Semen Storage

The method of preserving semen in a liquid form allows an intermediate storage of a few days only while the deep-frozen form permits storage for years without any significant decrease in semen quality. According to the length of storage planned, different semen extenders have been developed.

Methods of semen storage:

1- At **ambient temperature:** in this methods semen can be store for 2-3 days at 25 C by **provide CO2**, **energy**, **control ph changes** and **inhibit bacterial growth.**

The extender used for this method must be have energy substrate (fructose, glucose),buffering agent (citrate, Tris, phosphate), antibiotics.

Some extenders used in storage semen in ambient temperature:

Illini variable temperature extender (IVT), Cornell university extender (CUE), Caprogen extender.

2- **Cold or liquid semen(refrigerator temperature)**. In this method semen can be stored for 3-5 days by **decrease the metabolic rate of sperm** after stored it at 4 C . Sperm motility must be evaluated before used semen.

The extender used for this method must be have **energy** substrate (fructose, glucose),**buffering** agent(citrate, Tris, phosphate), **antibiotics** and **substrate that protect the sperm from the cold shock** (lipoprotein in egg yolk or milk).

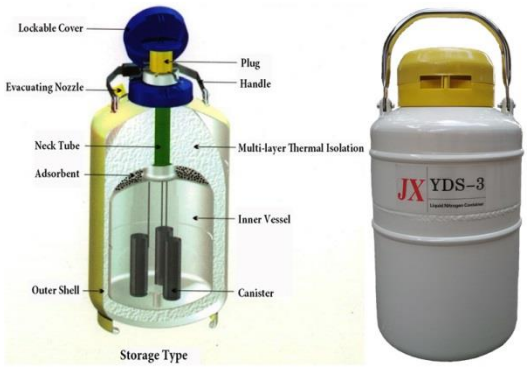
Some extenders used in storage semen in refrigerator temperature:

Tris-egg yolk, citrate-egg yolk, phosphate-egg yolk, homogenized milk extender.

3- **Frozen semen**: In this method semen can be stored by **inhibit sperm metabolism** through two ways:

a- **Freezing at -79 C** by using solid carbon dioxide at –79°C (dry ice), drops of diluted semen were placed directly on to the surface of a block of solid carbon dioxide where they froze in pellet form. Long-term storage at –79°C was not satisfactory, however, as deterioration occurred at that temperature Semen can be stored until 6 months.

b- **Freezing at -196 C in liquid nitrogen**: Storage in liquid nitrogen at –196°C has subsequently become established as the standard medium for long-term preservation of semen and, over the 40 years for which it has been practiced, has maintained sperm fertility unscathed. For this method we need a Double wall stainless steel or aluminum container with a vacuum between the walls.



Sperm motility and integrity after freezing and thawing it will be decreased there for the motility sperm concentration must be increased before freezing to insure that the inseminating unit have the adequate number of sperm to insure the fertilization take place. For example if the inseminating needs 10^6 motile sperm the inseminating unit must be have $15*1o^6$ motile sperm before freezing.
The extender used for this method characterized by same properties of liquid semen extenders except in the freezing the extender must have the substance to protect the sperm from the effect of freezing such as glycerol.
Some extender used in storage semen in freezing temperature:

Tris-egg yolk, citrate-egg yolk, phosphate-egg yolk, homogenized milk extender.

Packaging of semen:

There are many types of packaging semen which include:

1- Ampoule

Glass ampules with 0.5-ml, 0.8-ml, and 1.0-ml capacities were used almost exclusively for packaging from the onset of frozen semen until about 1970. The ampules were filled through a small opening and heat was used to melt the glass to form a seal

2- Pellets

Semen containing about 10 times the usual number of motile sperm is placed in the depression to freeze. The usual volume is about 0. I ml. After freezing, the pellets are transferred directly to liquid nitrogen for storage. For insemination, the pellets are thawed in enough warm diluter to provide adequate volume for insemination.
Two major disadvantages of the pellet are difficult identification of the individual pellet and microbial contamination incurred during handling. There is also some possibility of mixing sperm from different bulls from forceps used to handle the pellets. Difficult to handle and transport, dry ice must be replenished frequently.

3- straw

The 0.5-ml plastic straw has been the package of preference since about 1970. Plastic straws with 0.25-ml and 0.5 ml capacities are used One end of the straw contains a three-part plug. A small amount of **polyvinyl alcohol powder** is placed between two small cotton plugs. The straws are filled by applying a vacuum to the end with the plugs. The powder allows air to pass through as long as it remains dry, but when aqueous material (the semen) comes in contact with the powder it forms a seal which will not allow liquid or air to pass through. The opposite end of the straw is sealed ultrasonically after filling.

The standard procedure for freezing is to place a single layer of straws on a tray. The tray is placed about 5.5 cm above the liquid nitrogen level of a large storage unit. The cold nitrogen vapors in this area will freeze the semen at about the desired rate. Straws will reach the vapor temperature in about 2 minutes.

One major advantage of the straw over the ampule is the conservation of storage space. Up to three times as many straws can be stored in a field or storage unit as ampules. Most studies indicate that the straw offers the added advantage of increased sperm survival and a slight increase in conception rate over the ampule.