Semen Composition

Semen is composed of spermatozoa and seminal plasma. Its sources are the epididymides and vasa deferentia, which supply the cellular components (spermatozoa), and the accessory glands, which provide most of the fluid portion (seminal plasma). The relative contri-

14–1 SPERMATOZOA

The concentration (no./ml) of spermatozoa in an ejaculate of semen is approximately 150 million for stallions, 200 million for boars, 1.2 billion for bulls, and 2 billion for rams (Table 14–3). Theoretically, 50% of the spermatozoa in a given ejaculate will contain X chromosomes and 50% Y chromosomes, which on a population basis would result in equal numbers of male and female offspring. Approximately 60% to 70% of the spermatozoa in semen are expected to be progressively motile, with an average speed of 6 mm per minute. In high-quality semen, 80% to 90% of the spermatozoa will have normal morphology. Concentration, motility percent, and morphology are all important criteria in the evaluation of semen before use in artificial insemination (Chapter 15). Spermatozoa of bulls have an overall length of 60μ to 70μ. The head is 8μ to 10μ long, with the tail accounting for the remainder. The head is flattened, about 4μ wide and 0.5μ thick. Both boars and rams have sperm of similar size, while sperm of stallions are smaller (about 50μ in length).

14–1.1 *Normal Morphology*

The normal spermatozoon is composed of a head and a tail that is divided into a mid-piece, main-piece, and end-piece (Figure 14–1).

Table 14–3 *Characteristics of semen from farm animals*

	Cattle				
	Dairy	Beef	Sheep	Swine	Horses
Volume (ml)	6	4	1	125*	60*
Sperm concentration (billion/ml)	1.2	1.0	2.0	0.2	0.15
Total sperm (billion)	7	4	3	45	9
Motile sperm (%)	70	65	75	60	70
Morphologically normal sperm (%)	80	80	90	60	70
pH	6.5–7.0	6.5–7.0	5.9–7.3	6.8–7.5	6.2–7.8

*Gel-free portion.

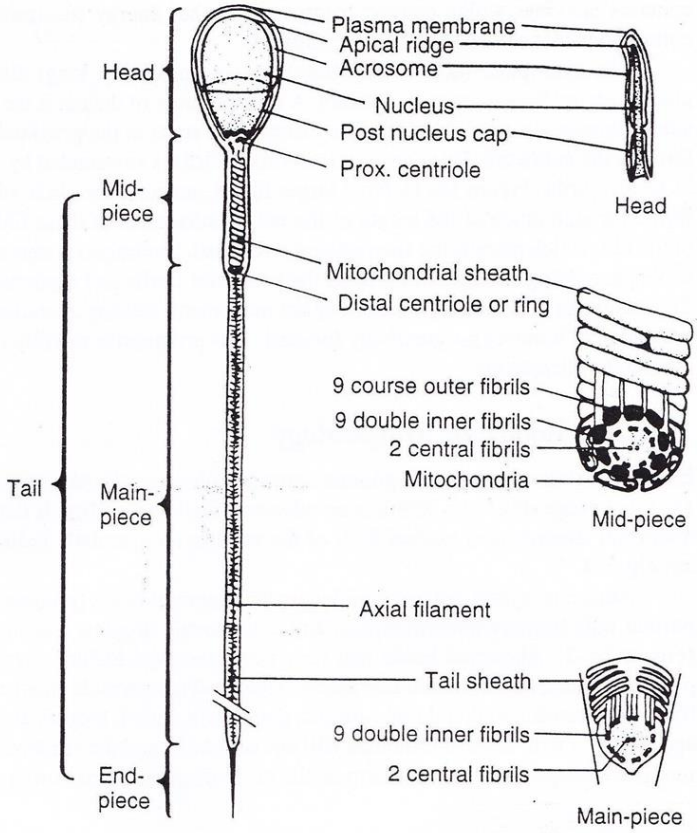

Figure 14–1 Structural diagram of spermatozoon. (Adapted from Wu. 1966. *AI Digest*, 14:7.)

