The Human Semen

11

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Learning Objectives
- Physical properties
- Composition
- Fructolysis
- Coagulation and liquefaction

11.1 Introduction

Human semen is a protein-rich body fluid produced by the male reproductive organs. It is a complex cell suspension in a fluid containing an array of heterogeneous substances produced by different male reproductive glands like the testis, epididymis, seminal vesicles, prostate, Cowper's gland (bulbourethral) and glands of Littre (periurethral glands). Its main function is to act as a buffered, nutrient-rich medium which transports the sperm through the male reproductive tract into the female reproductive tract.

During coitus, a heterogeneous ejaculate is deposited in the female tract. This is because the accessory sex glands discharge their secretions by contracting in an organ-specific sequence during emission/ejaculation. This ensures that the various components of semen are delivered in sequential order. The order of

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Table 11.1 Sequence of secretions during ejaculation

Order of secretion	Contributing gland	% of total ejaculate volume
Ist	Cowper's gland (bulbourethral) and glands of Littre (periurethral)	1–5
IInd – A	Testis/Epididymis	5–10
IInd – B	Prostate	20–30
IIIrd	Seminal vesicles	65–75

secretion and the relative contribution of each gland are listed in Table 11.1. The initial secretion, known as pre-ejaculate, comprises of secretions from the Cowper's and Littre glands. This mucinous secretion lubricates the urethra and neutralizes any traces of residual acidic urine. The next fraction results from the simultaneous contractions of the epididymis and prostate. It contains the maximum concentration of sperm along with epididymal and prostatic secretions. The final and largest fraction of the ejaculate is contributed by the seminal vesicles.

Two fates exist for the semen components that remain in the male tract after ejaculation: (i) passive resorption by surrounding tissue or (ii) expulsion during urination (Prins and Lindgren 2015).

11.2 Physical Properties

The volume of a typical human ejaculate is about 3 ml although it can range from 2 to 5 ml (Owen and Katz 2005). Normal semen is a greyish, opalescent fluid with a density between 1.043 and 1.102 g/ml. The colour may appear whitish due to the presence of high number of sperm or leukocytes. If red blood cells are present (hemospermia), the colour may appear reddish brown (WHO 2010).

Semen is slightly alkaline which helps in neutralizing the acidic environment of vagina. The pH measured can vary from 7.2 to 7.8 depending on the time elapsed since ejaculation. Decrease in pH of whole semen over time is attributed to fructolysis and production of lactic acid. However, semen has a buffering capacity much higher than other body fluids. This buffering capacity is contributed by bicarbonate/carbon dioxide (HCO_3/CO_2), high protein content and low molecular weight compounds like citrate, pyruvate and phosphate (Wolters-Everhardt et al. 1987). Another peculiar property of semen is its high osmolarity which is due to the presence of high concentration of organic components rather than inorganic ions.

11.3 Composition

The components of semen can be divided into 'cellular' and 'acellular' components (see Fig. 11.1). The acellular component, obtained after removal of the cells by centrifugation, is termed as seminal fluid and comprises >90% of the semen volume.

11.3.1 Cellular Components

An average human ejaculate has about 100 million sperm/ml though they contribute less than 1% of the ejaculate volume (Prins and Lindgren 2015). The total number of sperm per ejaculate correlates with the length of abstinence as well as the testicular volume (Schwartz et al. 1979; Cooper 2010). Detailed description of sperm is given in the subsequent chapters, The Sperm and Sperm Function Tests.

The other cellular components of semen are epithelial cells of the urogenital tract, leukocytes and even spermatogenic cells. The presence of immature germ

