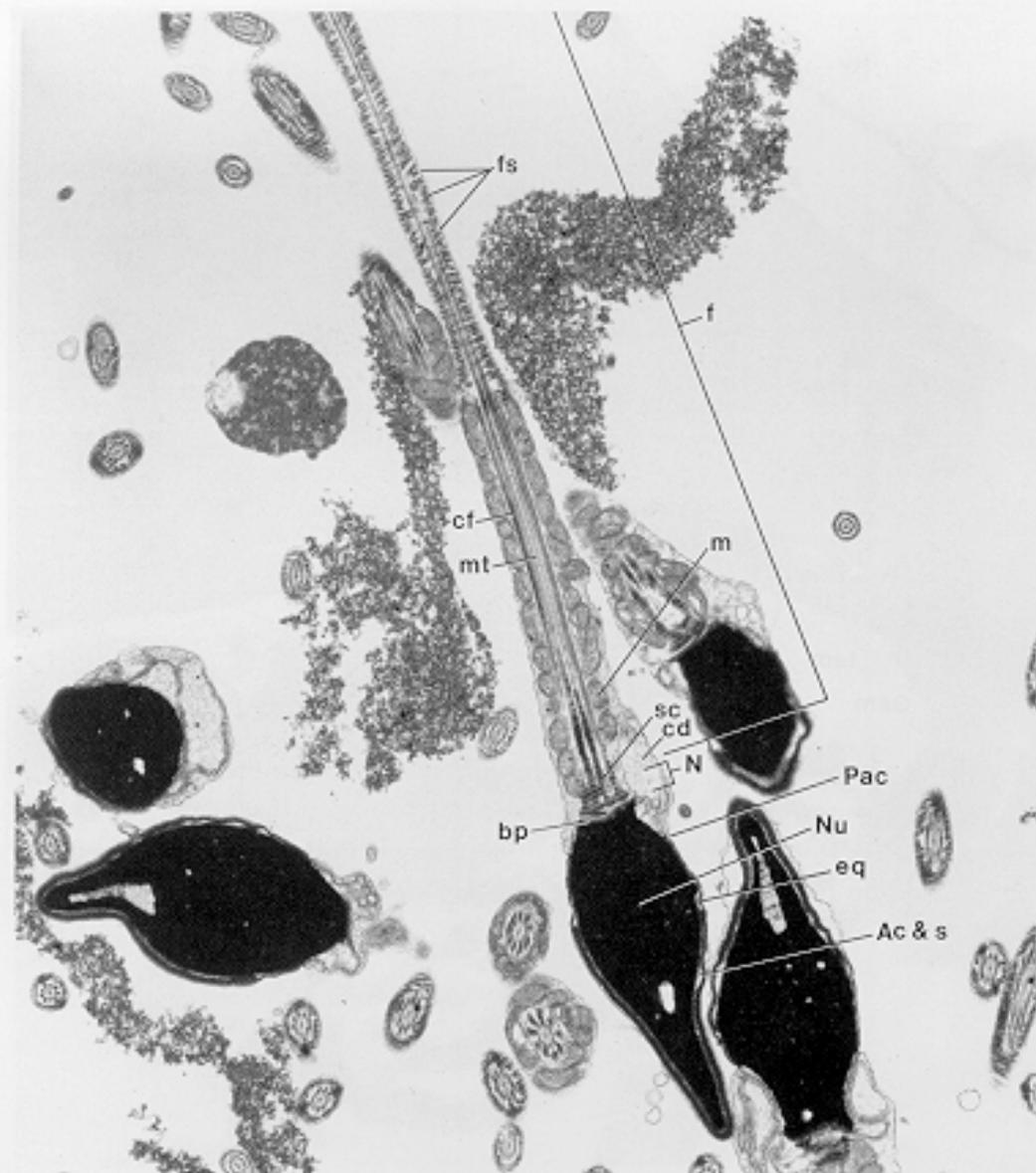


Figure 3.56. Longitudinal section of a normal spermatozoon. Nu = nucleus; Ac = acrosome; eq = equatorial region; Pac = postacrosomal region; cd = cytoplasmic droplet; sc = segmented columns of the proximal centriole; f = flagellum; bp = base plate; s = subacrosomal space; m = mitochondrion; N = neck;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

Axoneme of cilia and spermatozoa :

