Chapter 12

SEMINAL FLUID ANALYSIS

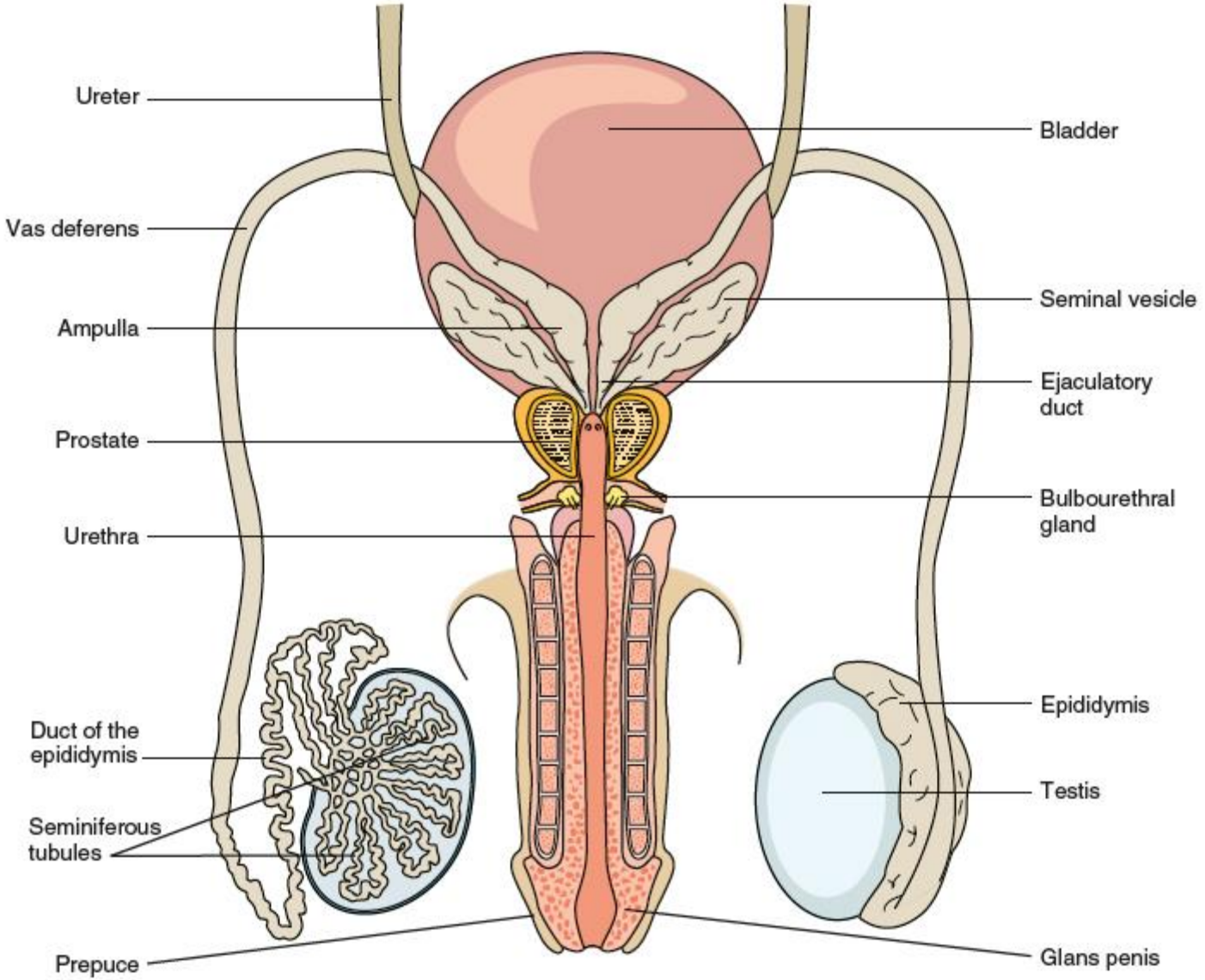
Semen Physiology

Semen composed of secretions from testes, epididymis, seminal vesicles, prostate, and bulbourethral glands

Testes secrete testosterone and produce sperm regulated by follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from anterior pituitary

Sperm production regulated by Sertoli cells in seminiferous tubules of testes

Germ cells undergo meiosis to form spermatids, which differentiate into sperm



Pathway of Sperm

Mature and stored in epididymis

Enter vas deferens, which leads to urethra

Fluids added by accessory glands (prostate, two seminal vesicles, and two bulbourethral glands)

