

# **Artificial insemination**

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# Sperm Metabolism

## (Fructose Utilization)

Anaerobically: Fructose  $\xrightarrow{O_2}$  lactic acid + H<sup>+</sup> + 2 ATP (net yield)

• Aerobically : Fructose  $\xrightarrow{O_2}$  CO<sub>2</sub> + H<sub>2</sub>O + 38 ATP (net yield)

ATP + H<sub>2</sub>O  $\xrightarrow{\hspace{1cm}}$  ADP + H<sub>3</sub>PO<sub>4</sub> + 7,000 calories /mole

### • Measurement of metabolism rate

a. Under aerobic condition: 1). O<sub>2</sub> consumption 2). CO<sub>2</sub> liberation  
b. Under anaerobic condition: 1) Increase in lactic acid 2) Decrease in fructose 3) Methylene blue reduction time

## **Relationship of fertile life to metabolic rate of sperm:**

- a. Reduced metabolic rate : extend the storage life of semen;**
- b. Reduced metabolic rate in epididymis: extend life of epididymal sperm**
- c. Sperm in fresh ejaculate of semen: fertile for a few hours under high rate of metabolism**

# Factors affect the rate of metabolism:

## 1- Temperature :

a) Increase temperature leads to increase the rate of metabolism and decrease sperm life span

\*if temperature reach 50 C this leads to irreversible loss of motility

\*causes of death are due to exhaustion of substrate and decrease pH.

b) Decrease temperature leads to slow metabolic rate and extend fertile life if cold shock avoided

2-pH: optimum activity of sperm enzymes at pH 7.0.

3- Osmotic pressure

# Standard Technique of Freezing Bull Semen

## 6- Freezing

- A. Freezing procedure: A single layer of straws are placed on a tray at 5.5 cm above the liquid nitrogen level; Straws will reach the temperature of liquid nitrogen vapor in about 2 min.**
- B. Optimum freezing rate:: -50 C/min to -7 C/ min.: satisfactory results: influenced by the type of package, glycerol level, thawing rate and diluter composition**

## 7- Storage of bull semen:

**Semen storage in liquid nitrogen container: Double wall stainless steel or aluminum container with a vacuum between the walls:  
Large storage unit and small field unit**