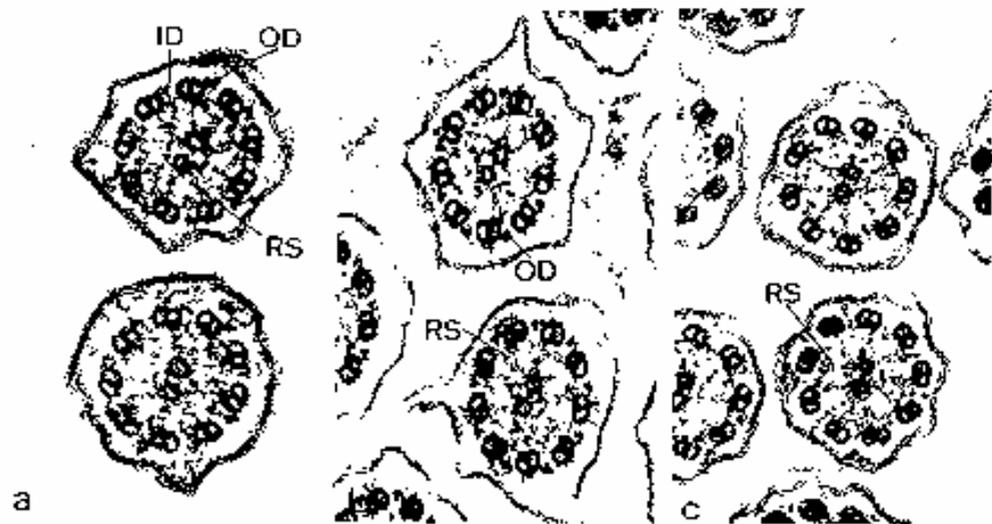
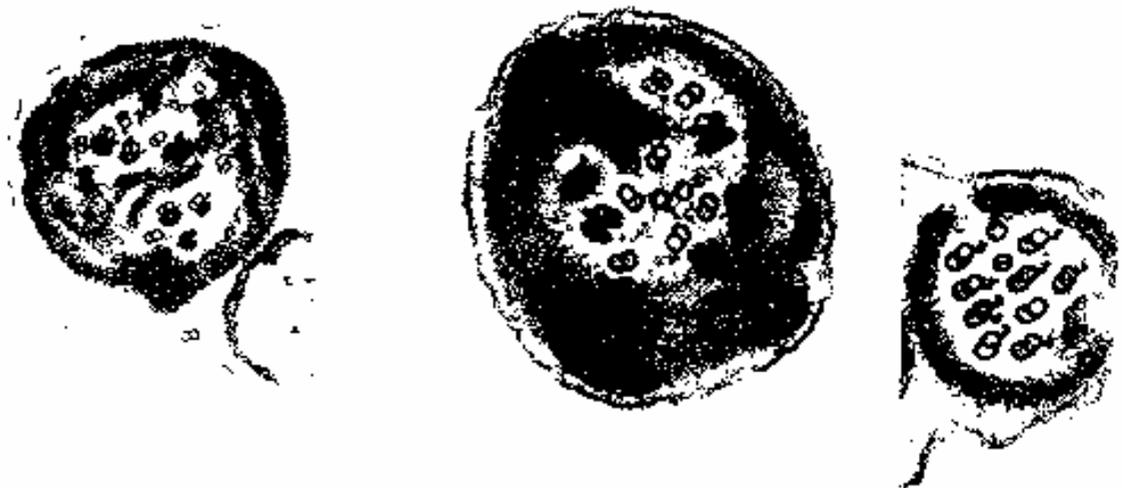


Figure 2 – Electron micrographs of sections through normal and diseased human bronchial cilia. a – Normal pattern with inner (ID) and outer (OD) dynein arms extending from the A-subunit of each of the nine microtubular doublets. b – Inner dynein arms are missing. c – Both inner and outer dynein arms are missing. Biopsy from a person with the irritable cilia syndrome. Note the presence of radial spokes (RS) in all three biopsy specimens. (x 100,000) (Courtesy of Dr. M. Baud, University of Geneva, Geneva, Switzerland)



