**Semen and its components**

**Semen is composed of spermatozoa and seminal plasma**. Its sources are the epididymis and vas deferens, which supply the cellular components (spermatozoa), and the accessory glands, which provide most of the fluid portion (seminal plasma). In terms of total volume, the contribution of the epididymis and vas deferens is relatively small. **In bulls, the greatest contribution to the fluid volume of semen is from the vesicular glands, with minor contributions from the prostate gland and bulbourethral glands. In boars, there are greater contributions from the prostate and bulbourethral glands with a smaller proportion from the vesicular glands.** These differences are reflected in the chemical composition of semen. **Bull semen is higher in fructose and sorbitol**, **which comes from the vesicular glands**, **whereas boar semen is higher in most minerals, the major source of these being the prostate gland.**

**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.** 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.

**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**. **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 (acrosin and hyaluronidase) needed for penetration of the corona radiate and zona pellucid during fertilization**. **If the acrosome is malformed, damaged, or missing, the spermatozoon will not be able to participate in fertilization. During aging, the acrosome becomes lossened 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.

The mid piece, a thickened portion of the tail is located just posterior to the proximal centriole. The mitochondria 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 and end piece differ in that the end piece does not have a protect 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. Nine larger fibrils surrounded 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.



**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 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 ).**

**Abnormal heads** that have been observed **include asymmetrical, tapering, pyriform, gaint, micro, and double heads.**

**Abnormal tails** **include enlarged, broken, bent, filifrom, 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 from on the neck of spermatozoa during spermiogensis. These are usually lost during maturation in the epididmis, if they are still present when 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 and the last to disappear are increase in cytoplsmic droplets.



**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 epididmis and vas deferens**. Seminal plasma serves as a buffered, nutrient medium which suspends and maintains the fertility of spermatozoa. Seminal plasma is **slightly acidic in bull 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 plasmas.

**Inorganic ions**

**Sodium and chloride are the principle inorganic ions in seminal plasma. Smaller quantities of calcium and magnesium are found also. Potassium, which is present in substantial amounts in whole semen, 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**. **The 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**.

**Buffering agents**

In addition to inorganic ions, organic ions are found in seminal plasma. **The principle 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.**

**Energy substrates**

Several organic compounds which serve primarily as energy substrates for spermatozoa are found in seminal plasma. **The principal ones are fructose, sorbitol, and glycerylphosphorylchloride (GPC)**. **Fructose (a simple sugar) and sorbitol ( a sugar alcohol) are produced by the vesicular glands, whereas GPC is produced in the epididymis**. 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, 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.**

**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. Ergthionine is in the semen of boars and stallions**. These compounds are not found in substantial amounts elsewhere in the body.

**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 spermatozoa. ATP is converted to ADP yielding 7,000 calories per mole of energy by the following reaction:**

**ATP + H2O ↔ ADP +H3PO4 + 7, 000 calories / ml**

If there were 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:**

**No O2**

**Fructose ↔ 2 lactic acid + 2 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 will soon lower the PH of the semen, adversely affecting the viability of spermatozoa.

**Under aerobic conditions, the metabolism of fructose is:**

**Fructose ↔ CO2 ± H2O + 38 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.

**Factors affecting rate of metabolism**

**Rate of metabolism is the rate at which spermatozoa utilze their energy substrates under aerobic conditions**, it can be monitored by measuring **oxygen consumption, 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 measure 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 epididymis.

**In epididymis, 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 measure used must be reversible without injury to spermatozoa if they are to be practical for semen handling.

**Temperature**

 **Metabolic rate increase and the life span of spermatozoa decrease 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 either exhaustion of available energy substrates, drop in PH due to buildup of lactic acid, or 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.**

 Spermatozoa of all species studied are susceptible to cold shock if they are cooled too quickly. The most obvious indication of cold shock is an irreversible loss of motility. **The most critical range for cold shock protection is provided for bull, ram, and stallion semen by cooling slowly after addition of an egg yolk or milk diluter. Both egg yolk and milk contain lecithin and lipoprotein, which give protection against cold shock.**

**The second problem in reducing metabolic rate to extend the fertile life of semen is freeze kill. Spermatozoa may be killed during the freezing and or thawing process, apparently by a disruption of the sperm cell membrane.** **Equilibration of bull semen in a diluter containing glycerol will give adequate protection**. Some freeze kill will still occur, but fertility will not be affected if sufficient motile spermatozoa are placed into the semen unit before freezing. While there has been less success in freezing ram, buck, and stallion semen, glycerol is beneficial when freezing semen from these species. **Excess glycerol is detrimental to boar and ram spermatozoa during freezing**.

 **Reducing the temperature of semen to lower its metabolic rate has been the most useful means of extending the fertile life of semen because it permits restoration of metabolic rate before insemination**. Also, greater reduction of metabolic rate can be achieved without reducing fertility. **When bull semen is frozen in liquid nitrogen at – 196 C, its metabolic activity is reduced to less than 0.02% of the metabolic rate at body temperature. Further, fertility can be maintained in the semen of decades.**

**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.

**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 spermatozoa membrane is a semipermeable membrance. **Both hypotonic and hypertonic diluters will alter transfer of water through this membrance, 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.**

**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.

**Hormones**

**Testosterone and other androgen 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 increase motility, which increase the frequency of collisions between spermatozoa and the oocyte in the oviduct.**

**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 epididymis.** **Oxygen is necessary for aerobic 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.

**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 with 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 sun light.**

**Antibacterial agents**

**Gentamicin, tylosin, and lincospectin 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.

**A lecture prepared by Dr. Jawad kadhim**