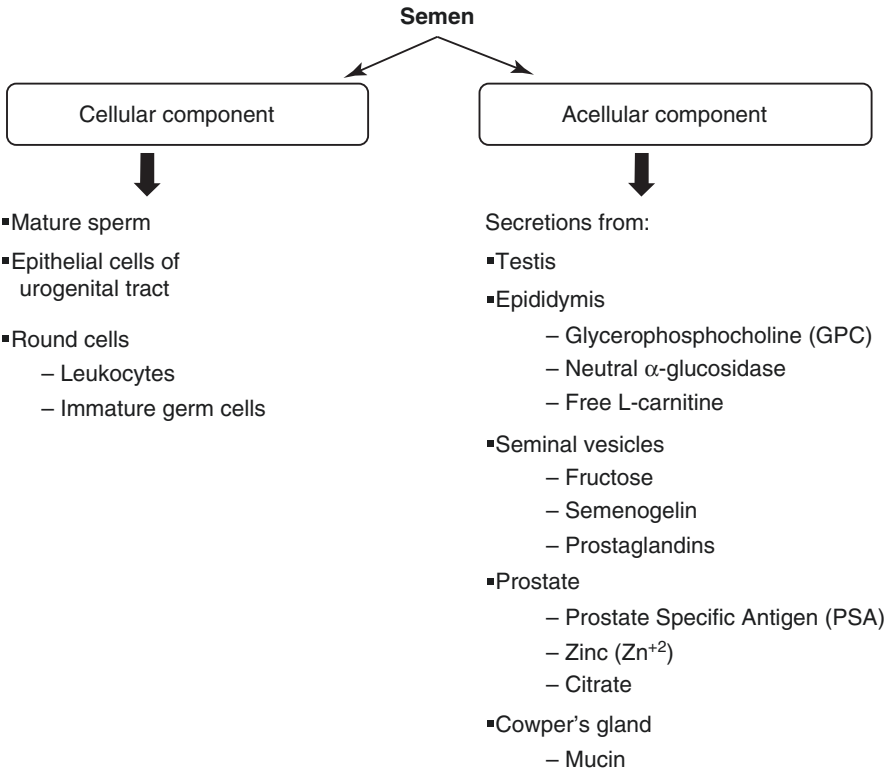

Fig. 11.1 Composition of human semen

cells in the semen may indicate testicular damage or defective spermatogenesis, while the presence of leukocytes may be suggestive of inflammation in the accessory glands (WHO 2010).

A simple microscopic examination of semen is not able to differentiate between leukocytes and spermatogenic cells, which are collectively labelled as 'round cells' (Johanisson et al. 2000). Most human ejaculates show the presence of leukocytes with a predominance of granulocytes. The leukocytes present in the semen are predominantly peroxidase-positive granulocytes (polymorphonuclear leukocytes). They can be easily distinguished from the peroxidase-free multinucleated spermatids by histochemical staining for peroxidase. However, activated granulocytes, which have lost their granules, and other leukocytes, e.g. lymphocytes, monocytes and macrophages, are peroxidase negative. They can be differentiated by immunostaining for CD45 which is the common leukocyte antigen (WHO 2010). If the number of leukocytes present in the ejaculate is greater than the threshold value (1.0×10^6 peroxidase-positive cells/ml), it is termed as leukospermia. An increase in the total number of leukocytes present in the ejaculate correlates with the severity of the inflammatory condition (Wolff 1995).

11.3.2 Components of Seminal Fluid

Seminal fluid comprises of secretions from the seminal vesicles, prostate, testes, epididymides and Cowper's and Littre glands with the greatest molecular content being provided by seminal vesicles (see Fig. 11.1). Some of the constituents are found in the serum and are probably exudates from the circulation, but many others are produced exclusively by the reproductive organs and are unique to seminal fluid. Individual seminal fluid constituents are not essential for fertilization as evidenced by the fact that epididymal/testicular sperm, obtained by testicular sperm extraction (TESE), can be used for assisted reproductive technology (ART) to achieve normal fertilization rates in vitro. However, they may be important under normal conditions for transport/maturation of sperm and greatly enhance the fertilization capacity of sperm in vivo.

The precise function of all individual constituents of the seminal fluid has not yet been determined. They are presumed to be important for the sperm function, during and/or after ejaculation. Qualitative and/or quantitative assessment of specific semen components can serve as marker of the proper functioning of each accessory sex gland, for example, measurement of citric acid, zinc and acid phosphatase to assess the prostatic gland function; fructose and prostaglandins for seminal vesicle; free-L-carnitine, glycerophosphocholine (GPC); and neutral α-glucosidase for epididymal function.