Globozoospermia

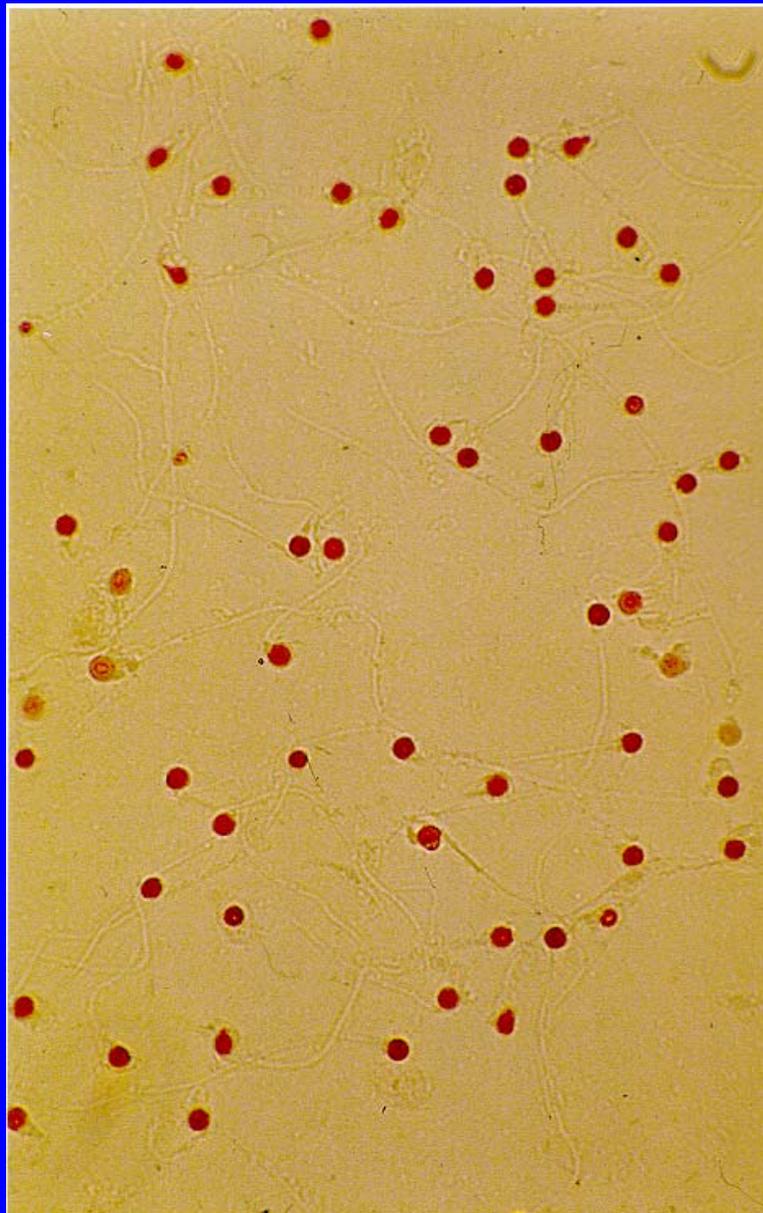


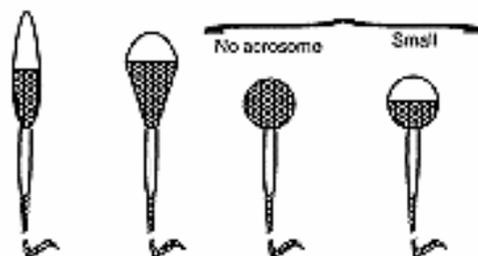
Figure 4.6. Transmission electron microscopy. Spermatozoa have spherically shaped nuclei, completely lacking both acrosome and postacrosomal sheaths.