Semen is complex and contains various substances such as fructose, flavin, proteins, prostaglandins, citric acid, zinc, enzymes (proteolytic enzymes and acid phosphatase)

Specimen Collection

For fertility assessment, several samples over a 3-month period, with abstinence between 2 and 7 days before collection of each sample

Entire ejaculate is collected in appropriate clean sterile container

Samples delivered to laboratory within 1 hour maintained between 20°C and 40°C

The collection container should be kept at room temperature or warmed to approximately body temperature

Note time of specimen collection

Physical Examination

Appearance

- Normal gray-white and opalescent
- Immediately coagulates but should liquefy within 30 minutes

Volume

- Normal 2 to 5 mL

Viscosity

- After liquefaction, normal specimen is watery and forms discrete droplets

TABLE 12.1 Semen Characteristics Associated With Fertility

Parameter	Reference Interval*	Lower Reference Limit†
Physical Examination		
Appearance	Gray-white, opalescent, opaque	
Volume	2–5 mL	1.5 mL (1.4–1.7)
Viscosity/liquefaction	Discrete droplets (watery) within 60 minutes	
Microscopic Examination		
Motility	50% or more with moderate to rapid linear (forward) progression	40% (38–42)
Concentration	20 to 250 $\times 10^6$ sperm per mL	15 $\times 10^6$ sperm per mL
Morphology	14% or more have normal morphology	4% normal forms
Vitality	75% or more are alive	58% (55–63)
Leukocytes	Less than 1 $\times 10^6$ per mL	
Chemical Examination		
pH	7.2–7.8	≥7.2
Acid phosphatase (total)	≥200 U per ejaculate at 37°C (*p*-nitrophenylphosphate)	
Citric acid (total)	≥52 µmol per ejaculate	
Fructose (total)	≥13 µmol per ejaculate	≥13 µmol per ejaculate
Zinc (total)	≥2.4 µmol per ejaculate	≥2.4 µmol per ejaculate

Microscopic Examination: Motility

Standardization of procedures very important as is using experienced personnel

WHO [World Health Organization] Laboratory Manual for the Examination and Processing of Human Semen is an excellent reference

Motility

- Very important, since immotile sperm are unable to reach and fertilize an egg
- Evaluated subjectively or by automated method
- 50% or more of sperm should show moderate to strong linear or forward progression

TABLE 12.2 Sperm Motility Grading Criteria

0	Immotile
1	Motile, without forward progression
2	Motile, with slow nonlinear or meandering progression
3	Motile, with moderate linear (forward) progression
4	Motile, with strong linear (forward) progression

