Dilution of semen

- The ejaculates of most domestic animals contain more sperm than are needed for achieving a pregnancy. Hence, by diluting the semen, it can potentially be used for several inseminations. In species such as the dog and the horse, the whole sperm-rich fraction of the ejaculate is diluted and chilled, then used either for sequential inseminations of the same female over her extended oestrous period or after various determinations of the fertile period. In either case, the maximum degree of dilution is determined from the **minimum number of spermatozoa and the volume of inseminate that is required to achieve acceptable pregnancy rates.**

- These factors are themselves determined by the site of insemination, the survival of sperm in diluent and the characteristics of individual species and sires. In general, where an intrauterine insemination can be achieved, the minimum numbers of sperm are one or two orders of magnitude lower than for an intracervical insemination, which is itself one or two orders of magnitude lower than for an intravaginal insemination. The major properties of semen diluents are:

1- Addition of volume

- Insemination doses must be prepared in a volume that is a compromise between ease of handling and an appropriate volume for the site of insemination. Thus, for ovine intracervical inseminations, minimizing volume is important to reduce retrograde loss from the cervix.

- Diluents exhibits an initial increase in motility, which is then rapidly followed by a loss of motility. This phenomenon, known as the 'dilution effect', represents a loss of cell viability, probably through leaching of structural components of the cell membrane. Although it was of great concern among the early practitioners of AI, the use of diluents containing macromolecules such as proteins or polyvinyl alcohol was found to abrogate the dilution effect.

2- Buffering

- Spermatozoa have a narrow range of tolerance to changes in pH, so provision of buffering capacity is necessary. Buffering is especially important where the semen is only to be chilled and not cryopreserved, as the metabolic activity of cooled spermatozoa remains appreciable. Simple buffers are effective, with citrate being widely used. Phosphate-buffered saline is rather less suitable, as it predisposes to head-to head agglutination of sperm. More recently, organic buffers have been used, Tris (hydroxymethyl aminomethane) is probably the most widely employed of such buffers. The proteins contained in skimmed milk products also provide considerable buffering capacity to diluents.

3- Maintenance of osmotic pressure

- Sperm can tolerate a moderate range of tonicity. Some debate has centered on whether sperm respond better to a slightly hyperosmotic or isosmotic diluent, with the former being generally favored. Apart from the osmotic activity of the ionic component of diluents, a substantial contribution is made by **proteins** and, particularly, by **sugars**, which are added to provide nutrition for the sperm or to contribute to the cryoprotective properties of the diluent.

4- Provision of an energy substrate

- Most diluents make some provision of energy substrates for sperm. In general, simple sugars such as glucose, fructose, mannose and arabinose are suitable substrates, although the rate at which these sugars are metabolized varies substantially between species.
- Lactose, which is present in milk-based diluents, is not metabolizable to any appreciable extent. However, egg yolk also a component of many diluents, provides many substrates for sperm metabolism. The provision of energy is relatively less important where sperm are to be frozen, for they will only remain active for a few hours at most before freezing suspends metabolic activity. However, if semen is to be used

chilled, when sperm metabolism has to be sustained for several days, provision of energy is important.

5- Antimicrobial activity

- Antibiotics are added to most semen diluents as a prophylactic measure against the transmission of pathogenic bacteria and to reduce the load of nonpathogenic organisms that contaminate the semen. In cattle Al, benzylpenicillin and streptomycin are the most widely used antibiotics for these are efficacious against C. *fetus*. Most other antibiotics either fail to control this organism or are directly detrimental to sperm. Concern over the potential transmission of *Mycoplasma* and *Ureaplnsma* species in bovine semen has led to the incorporation of lincomycin and spectinomycin into semen diluents in an effort to control these organisms. There is evidence that the efficiency of antibiotics may be reduced in the presence of some components of diluents, notably egg yolk.

Effective diluters for semen:

1- Tris Buffer Solution. 2- Milk diluent.

3- Citrate Buffer Solution. 4- Cornell University Extender (CUE).

Constituent (litre)	*Skimmed milk*	*Egg yolk-citrate*	*Reading diluent*	*Egg yolk-Tris*	*CUE extender*
Egg yolk	100 ml	200 ml	200 ml	200 ml	200 ml
UHT skimmed milk	870 ml				
Fructose	12.5 g				
Lactose			82.8		
2.9% sodium citrate buffer[a]		770 ml			
Tris buffer[b]				800 ml	
Citrate-HCO³ buffer[c]					753 ml
Glycerol			47 ml		47 ml
Stage 1	30 ml	30 ml		Nil	
Stage 2	110 ml	110 ml		140 ml	
Antibiotics	Typically, 1000 IU penicillin + 1000 μg streptomycin/ml				

[a] Trisodium citrate dihydride
[b] 30.28 g Tris, 17.30 g citric acid monohydrate/litre
[c] 14.5 g trisodium citrate dihydride, 2.1 g $NaHCO_3$, 0.4 g KCl, 3.0 g glucose, 9.4 g glycine, 0.9 g citric acid, 185 g raffinose/litre
Stage 1 diluents are added at 30–37°C, after which the semen is cooled to 4°C; stage 2 diluents are added at 4°C. Single-stage diluents are added at 30–37°C, after which the semen is cooled to 4°C