The important components of the head include the *nucleus*, containing the genetic code, which is the sire's contribution to a new offspring, the *postnuclear cap*, covering the posterior portion of the nucleus, and the *acrosome*. The acrosome covers the anterior part of the nucleus and contains enzymes needed for penetration of the corona radiata and zona pellucida during fertilization (Chapter 8). If the acrosome is malformed, damaged, or missing, the spermatozoon will not be able to participate in fertilization. During aging, the acrosome becomes loosened from the nucleus starting at the apical ridge.

The point where the tail joins the head contains the *proximal centriole* and is called the implantation region. The head and tail become separated at this point during fertilization. Similar separation is sometimes seen in heat-damaged semen.

The mid-piece, a thickened portion of the tail some 8μ to 10μ long in bulls, is located just posterior to the proximal centriole. The *mitochondrial sheath*, which forms from the mitochondria of the spermatid, is a part of the mid-piece. The mitochondrial sheath

contains enzymes which convert fructose and other energy substrates into high-energy compounds that can be used by spermatozoa.

The main-piece (40μ to 50μ long) and end-piece (3μ long) differ in that the end-piece does not have a protective sheath. A major feature of the tail is the *axial filament*. The axial filament is a small bundle of tiny fibrils that starts at the proximal centriole and runs through the entire tail. One center pair of small fibrils is surrounded by a circle of nine pair of small fibrils (Figure 14–1). Nine larger fibrils surround the circle of nine pair of small fibrils through much of the length of the tail. Contractions of these fibrils cause a lashing of the tail, which propels the spermatozoon forward. Contractions start at the proximal centriole proceeding sequentially around the perimeter fibrils and rhythmically down the tail. This results in an urn-shaped pattern of tail movement, causing a rotation of the entire spermatozoon as it moves progressively forward. This progressive motility can be observed under a light microscope.

14–1.2 *Abnormal Morphology*

Every ejaculate of semen will contain some morphologically abnormal spermatozoa. The expected range of 8% to 10% has no adverse effect on fertility. If the accumulated total abnormal spermatozoa exceed 25% of the total in an ejaculate, reduced fertility can be anticipated.

Abnormal sperm can be classified under abnormal heads (primary abnormalities), abnormal tails (tertiary abnormalities), and cytoplasmic droplets (secondary abnormalities) (Figure 14–2). Abnormal heads that have been observed include asymmetrical, tapering, pyriform, giant, micro, and double heads. Abnormal tails include enlarged, broken, bent, filiform, truncated, and double mid-pieces, along with coiled, looped, and double tails. Most spermatozoa with tail abnormalities will not be motile, and the remainder exhibit abnormal motility. Cytoplasmic droplets form on the neck of spermatozoa during spermiogenesis. As