**Spermatozoa morphology,
classification according to
Tygerberg criteria (Kruger)
Ref: WHO manual, 1999.**



A. Head defects

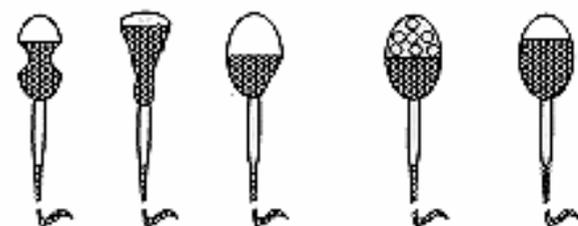
(a) Tapered (b) Pyriform (c) Round



(d) Amorphous

(e) Vacuolated

(f) Small
acrosomal area



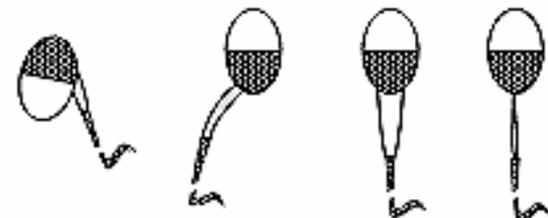
B. Neck and midpiece defects

(g) Bent neck

(h) Asymmetrical
insertion

(i) Thick

(j) Thin



C. Tail defects

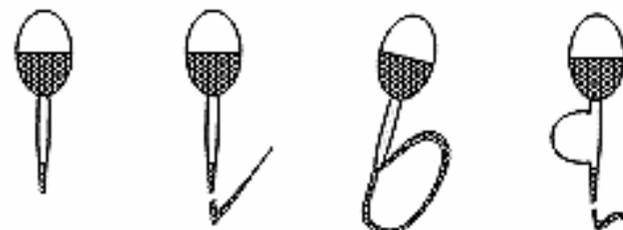
(k) Short

(l) Bent

(m) Coiled

D. Cytoplasmic droplet

(n) >1/3rd head



Morphologie des spermatozoïdes humains.

Têtes normales

1.



2.



3.



4.



Anomalies de la tête

5. Macrocéphale



6. Microcéphale



7. Pyriforme



8. Tapering



9. Ronde (avec acrosome)



10. Ronde (sans acrosome)



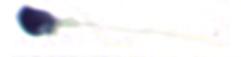
11. Double tête (amorphe)



12. Amorphe



13. Amorphe



14. Amorphe



15. Amorphe



16. Amorphe



Anomalies de la pièce intermédiaire

17. Reste cytoplasmique



18. Angulation



Anomalies du flagelle

19. Double



20. Court



21. Enroulé



22. "



23. "



24. Absent



25. Angulé



Certaines anomalies morphologiques peuvent compromettre la capacité fécondante des spermatozoïdes

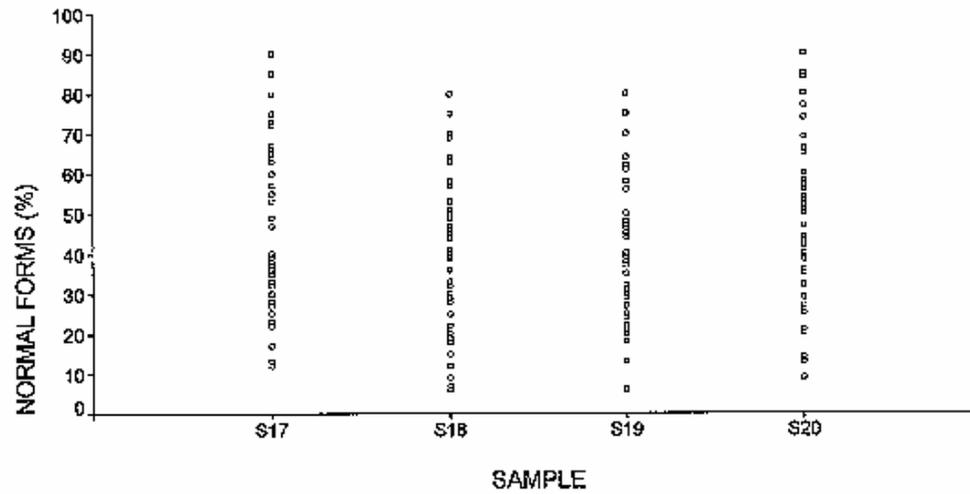
- Une anomalie de la pièce intermédiaire ou du flagelle peut gêner la mobilité
- Un acrosome incomplet ou absent peut empêcher la pénétration de la zone pellucide de l'ovocyte.
- Une tête trop volumineuse est un signe que la compaction de l'AND du noyau est incomplète
- En cas d'anomalies polymorphes de la tête des spermatozoïdes, il est impossible de présumer de leur fécondité

Le rapport entre la morphologie des spermatozoïdes et la fertilité dépend d'une évaluation à **deux niveaux distincts** :

- 1 Chaque spermatozoïde est évalué individuellement et classifié comme normal ou anormal suivant une **définition de la morphologie normale du spermatozoïde**.
- 2 Le pourcentage de spermatozoïdes de morphologie normale présents dans le sperme est évalué et relié à la fertilité suivant une **valeurs limite, au-dessous de laquelle la fertilité diminue**.

