Saline Solution For I.I.

Simple Saline Solution- 100 ml.

100 ml. distilled water
1.0 g. sodium chloride
0.25 g. dihydrostreptomycin or gentamicin

Tris-Buffer Diluent- 100 ml.
(Harbo & Williams 87)

100 ml. distilled water
1.11 g. sodium chloride
0.10 g. glucose
0.01 g. L - lysine
0.01 g. L - arginine
0.01 g. L - glutamic acid
0.329 g. Trizma HCL
0.329 g. Trizma base
0.25 g dihydrostreptomycin or gentamicin

Water Source

The water source used to make up solutions is important. Use double distilled water. When mixing or storing semen it is best to use HPLC (high pressure liquid chromotography) water.

Ph

The pH should be adjusted to 8.6. If the pH is too high use dilute HCL to adjust. If the pH is too low use NaOH to adjust.

To Sterilize

Bacteriological filtering is recommended. Use filter with a pore size of 0.2 um. Solutions can also be sterilized by heating to 350 F for 30 minutes. Note that heating will denature the antibiotic, add this after the solution has cooled.

1.1. Saline Diluent Formulas

Two saline diluent formulas are recommended. The simple formula is for insemination with fresh collected semen. The second formula is for storage and mixing of semen (Hopkins et al, 2012).

- Use double distilled water to make all solutions.
- To sterilize, use bacteriological filtering (pore size 0.2μm).

    - Solutions can also be heat sterilized, 350ºF for 30 minutes
        - Add amino acids and antibiotic <u>only after</u> heating
- Adjust the pH of the final product to 8.6.
    - To increase the pH use NAOH, sodium hydroxide
    - To decrease the pH use HCl, hydrochloric acid

1.1.1. Simple Formula

The Simple saline diluent formula (Table 1) is for same-day semen collection and insemination. Add distilled water to make up 100 ml. Items marked in bold are essential; the others are beneficial but optional. Sugars (glucose, fructose, and trehalose) are essential for semen motility and occur naturally in the seminal fluid. The use of sugars in the diluent is only necessary when performing I.I. using semen that has been stored. Sugars can be beneficial in boosting motility when using saline to collect and perform inseminations immediately.

Table 1: ******	
Simple Saline Formula	g/100 mL
Penicillin	**0.08**
Streptomycin	**0.08**
TES (acid)	**0.6879**
Tris (base)	**0.3635**
Sodium Phosphate Dibasic	**0.0142**
Sodium citrate	**0.02942**
KCl	**0.61131**
NaCl	**0.4847**
$NaHCO_3$	**0.042**
Fructose	0.05
Trehalose	0.02
Glucose	0.05
Arginine	0.01
Glycine	0.001
Proline	0.05
Catalase	**0.002**
BSA (lipid rich)	0.002

1.1.2. Formula for Semen Storage and Mixing

A more complex saline diluent formula is recommended when mixing and storing semen, including storage at temperatures above freezing and in liquid nitrogen. As a few of the components required are at very small quantities and difficult to measure with precision, it is necessary to make a 100X solution that can be added back to make up the finished product.

To prepare the following solution:

1. Add the marked components (Table 2, column ???) to the volumetric flask and fill to 100 ml with distilled water.
2. Use 10 ml of the base solution in the volumetric flask to make a 100X solution of the minor components.
3. Add 0.9 ml of the 100X back to the 90 ml of base solution.
4. Filter sterilize, as in 2.2.1.

Table 2. *****			
HHBSE Saline Formula For Storage and Mixing	g/100 mL	In 100 mL Volumetric	g/10mL (100X)
Penicillin	0.0125	x	
Streptomysin	0.011	x	
Kanomyosin	0.015	x	
Tylosin	0.0008		0.008
EDTA (ultra pure)	0.0002923		0.003
TES (acid)	0.6879	x	
Tris (base)	0.3635	x	
Sodium Phosphate Dibasic	0.0142	x	
Sodium citrate	0.02942	x	
Arginine	0.01	x	
Glycine	0.00075		0.0075
Proline	0.05	x	
Catalase	0.002		
BSA (lipid rich)	0.002	x	
KCl	0.61131	x	
NaCl	0.4847	x	
NaHCO3	0.042	x	

2.