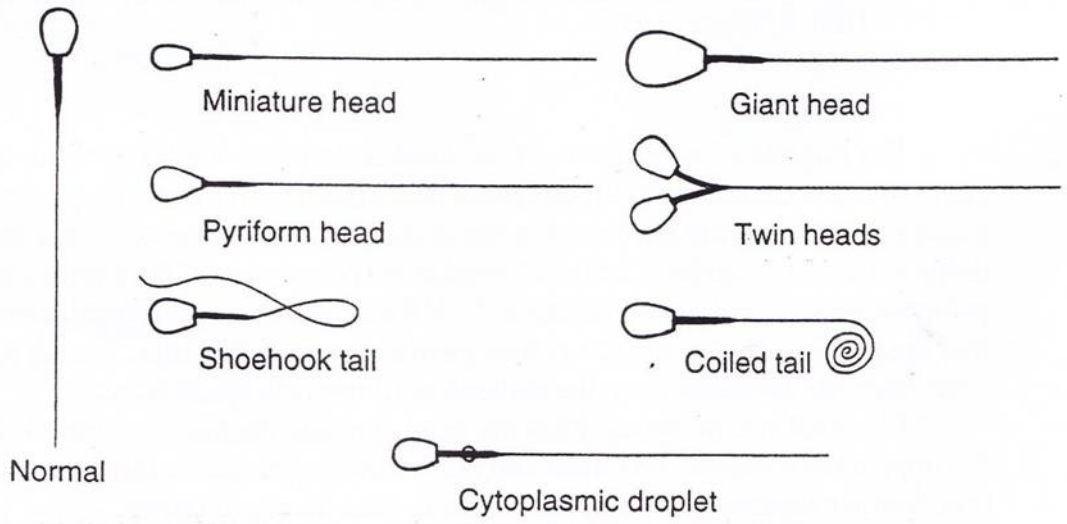

Figure 14–2 Morphological abnormalities of spermatozoa identified through examination of semen for quality.

discussed in Chapter 3, these are usually lost during maturation in the epididymis. If they are still present after spermatozoa are ejaculated, they are considered an abnormality and, as with other abnormalities, too high a percentage will reduce the fertility of the semen. Stress causes an increase in abnormal sperm. Abnormalities of all types increase, but the first to appear and the last to disappear are increases in cytoplasmic droplets. Cytoplasmic droplets on the tail of ejaculated sperm is an indication that the maturation process is not completed.

–2 SEMINAL PLASMA

The fluid portion of semen is seminal plasma. The accessory glands contribute most of this, but a small amount of fluid is a part of the spermatozoa concentrate which comes from the epididymides and vasa deferentia. Seminal plasma serves as a buffered, nutrient medium which suspends and maintains the fertility of spermatozoa. Seminal plasma is slightly acidic in bulls and rams and slightly alkaline in boars and stallions. The osmotic pressure of seminal plasma is similar to blood (equivalent to physiological saline—0.9% sodium chloride). A number of organic and inorganic compounds are in solution in seminal plasma.

14–2.1 *Proteins*

Several proteins that have a relationship to fertility have been found in seminal plasma. The role of glycosaminoglycan (GAG)-binding proteins in capacitation has been discussed previously (Section 6–4). The binding affinity of ejaculated sperm for heparin and other glycosaminoglycans (via binding proteins) corresponds to fertility in bulls. These GAG-binding proteins are found in seminal plasma, as well as on the sperm membrane, with their source being the fluids of the vesicular glands. Studies of protein profiles in seminal plasma have

14–2.2 *Inorganic Ions*

Sodium and chlorine are the principal inorganic ions in seminal plasma. Smaller quantities of calcium and magnesium are found, also. Potassium, which is present in substantial amounts in whole semen, is more concentrated in spermatozoa than in the fluid suspending the spermatozoa. Thus, when spermatozoa are concentrated, as in the epididymis, the potassium-to-sodium ratio is higher. These inorganic ions are important to the viability of spermatozoa, possibly through their effect on the integrity of the sperm cell membrane.

Along with the organic molecules in solution in seminal plasma, the inorganic ions help maintain an osmotic pressure that is optimum for the survival of spermatozoa.

14–2.3 *Buffering Agents*

In addition to inorganic ions, organic ions that serve as buffering agents are found in seminal plasma. The principal organic ion is bicarbonate. It is produced by the vesicular glands and functions as a buffering agent, guarding against changes in the pH of semen. Buffers are not found in sufficient quantities to prevent a reduction in pH when semen is maintained in storage. Therefore, good semen diluters must be used to provide sufficient buffering capacity for long-term storage (Chapter 16).

14–2.4 *Energy Substrates*

Several organic compounds that serve primarily as energy substrates for spermatozoa are found in seminal plasma. The principal ones are fructose, sorbitol, and glycerylphosphorylcholine (GPC). Fructose (a simple sugar) and sorbitol (a sugar alcohol) are produced by the vesicular glands, whereas GPC is produced in the epididymides. All are unique in that they are not found in substantial quantities elsewhere in the body.