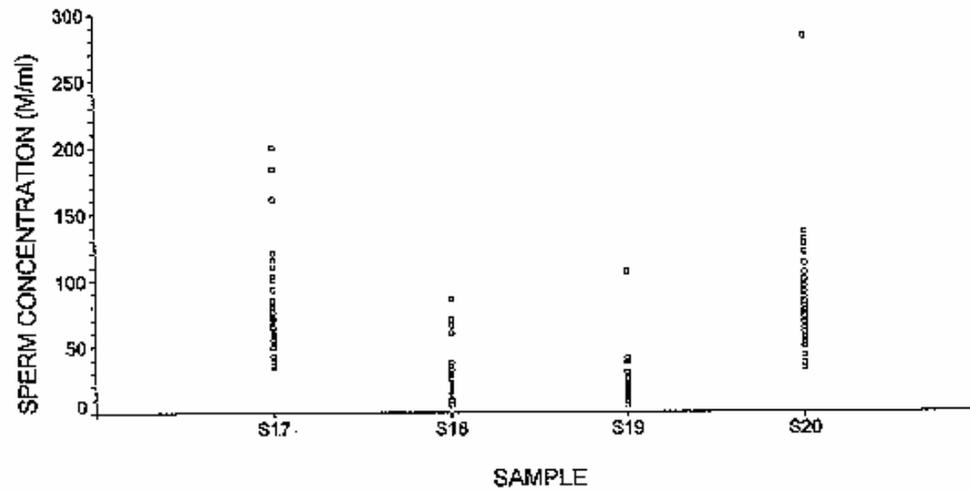
UK NEQAS - NORMAL MORPHOLOGY

DISTRIBUTION 95/96 - 1



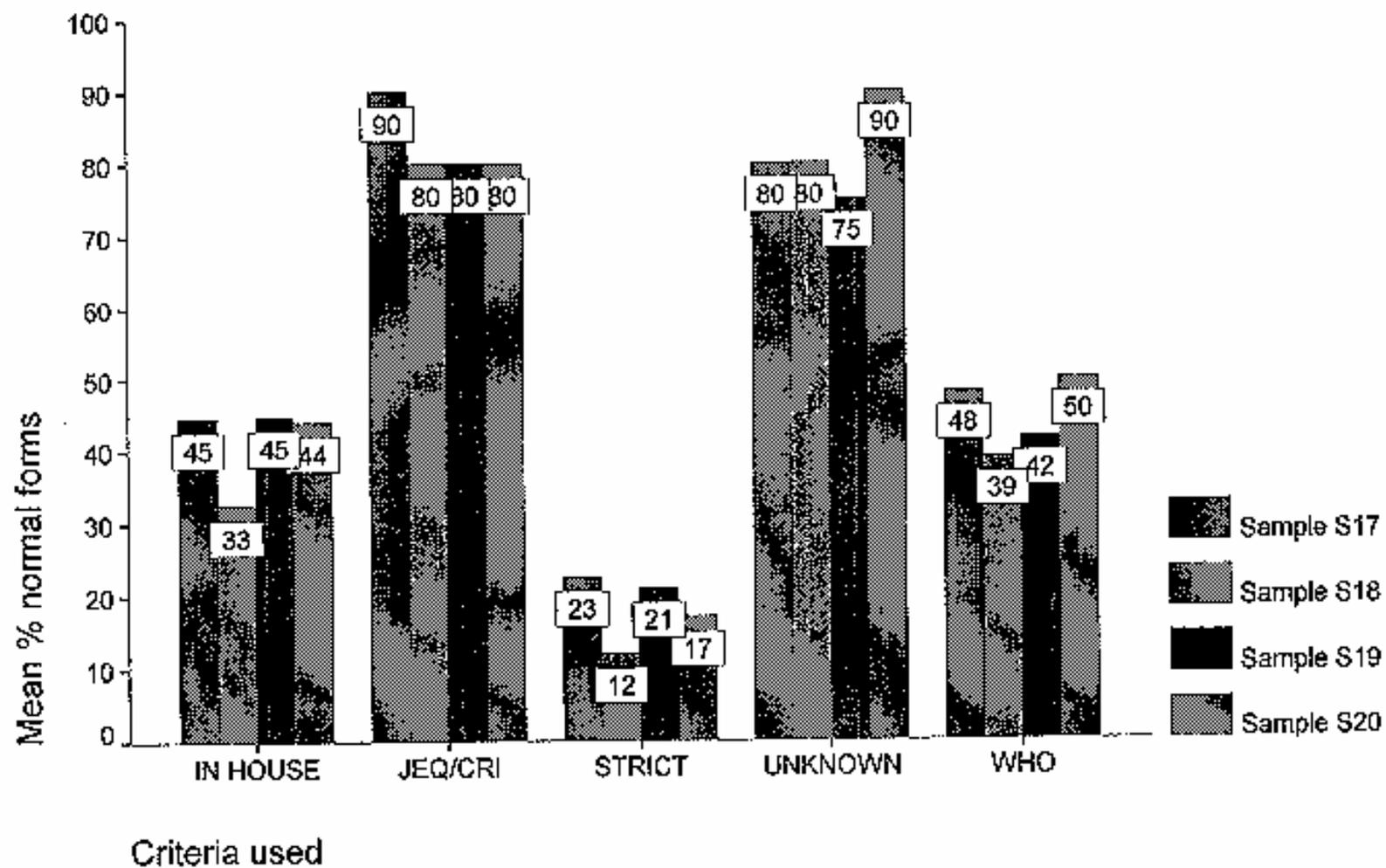
UK NEQAS - SPERM CONCENTRATION

DISTRIBUTION 95/96 - 1



UK NEQAS - NORMAL MORPHOLOGY COMPARISON OF CRITERIA

Distribution 95/96 - 1



Etablissement de normes valides pour les paramètres du sperme

- De vastes études multicentriques ont été lancées depuis deux ans, les premiers résultats ont été récemment publiés.
- Ils permettent de valider les limites de normalité chez des hommes fertiles.

Variable	Semen Measurement		
	Concentration (x 10 ⁶ /ml)	Motility (%)	Morphology (% normal)
Fertile range	> 48.0	> 63	> 12
indeterminate range	13.5 - 48.0	32 - 63	9 - 12
Univariate odds ratio for infertility (95% C.I.)	1.5 (1.2 - 1.8)	1.7 (1.5 - 2.2)	1.8 (1.4 - 2.4)
Subfertile range	< 13.5	< 32	< 9
