Semen dilution:

Sperms in normal ejaculate are very active, and this activity of sperm cells exhaust the energy sources and enzymes that found in seminal plasma which leading to death of these sperms after a short time of ejaculation. So in order to use the semen in artificial insemination programs, it must be diluted by proper diluents that can be preserve the sperms lifespan for a longer period and to protect sperms from cold shock during storage.

benefits of diluents:

1- increases the ejaculate volume

2- prolongation the lifespan of sperm cells because it contains nutrients.

3- contain substances that maintains the media PH.

4- contain substances that preserve the media osmotic pressure.

5- contain substances that protects the sperms from cold shock

6- contain substances that prevents ice crystals formation

7- diluents contain antibiotics, so it can controls bacterial pollution.

Properties of good semen diluter:

1- isotonic: a diluter must be isotonic with semen.

2- buffering capacity: must be added to diluters in order to prevent PH changes.

3- protection from cold shock during cooling process.

4- energy source: such as some sugars (fructose, glucose).

5- antibiotics.

6- cryoprotectants: such as glycerol

7- cheap and easy to prepare.

Semen processing: this term means all operations that deals with semen in artificial insemination centers -which include; dilution, cooling, glycerolization, equilibration, packaging, storage and thawing.

●Dilution: the collected semen should be placed with the prepared diluter at the same water bath at 30-35°C.

●Cooling: is the process when the semen is slowly cooled from body temperature 37°C to 5°C.

●Glycerolisation: means addition of glycerol to diluted semen.

●Equilibration: the glycerolized diluted semen is left at 5°C for 2-4 hours which called (equilibration period).

●Packaging: the diluted glycerolized semen can be packed before freezing in many packages.

●Thawing: ampoules and straws thawed in a water bath 35-38°C for 15-30 seconds.

Preservation of semen (semen storage): the idea of preserving sperms depends on inhibition of sperms metabolic activity. For artificial insemination, the semen can be preserved in three ways:

I- at 20-37°C.

II- in refrigerator at 4-5°C.

III- cryopreservation means storage of semen under freezing degree which can be done by dry ice -79°C, by liquid air -183°C and by liquid nitrogen -196°C.

Buffer solutions used in semen diluters

A. To prevent changes in pH of semen by neutralizing lactic acid produced by metabolic activity of sperm

B. To provide isotonic environment for sperm

C. To give no toxicity to sperm at the level required for isotonicity

1- Phosphate buffer solution

Preparation: $Na_2HPO_4.12H_2O$: 2.0 g and KH_2PO_4 : 0.2 g

2- Citrate buffer solution

Preparation:$Na_3C_6H_5O_7.2H_2O$: 2.9 g

3- Tris Buffer solution Prepartion:

Tris (hydroxymethyl) aminomethane : 3.028 g = to be 0.2 mol., 10% citric acid : add to have pH 6.5 and Fructose : 1.0 g.

Antimicrobial agents for semen diluters :

Antibiotics used: Penicillin : 1000 IU/ ml , Streptomycin: 1000 mg/ ml.

Nutrient media

1- Yolk-phosphate

Phosphate buffer: egg yolk = 1: 1

2- Yolk-Citrate

A. For liquid semen: citrate buffer: egg yolk = 1: 1

B. For frozen semen: citrate buffer: egg yolk = 4: 1

2- Tris buffered-Yolk

3- Tris buffer: egg yolk = 4: 1

4- Whole homogenized milk and skim milk

A. Milk heated to normal pasteurization temperature contains spermicidal material **LACTENIN**

B. Heating milk to 90 - 9°C for 10 min. inactivates lactenin

5 Other diluters:

A. Addition of simple sugars, amino acids and/or enzymes

B. Various concentrations of egg yolk.