Fructose can be used by spermatozoa as an energy substrate under the anaerobic (oxygenless) conditions of storage and the aerobic (oxygenated) conditions found in the female tract. Sorbitol and GPC can be utilized only aerobically. In addition, GPC must be acted on by an enzyme found in the female tract before it can be utilized. This enzyme splits the choline from the rest of the molecule, forming glycerylphosphate, which can be metabolized as an energy substrate. Lactic acid, a by-product of the anaerobic metabolism of fructose (Section 14–3), builds up in semen that is being stored and theoretically can be used as an energy substrate when placed in aerobic conditions.

Fructose is found in high concentrations in bull and ram semen but is much lower in both boar and stallion semen. The low concentration of fructose in boar and stallion semen may contribute to the problems of storing semen from these species.

14–2.5 *Other Organic Compounds*

Compounds found in seminal plasma in rather large concentrations but not used as energy substrates are inositol and citric acid. Both are produced by the accessory glands. Ergothionine is found in the semen of boars and stallions. These compounds are not found in substantial amounts elsewhere in the body.

14–3 Energy Metabolism by Spermatozoa

Energy metabolism is the means by which spermatozoa convert energy substrates into usable forms of energy. Enzymes for this conversion are in the mitochondrial sheath. In addition to fructose, sorbitol, and GPC, which are present in seminal plasma, plasmalogen, a lipid found within the spermatozoon is an energy reserve that can be used when other substrates are limiting.

Adenosine triphosphate (ATP), a high-energy compound, is the form of energy that can be used by spermatozoa. It is converted to ADP yielding 7,000 calories per mole of energy by the following reaction:

$$'ATP + H_2O \leftrightarrows ADP + H_3PO_4 + 7{,}000 \text{ calories/mole}$$

If there were no means of regenerating ATP, the spermatozoa would not survive due to lack of energy. Energy substrates provide a means by which ATP can be regenerated from ADP plus inorganic phosphorus. Fructose serves as a good example, since it can be utilized anaerobically and aerobically. The anaerobic reaction is as follows:

$$\text{Fructose} \xleftrightarrows{\text{No } O_2} 2 \text{ lactic acid} + 2 \text{ ATP (net yield)}$$

Fructose metabolized anaerobically yields a net of 2 ATP, or 14,000 calories. This reaction provides energy to maintain the viability of spermatozoa during storage. However, an end product of this metabolism is lactic acid. If steps are not taken to slow metabolism during storage, the buildup of lactic acid will soon lower the pH of the semen, adversely affecting the viability of spermatozoa.

Under aerobic conditions, the metabolism of fructose is

$$\text{Fructose} \xleftrightarrows{O_2} CO_2 + H_2O + 38 \text{ ATP (net yield)}$$

When oxygen is present, metabolism of fructose is 19 times more efficient in terms of energy yielded. The net energy from 38 ATP is 266,000 calories. When sufficient oxygen is present, the fructose molecule is metabolized completely to carbon dioxide and water. There is no buildup of lactic acid. In addition, sorbitol, plasmalogen, and, if in the female tract, GPC are available for metabolism and regeneration of ATP. Sorbitol and GPC are metabolized through the same biochemical pathways as fructose. Plasmalogen, a lipid rather than a carbohydrate, utilizes different metabolic pathways, but the needed enzymes are in the mitochondrial sheath.

14–4 Factors Affecting Rate of Metabolism

Rate of metabolism is the rate at which spermatozoa utilize their energy substrates. Under aerobic conditions, it can be monitored by measuring oxygen consumption, by measuring liberated carbon dioxide, or by methylene blue reduction. Under anaerobic conditions, the rate of reduction of pH or chemical determination of lactic acid buildup and/or fructose disappearance can be used as measures of metabolic rate. Control of metabolic rate is of interest because a reduction in metabolic rate is necessary to extend the storage life of semen. A number of factors contribute to reduced metabolic rate and extended life of spermatozoa in the epididymides (Chapter 3). In the epididymides, spermatozoa may remain fertile for up to 60 days. However, spermatozoa in a fresh ejaculate of semen will be fertile only for a few hours if steps are not taken to reduce their metabolic rate. The measures used must be reversible without injury to spermatozoa if they are to be practical for semen handling.