Univariate odds ratio for infertility (95% C.I.)	5.3 (3.3 - 8.3)	5.6 (3.5 - 8.3)	3.8 (3.9 - 5.0)

TABLE 3. ODDS RATIOS FOR INFERTILITY FOR COMBINATIONS OF SPERM MEASUREMENTS.*

SPERM MEASUREMENT RANGE			ODDS RATIO (95% CI)
MORPHOLOGIC FEATURES	MOTILITY	CONCENTRATION	
Fertile	Fertile	Fertile	1.0
Subfertile	Fertile	Fertile	2.9 (2.2–3.7)
Fertile	Subfertile	Fertile	2.5 (1.6–4.2)
Fertile	Fertile	Subfertile	2.2 (1.3–3.6)
Subfertile	Subfertile	Fertile	7.2 (4.3–12.2)
Subfertile	Fertile	Subfertile	6.3 (3.8–10.3)
Fertile	Subfertile	Subfertile	5.5 (3.0–10.2)
Subfertile	Subfertile	Subfertile	15.8 (8.7–29.0)

• Guzik et al. NEJM, 2001

Sensitivity : % of the infertile men with abnormal semen analysis results

Specificity : % of the fertile men with normal semen analysis results

TABLE 4. SENSITIVITY AND SPECIFICITY OF SPERM MEASUREMENTS FOR IDENTIFYING INFERTILE MEN AT VARIOUS THRESHOLDS.

