**Department of Animal Resources**

**Reproductive Physiology and Artificial Insemination (Practical 5) Third Class**

**Dilution and cooling semen for artificial insemination**

must meet the conditions appropriate for the dilute and the most important of these conditions: -

1 - Do not have a detrimental effect on sperm.   
 2 - The Osmosis pressure is equal to the pressure of the blood Osmosis.  
3 -Has a degree (PH )appropriate for the continued activity of sperm.  
4 - Has a viscosity appropriate for Semen plasma.  
5 - His ability to organize the constant changes in the degree of (PH) resulting from the activity of sperm.  
6 - keeps the activity of the sperm for a long time without harm in fertility degree.   
7 - low-cost and easy preparation.  
8 - To keep the sperm from the impact of the cold thermal shock

**Effective Diluter for semen**

1 - Solution of sodium citrate with egg yolk.  
This dilute solution consists of the following: -   
2.9 g of sodium citrate or 3.6 grams of sodium citrate water Na3C6H507.5.5H2O in 100 **cm3** of distilled water and add the egg yolks by 1:3 diluted solution are added as the normal amount of antibiotics.

And egg yolk many Interest, including:   
A - has a protective effect with thermal shock of low grades Cold shock during storage. and that this effect is due to the lecithin and Lipoprotein, which are based on keep the quality and strength of unity of the cover of sperm.  
B - Contains materials to boost work on the metabolism of sperm whereas sperm can take advantage of the sugars found in egg yolks, especially glucose.  
C - Play a major say in the process of sperms storage as it protect from the impact of detrimental materials resulting from the decomposition of some types of amino acids in semen.

2 - solution with milk, egg yolks: I have used many of the countries Dilutes milk as an alternative to citrate with egg yolk and egg yolk by 20-25% is added to milk, it should be noted the following points when using the milk:-  
A - not to prolong the period of heating (5) minutes and the temperature of 95-97 °C.  
B - to be a source of sure milk.  
C - Do not use milk is not sterilized.  
There are also many merchant Dilutes such as SPV.161, Sperm sol, Seminal, Laiciphos 231.

**Conventional method for deep freezing of semen**

1. Maintain extended semen and glycerolated diluting fluid at 4°C for about 4 hours.
2. After 4hours of refrigeration add glycerolated dilutor to extended semen in three parts:
   1. Add 20 percent of the glycerolated dilutor at zero hour.
   2. Add 30 percent at 15 minutes.
   3. Add 50 percent at 30 minutes.
3. The final glycerolated semen, which contains 7.5 percent glycerol is maintained at 5°C for 12 at 18 hours for equilibration.
4. Immediately after equilibration semen is packed in 0.5 ml in straws or ampoules .It is important in filling to leave a small open portion of the straw for sealing and expansion.
5. Straws may be sealed with small plastic plugs or polyvinyl powder. Automatic sealers are nowadays available, ampoules are flame sealed.
6. Place the sealed ampoules in an ethyl alcohol or acetone bath.
7. Freeze semen in ampoules at the rate of -1 to 2°C per minute for +5 to -15°C by adding solid carbon dioxide gradually.
8. When temperature has fallen down to -15°C the rate of freezing is increased -4 to -5°C per minute up to -45°C.
9. Semen is then rapidly frozen to -79°C.The total time taken in deep freezing is approximately 40 minutes.
10. Ampoules of frozen semen are maintained in dry ice chests for longer storage at -79°C and in liquid nitrogen at -196°C.

**Methods used in calculating of dilution ratio**

1- The multiply individual with the movement of the sperm and concentration of ejaculation divided on100 multiply the concentration of sperm in inseminated.  
                      The value of the movement of individual sperm × sperm concentration of the ejaculation   
Dilution ratio = ----------------------------------------------------------------------------  
                      100 × concentration of sperm in inseminated   
  
Example / if ejaculation size = 8 **cm3**, the value of individual movement = 80, concentration of sperm per ejaculation = 1200 million sperm, sperm concentration for the inseminated of one = 25 million sperm.  
                           80 × 1200 million  
Dilution ratio = ----------------------- = 38.4:1   
                         100 × 25 million   
   
And the percentage of Dilution of the final ejaculation = 8 × 38.4 = 307.2 **cm3**

2 - **The number of inseminations that can be obtained from the semen one ejaculation.**  
                        Ejaculation volume × sperm concentration × Ratio of movement × ratio of sperms normal   
The number of inseminations = ----------------------------------------------- ---------------------  
                          Number of normal sperms that must be met in insemination   
   
Example / in the examination of the semen volume was ejaculation = 6 ml, sperms concentration = 800 million sperm, the ratio of individual movement = 85%, the ratio of sperms normal = 95%, note all insemination should contain 20 million sperm.  
  
                       6 × 800 million sperm × 0.85 × 0.95  
The number of inseminations = ---------------------------------------------- = 193.8  
                                     20 million sperm  
  
= semen size diluter - the size of ejaculated semen Volume of diluter   
= 193.8 - 6 = 185.8 ml Volume of diluter

The ratio of egg yolk to the sodium citrate 4:6

Volume of diluter × 4

The Volume of egg yolk = -------------------------------

                                     10

                               185.8 × 4  
The Volume of egg yolk = ------------------ = 74.3 ml  
                                     10

Volume of diluter × 6

The Volume of sodium citrate = ----------------------------

10

185.8 × 6

The Volume of sodium citrate = ---------------------------- = 111.48 ml

10

Or The Volume of sodium citrate = Volume of diluter - Volume of egg yolk

The Volume of sodium citrate = 185.8 -74.3 = 111.48 ml

The amount of antibiotic per ml of a solution diluter = 1000 IU of penicillin.

The amount of antibiotics = 185.8 × 1000 = 185800 IU of penicillin

Example (1): in the examination of the semen of bulls was the volume of ejaculate seminal (10) ml, concentration of sperm (900) million sperm, the ratio of the movement of individual (75%), the ratio of sperm normal (90%) with the knowledge that all inseminated contains (20) million sperm. calculate all of the following: -  
1 - the number of inseminations that can be obtained from the ejaculate seminal?  
2 - volume diluter used in dilution?  
3 - volume of the egg yolk and sodium citrate?   
 4 - the amount of antibiotics?

Example (2): in the examination of the semen was sperm concentration of ejaculation and one (800) million sperm, the ratio of the movement of individual (90%), the ratio of sperm normal (90%) concentration of sperm for inseminated one (20) million sperm. Calculate the ratio of diluter that we need to diluted the ejaculate?