Human seminal fluid contains a diverse set of molecules ranging from organic constituents like proteins, peptides, sugars and lipids to inorganic ions like zinc (see Table 11.2). Average protein concentration of human seminal fluid is 25–55 g/L with albumin making up about one third of the total protein present (Owen and Katz 2005; Rodriguez-Martinez et al. 2011). Albumin in semen is mainly of prostatic

Table 11.2 Important constituents of human seminal fluid

Name of the constituent	Concentration (mg/ml)	Major source
Phosphorylcholine	10.0	Epididymis
Prostate-specific antigen (PSA)	0.5–5.0	Prostate
Citric acid	3.76	Prostate
Spermine	0.5–3.5	Prostate
Prostatic acid phosphatase (PAP)	0.3–1.0	Prostate
Zinc	0.14	Prostate
Fructose	2.0	Seminal vesicles
Total lipids (cholesterol + phospholipids)	1.85	Seminal vesicles
Prostaglandins	0.1–0.3	Seminal vesicles

origin, but the majority of other proteins present are contributed by seminal vesicles (Hirsch et al. 1991). Some of the most important components of the human seminal plasma are listed in Table 11.2 and discussed in subsequent section of the chapter.

11.3.2.1 Originating from Seminal Vesicles

The most important constituents in the seminal vesicle secretions include fructose, semenogelin and prostaglandins. Fructose serves as the primary energy source for sperm in semen. It is produced exclusively by the seminal vesicles, and, hence, its absence in semen is a sign of potential ejaculatory duct obstruction. Semenogelin is a 52-kDa protein which is involved in coagulation of semen. The cleavage products of semenogelin formed following liquefaction have biological functions, such as inhibition of sperm motility and antibacterial activity. The seminal fluid contains about 15 different prostaglandins, predominantly prostaglandin E. The prostaglandins induce smooth muscle contractions in the female genital tract, thereby helping in rapid sperm transport independent of sperm motility. Seminal vesicles are also the major contributor of phospholipids present in semen. The ratio of cholesterol to phospholipids in the semen is proposed to help stabilize the sperm against temperature and environmental shock (White et al. 1976). The other proteins secreted by seminal vesicles include fibronectin, lactoferrin, protein C inhibitor and prolactin-inducible protein (Rodriguez-Martinez et al. 2011; Drabovich et al. 2014).

11.3.2.2 Originating from Prostate

The major proteins secreted by the prostate include prostate-specific antigen (PSA), prostatic acid phosphatase (PAP) and cysteine-rich prostate-specific protein-94 (PSP94). PSA is a zinc-binding serine protease of the Kallikrein family, which hydrolyzes semenogelin leading to liquefaction of the coagulum. PAP is a 102-kDa glycoprotein dimer with enzymatic activity. The main substrate for PAP in seminal fluid is phosphorylcholine phosphate. Prostate also produces spermine which gives semen its unique odour. Spermine has four positive charges and can bind to acidic or negatively charged molecules like phosphate ions, phospholipids or nucleic acids. Enzymatic oxidation of spermine by diamine oxidase, which is present in the

seminal fluid, yields aldehyde products which are toxic to both sperm and bacteria. Hence, prolonged exposure of sperm to seminal fluid reduces their fertilization capability (Folk et al. 1980; Prins and Lindgren 2015).

Concentration of zinc in the normal human seminal fluid is more than 100 times compared to concentration in serum. It is involved in regulating liquefaction by binding to semenogelin. It also has an antibacterial activity. Similar to zinc, the concentration of citrate in the semen is 500–1000 times higher than that in blood. It is a potent binder of metal ions, and its concentration (20 mM) compares to the combined concentration of divalent metals (calcium, 7 mM; magnesium, 4.5 mM; and zinc, 2.1 mM).

11.4 Fructolysis

Due to the high motility of sperm, their energy requirement is very high. The major energy source for sperm in the semen is fructose which is produced by the seminal vesicle. Typical concentration of fructose in human semen is 200 mg/dl. To maintain a high adenosine triphosphate/adenosine diphosphate (ATP/ADP) ratio, the sperm utilize anaerobic glycolysis of fructose termed as fructolysis. The process of fructolysis has been described in the chapter, Seminal Vesicles. Each fructose molecule yields 2 lactate ⁻ ions and 2 hydrogen ions (H^+).