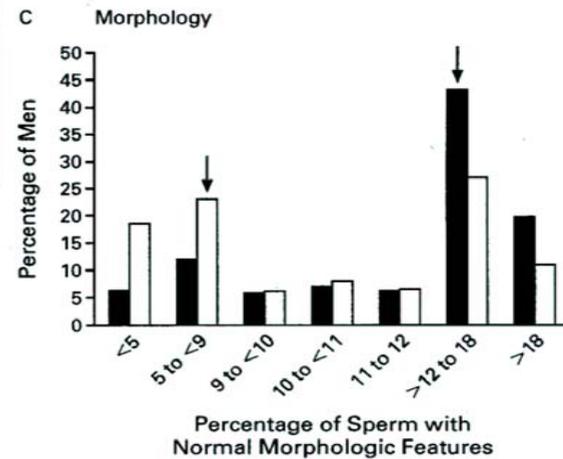
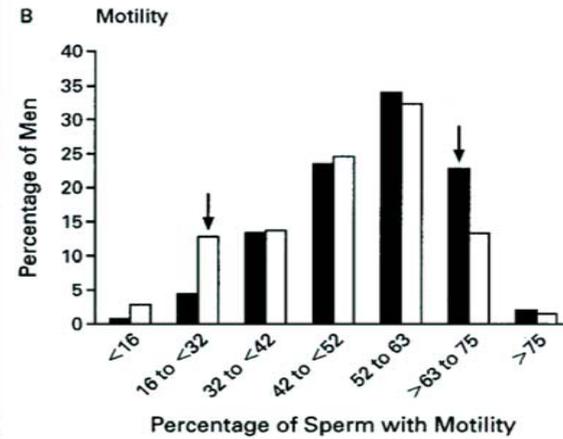
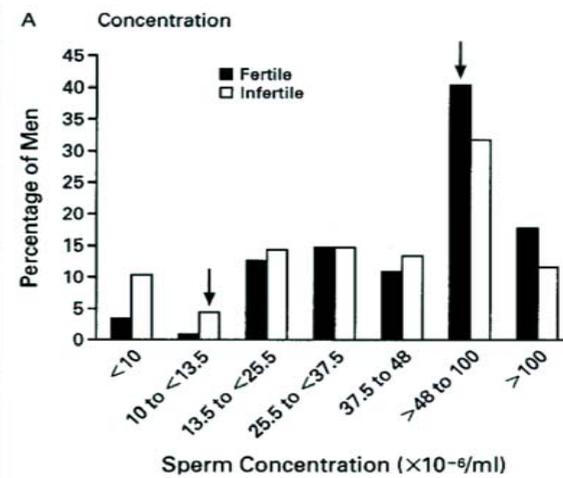
SPERM MEASUREMENT	SENSITIVITY SPECIFICITY	
	percent	
Concentration		
10.0×10 ⁶ /ml	10.2	96.8
13.5×10 ⁶ /ml*	14.8	96.1
48.0×10 ⁶ /ml†	57.1	58.0
60.0×10 ⁶ /ml	67.7	45.0
Percentage of motile sperm		
25	8.1	97.7
32*	15.3	95.1
63†	84.6	26.2
75	98.6	2.7
Percentage with normal morphologic features		
5	18.6	93.8
9*	43.3	81.4
11†	60.8	65.3
15	75.6	41.0

*This value was defined by classification-and-regression-tree analysis as the threshold between the subfertile range and the indeterminate range.

†This value was defined by classification-and-regression-tree analysis as the threshold between the indeterminate range and the fertile range.

Percentage of Men from Infertile and Fertile Couples

Guzic et al. NEJM, 2001



Regional differences in semen quality in Europe

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

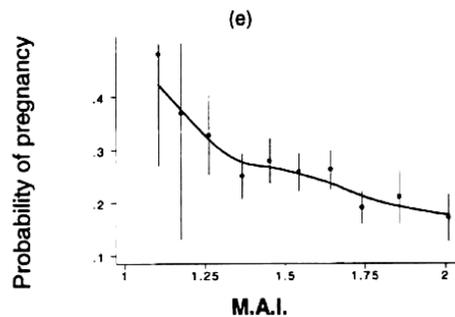
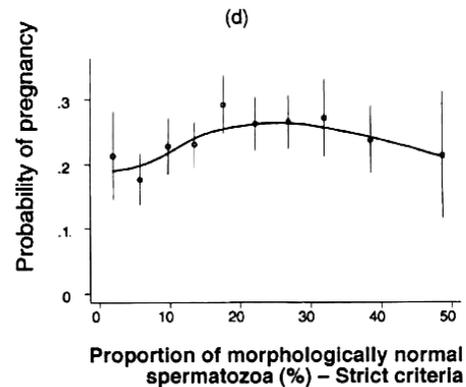
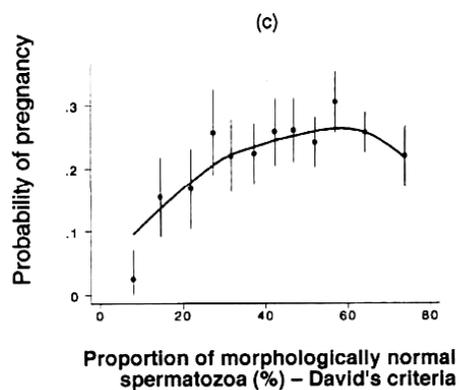
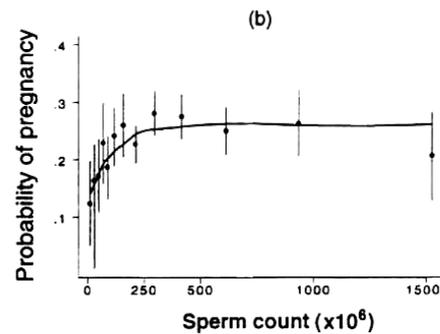
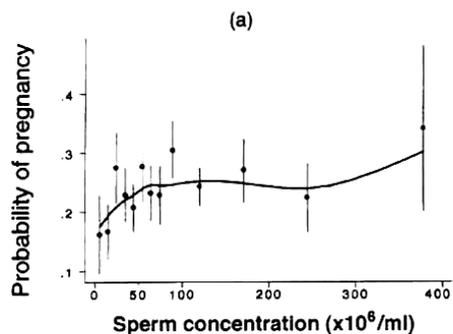
Region	Rank		Seasonal variation (%) winter / summer	Concentration range (million/ml)	
	Concentration and Total sperm count	Motility	Concentration and Total sperm count	winter	summer
Turku	4 [#]	3 [#]	100 % / 70 %	132	93
Edingurgh	3*	4*		119	84
Paris	2* #	1* #		103	73
Copenhagen	1	2		98	69