Concentration and Sperm Count

Normal sperm concentration 20 to 250 million/mL

Concentration determined manually by hemacytometer

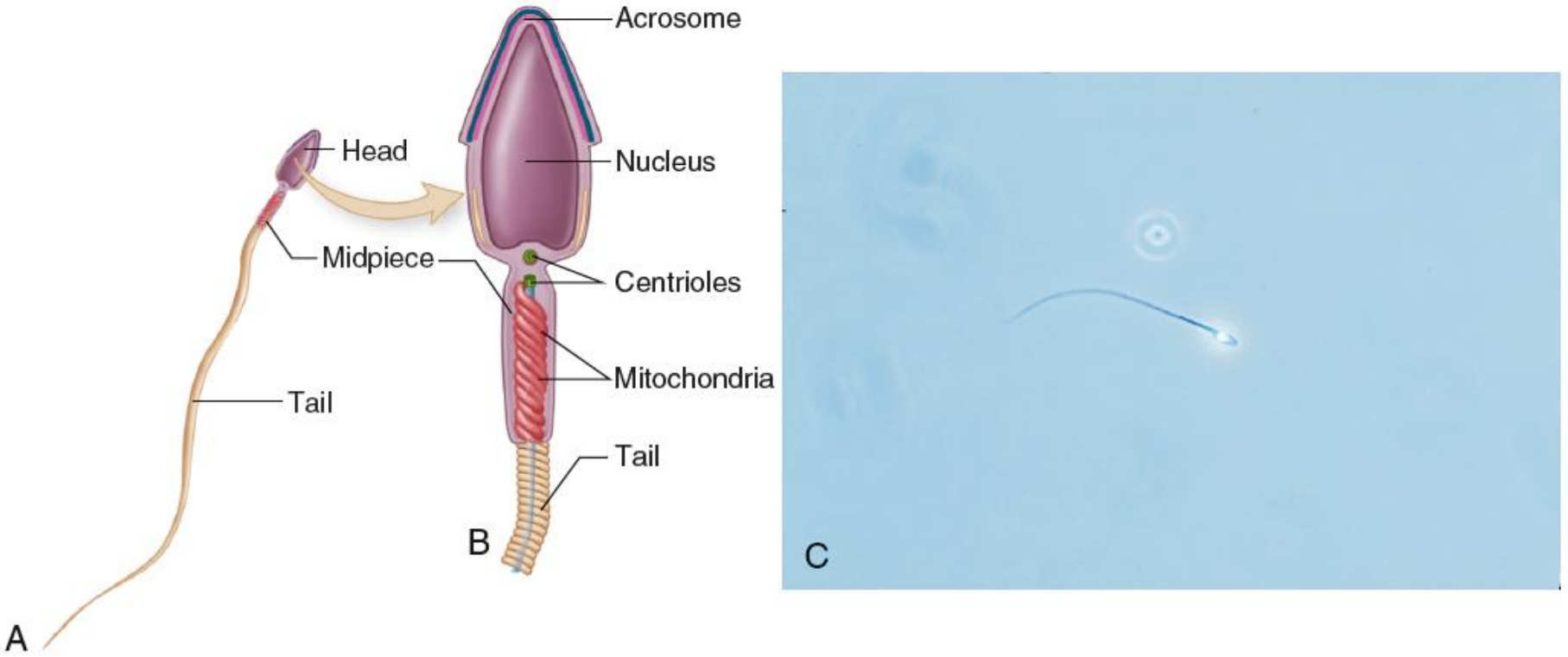
Sperm count determined by multiplying concentration per milliliter by volume of ejaculate

Postvasectomy sperm counts should be 0 within 12 weeks after procedure; any remaining sperm should be nonmotile

Microscopic Examination: Morphology

Subjective evaluation of three distinct areas

- Head
  - Oval, 2.5 to 3.5 µm wide and 4.0 to 5.0 µm long
  - Head length-to-width ratio should be 1.50 to 1.75
- Midpiece
  - 6 to 7.5 µm long and thicker than tail but less than 1 µm
- Tail
  - Slender, uncoiled, at least 45 µm long

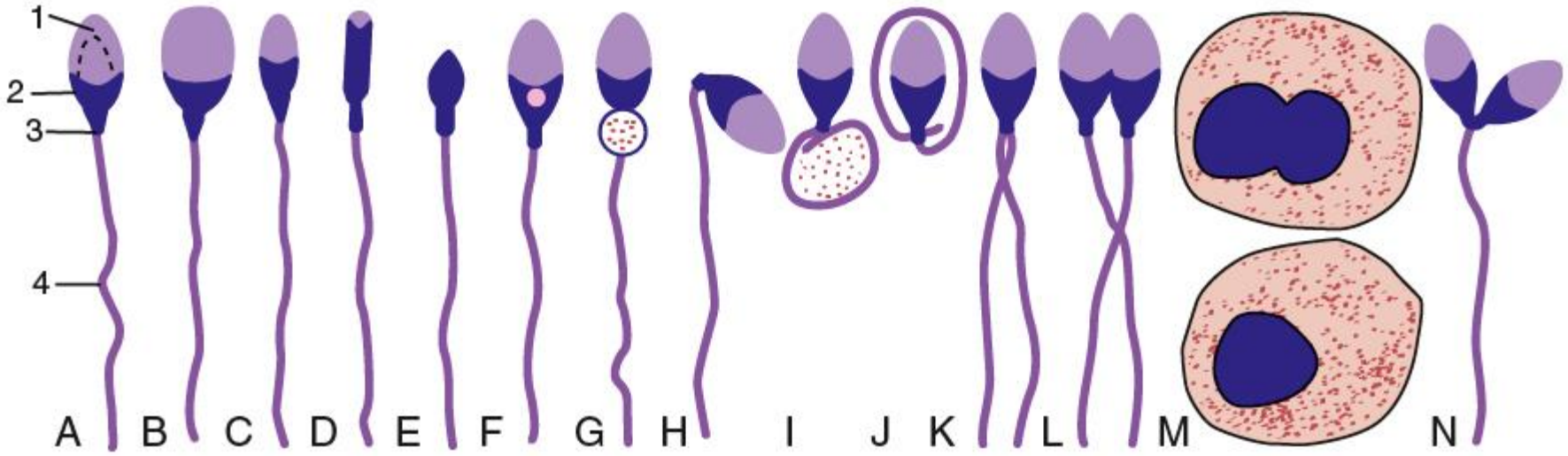


A and **B** from Patton KT, Thibodeau GA: Anatomy and physiology, ed 9, St Louis, 2016, Mosby.