A positive correlation exists between the degree of sperm motility and the rate of fructolysis in human semen (Peterson and Freund 1976). However, immobilization by spermicidal agent (lipid peroxidase) leads to irreversible loss of fructolytic ability of the sperm (Mann et al. 1980).

11.5 Coagulation and Liquefaction

Human semen coagulates spontaneously after ejaculation and subsequently liquefies within 15–60 min at room temperature. Although the exact mechanism underlying the process of semen coagulation/liquefaction is not clearly understood, it is believed to be regulated through a series of enzymes, mainly proteases, inhibitory factors and metal ions (Emami et al. 2008). Components of the semen are stored in separate glands and get mixed only upon ejaculation. The prostatic secretion containing Zn^{+2} and zinc-inhibited PSA are mixed with the seminal vesicle-produced semenogelin proteins and protein C inhibitor (PCI). Since zinc has a higher affinity for semenogelins in comparison to PSA, it preferentially binds to semenogelins after ejaculation. This induces a conformational change of semenogelin leading to formation of an insoluble, fibrous coagulum. Sperm are immobilized in this coagulum. Chelation of zinc ions diminishes the concentration of free Zn^{+2}, thus activating PSA. Activated PSA cleaves the semenogelins resulting in liquefaction of the gel and release of motile sperm (Malm et al. 2007). Zinc and PCI are also released into solution, and these in turn bind to PSA, preventing further undesirable proteolysis. The details of the coagulation and liquefaction are shown schematically in

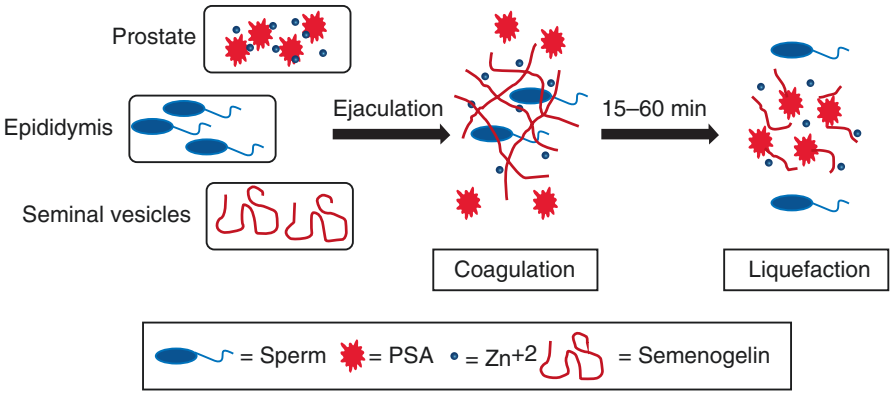

Fig. 11.2 Schematic diagram showing the coagulation and liquefaction process of human semen

Fig. 11.2. The coagulation/liquefaction process allows suitable exposure of sperm to seminal fluid that stimulates motility, increases fertilizing ability and also permits orderly entry of sperm into the female genital tract (Hafez 1976). Absent or incomplete liquefaction process correlates with reduced fertilizing capability (Prins and Lindgren 2015).

11.6 Future Directions

It is important to understand the salient physical and chemical properties of normal human semen in order to formulate a standardized semen simulant. This simulant fluid would be helpful in research related to intravaginal drug delivery for contraceptive and prophylactic drugs (Owen and Katz 2005).

Another area with untapped potential is the use of seminal fluid as a non-invasive clinical sample to identify biomarkers for infertility as well as reproductive tract diseases like prostatitis, cancer, etc. Seminal fluid contains many molecules which are produced by specific male reproductive organs/glands, and, hence, any pathological condition of these organs would influence the molecular composition of semen. Discovery of PSA as a marker of prostatic diseases, both benign prostatic hyperplasia and prostate cancer, is the best example to illustrate this point.

Key Questions

- In what sequence are the various fractions of semen secreted during ejaculation?
- Name the constituents of seminal fluid and discuss their function.
- Describe the key steps in the process of coagulation and liquefaction of human semen.

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