14–4.1 *Temperature*

Metabolic rate increases and the life span of spermatozoa decreases as the temperature of the semen rises. When the temperature rises above 50°C, spermatozoa suffer an irreversible loss of motility. If maintained at body temperature, spermatozoa will live for only a few hours due to exhaustion of available energy substrates, drop in pH due to buildup of lactic acid, or a combination of these factors. Reducing the temperature of the semen will slow metabolic rate and extend the fertile life of semen if precautions are taken to protect against *cold shock* and *freeze kill.*

14–4.2 *pH*

A pH of about 7.0 (6.9 to 7.5 for different species) falls in the optimum activity range of most of the enzymes in spermatozoa. Therefore, a higher metabolic rate is expected when the pH of semen is maintained near neutrality (7.0). If the pH of semen deviates toward alkalinity or acidity, metabolic rate will be reduced. The practicability of altering the pH of semen to extend its life is limited by the narrow range over which pH can be altered without permanently reducing activity. Research in this area has established the importance of diluting semen in a buffered medium that resists changes in pH, so that maximum fertile life of the semen can be maintained.

14–4.3 *Osmotic Pressure*

Semen maintains maximum metabolic activity when diluted with an isotonic diluter. Either hypotonic or hypertonic diluters will reduce metabolic rate, but neither will extend the life of the semen. The spermatozoon membrane is a semipermeable membrane. Both hypotonic and hypertonic diluters will alter transfer of water through this membrane, disrupting the integrity of the cell. It is very important that only isotonic diluters be used. Spermatozoa remain motile longest when suspended in isotonic media.

14–4.4 *Concentration of Spermatozoa*

Increasing the concentration of spermatozoa above that found in the normal ejaculate will decrease metabolic rate. Potassium is the principal cation in the sperm cell, whereas sodium is the major cation in seminal plasma. Increasing the cellular concentration will increase the potassium-to-sodium ratio in the semen. Potassium is a natural metabolic inhibitor. Increasing its concentration will reduce the metabolic activity in the semen.

Generally, moderate dilution of semen in a buffered, isotonic medium containing fructose will not greatly alter metabolic rate but will extend the life of the semen. Dilution such as this is usually done before lowering the temperature of semen. Some caution must be observed. If dilution is excessive (> 1 to 1,000), motility and metabolic rate will be depressed.

14–4.5 *Hormones*

Testosterone and other androgens depress metabolic rate, but those concentrations found in the male system have no permanent effect. Fluids from the female tract increase the metabolic activity of spermatozoa. This is thought to be primarily an effect from estrogens, but other unidentified factors may be involved. The increased metabolic activity in the female tract likely increases motility, which increases the frequency of collisions between spermatozoa and the oocyte in the oviduct.

14–4.6 *Gases*

Low concentrations of carbon dioxide stimulate aerobic metabolism of spermatozoa. If the partial pressure of carbon dioxide exceeds 5% to 10%, metabolic rate is depressed. Carbon dioxide has been identified as a factor in regulating metabolic rate in the epididymides. Oxygen is necessary for aerobic metabolism. On the other hand, too high a level of oxygen is toxic and will depress metabolic rate. This is not likely to be a factor in the laboratory unless oxygen or air is being bubbled through the semen. Anaerobic metabolism can proceed under nitrogen, hydrogen, or helium gases with no effect on metabolic rate.

14–4.7 *Light*

Light intensities that are normally found in the laboratory can depress metabolic rate, motility, and fertility in spermatozoa. The harmful effect is observed only if semen is in contact with oxygen. The enzyme catalase will prevent the harmful effect of light, which suggests that light causes a photochemical reaction in the semen that results in the production of hydrogen peroxide. Semen should be protected from light and never exposed to direct sunlight.

14–4.8 *Antibacterial Agents*

Gentamicin, Tylosin, and Linco-Spectin (Section 16–3) are added to semen during processing to control bacterial growth. None have a demonstrated effect on metabolic rate. They sometimes increase fertility of semen from low fertility bulls. Also, these antibacterial agents may extend the fertile life of the semen by controlling bacteria, thus sparing energy substrates for spermatozoa.