Evaluation of Sperm Morphology

Each sperm classified using five categories:

- Normal
- Head defects
- Midpiece defects
- Tail defects
- Cytoplasmic droplet present (located in midpiece region and is abnormal if this region is more than one-third the area of a normal sperm head)



Microscopic Examination

Vitality

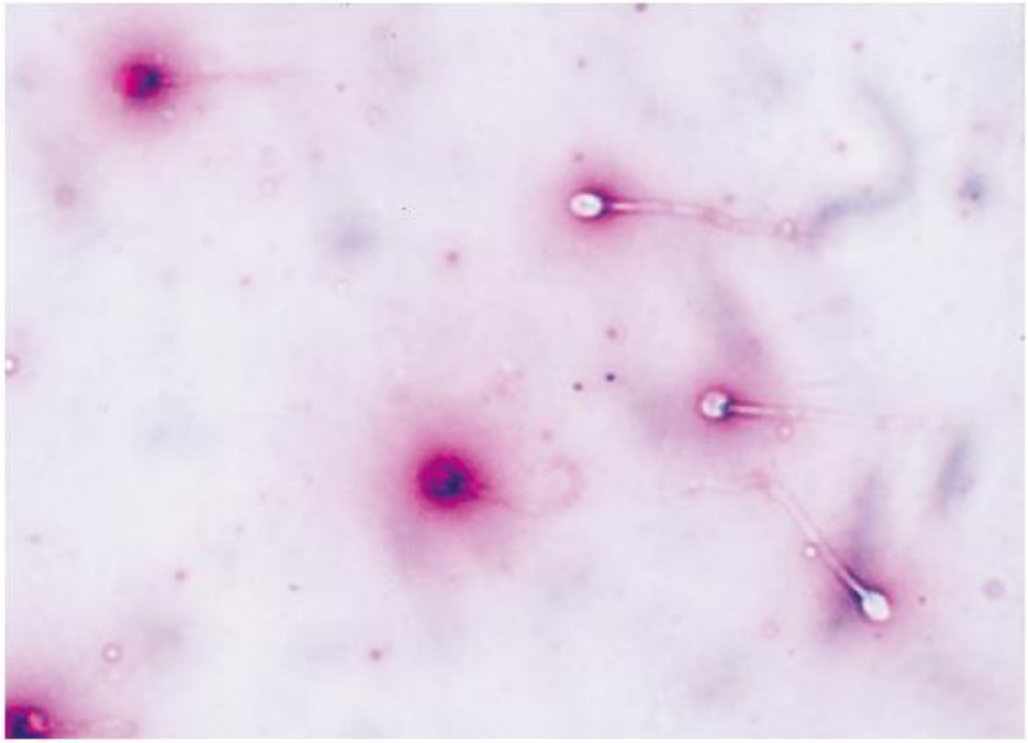
- Vital staining differentiates between live and dead sperm; dead sperm take up stain; live ones do not

Other cells

- More than 1 million white blood cells (WBCs) per milliliter indicates an inflammatory process
- Immature spermatogenic cells may resemble WBCs
- Red blood cells (RBCs) are abnormal

Agglutination

- Even a small amount is abnormal if sperm are alive



Chemical Examination

pH

- Normal ranges from 7.2 to 7.8
- Below 7.2 in abnormalities of epididymis, vas deferens, or seminal vesicles
- Above 7.8 suggests infection

Fructose

- Produced and secreted by seminal vesicles
- Most often measured when no sperm seen on count
- Normal levels greater than or equal to 13 µmol per ejaculate

Other Biochemical Markers

Zinc

- Used to evaluate prostate function
- Normal greater than or equal to 2.4 mmol per ejaculate

Citric acid

- Used to evaluate prostate function
- Normal greater than or equal to 52 mmol per ejaculate

Acid phosphatase

- From prostate
- Used in rape and sexual assault investigations to identify semen in vaginal fluid