Time to pregnancy and semen parameters in fertile couples

Table I. Number of contacted and included couples, time to pregnancy (TTP) and semen characteristics, according to city

City	Copenhagen	Paris	Turku	Edinburgh	Total
Number of eligible couples contacted	809	1368	1422	NA	NA
Men who delivered a semen sample (proportion among contacted couples)	348 (43.0)	208 (15.2)	275 (19.3)	250 (NA)	1081 (NA)
Use of a method to avoid pregnancy when the conception occurred ^a					
No	304 (87.4)	191 (91.8)	249 (90.5)	219 (87.6)	963 (89.1)
Yes	43 (12.4)	16 (7.7)	26 (9.5)	31 (12.4)	116 (10.7)
No answer	1 (0.3)	1 (0.5)	0 (0)	0 (0)	2 (0.2)
Couples with defined TTP ^b	302 (99.3)	191 (100.0)	237 (95.2)	212 (96.8)	942 (97.8)
Among men with defined TTP:					
Median TTP (proportion of couples who conceived within 3–6–12 months)	2 (55.8–71.8–79.9)	2 (51.0–72.6–82.7)	2 (57.5–71.6–77.5)	2 (54.4–70.4–78.8)	2 (55.0–71.6–79.6)
Mean semen volume (ml) (5–50–95th percentiles)	3.9 (1.0–4.0–7.0)	4.2 (2.0–4.0–8.0)	4.2 (2–4–7)	4.0 (1–4–8)	4.1 (2–4–7)
Mean sperm concentration ($\times 10^6$ /ml) (5–50–95th percentiles)	76 (12–60–178)	99 (17–78–232)	104 (18–81–259)	90 (19–76–192)	91 (15–73–229)
Total sperm count ($\times 10^6$) (5–50–95th percentiles)	279 (35–209–806)	406 (47–314–1228)	412 (54–324–1040)	349 (63–270–954)	354 (45–270–966)
Mean proportion of motile sperm (%) (5–50–95th percentiles)	60 (40–60–79)	56 (40–55–77)	66 (49–66–81)	67 (52–69–82)	62 (43–63–80)
Mean proportion of normal sperm (%), David's criteria (5–50–95th percentiles) ^d	49 (21–51–71)	50 (19–54–72)	52 (27–53–74)	49 (21–52–71)	50 (22–52–72)
Mean MAI (5–50–95th percentiles) ^e	1.60 (1.29–1.60–1.93)	1.51 (1.18–1.51–1.86)	1.63 (1.32–1.63–2.02)	1.58 (1.27–1.58–1.88)	1.58 (1.26–1.58–1.95)
Mean proportion of normal sperm (%), strict criteria (5–50–95th percentiles) ^f	20 (3–19–41)	23 (5–22–45)	19 (4–18–37)	19 (5–19–33)	20 (4–19–39)

Probability of pregnancy per month of attempt according to semen parameters in 942 fertile couples



CONCLUSION

L'analyse de sperme donne des renseignements précieux sur la fonction reproductive masculine

Elle permet :

- de tester la spermatogenèse et la fonction des glandes annexes
- de détecter des problèmes immunologiques, inflammatoires ou infectieux

Cependant, nous commençons seulement à obtenir des valeurs de référence adéquates, ce qui rend difficile l'évaluation de certains paramètres, comme la morphologie des spermatozoïdes

Les résultats des études multicentriques en cours, portant sur de grandes populations d'hommes fertiles, seront déterminantes pour établir des valeurs limites permettant de distinguer les cas associés à une fertilité réduite.