# Preparation for Freezing Placement of Straws in Racks



Picture from Select Sires  
Courtesy of Dr. Earl Aalseth

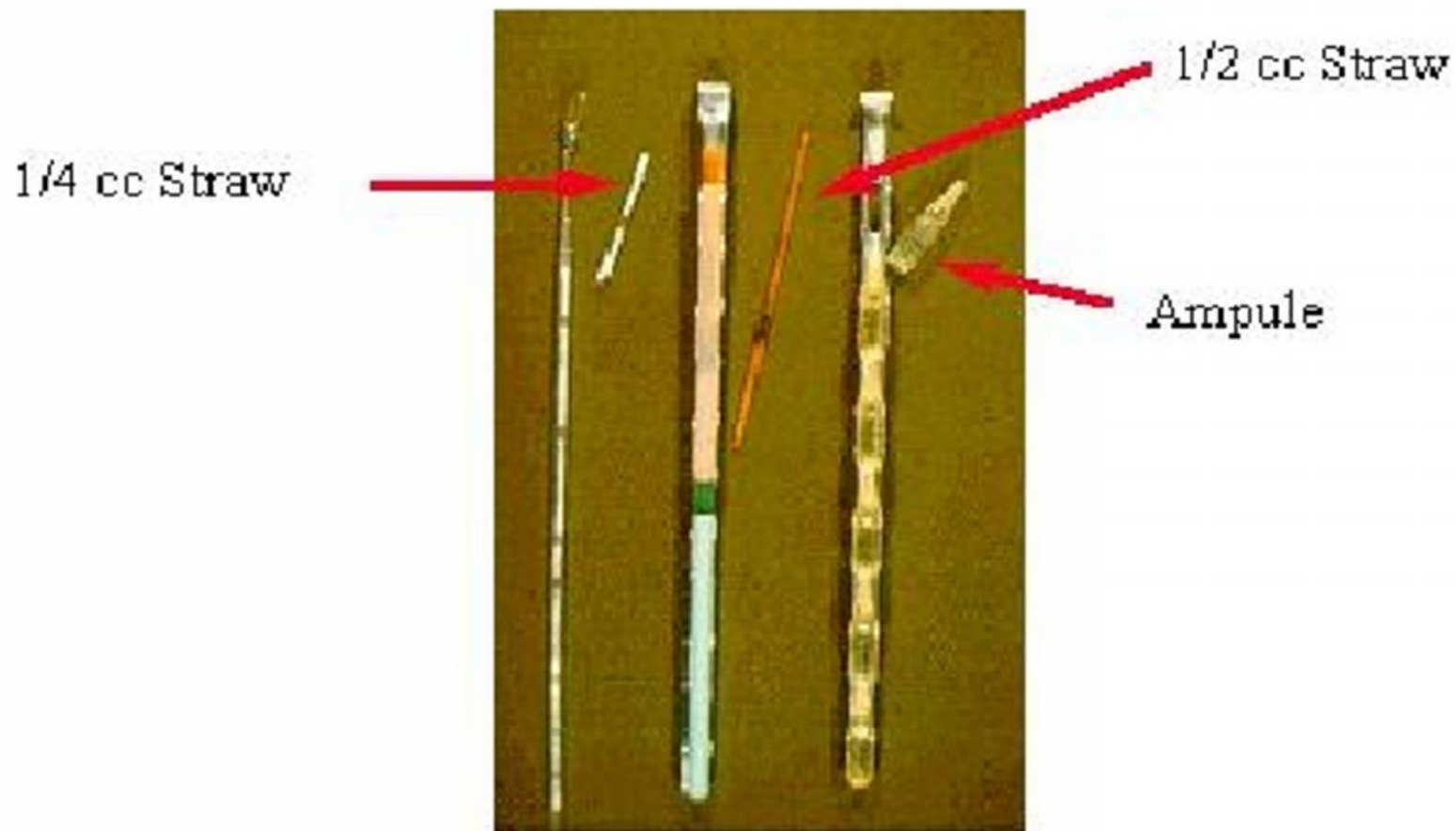
# Freezing of Straws

Initial Freeze in Liquid Nitrogen  
Vapor then Plunge into Liquid



Picture from Select Sires  
Courtesy of Dr. Earl Aalseth

# Storage Packages of Semen In Liquid Nitrogen



Picture courtesy of Dr. Earl Aalseth



# Inside Works of Nitrogen Tank



Picture courtesy of Dr. Earl Aalseth

# ASSESSMENT OF QUALITY OF FROZEN SEMEN

## METHODS USED TO EVALUATE FROZEN SEMEN:

**Motility: 2- Number of motile spermatozoa per inseminate 3- Acrosomal Integrity**

### **1-Motility:**

It is adopted 20% progressively motile spermatozoa as the minimum acceptable motility for frozen semen. The spermatozoa must show a reasonable rate of forward movement

## **2-Number of motile spermatozoa per inseminate:**

The number of motile spermatozoa per inseminate must be determined to know if too few or too many sperm are present for maximum reproductive efficiency. Final conclusion that for most bulls each inseminate should contain at least 10 million motile sperm

## **3-Acrosomal Integrity :**

Since the acrosome is involved in the fertilization process, it has been postulated that spermatozoa with deteriorated or damaged acrosome are unlikely to be capable of fertilization.

A relatively high positive correlation has been reported for acrosomal integrity and fertility of Holstein bulls.

## **Handling Frozen Bovine Semen in the Field**

Most of fertility problems associated with the use of frozen semen are due to improper handling or deposition of semen by the technician. Herewith, we will discuss how to minimize the reduction in fertility resulting from improper handling techniques for frozen semen packaged in straws.

### **1-Maintaining temperature of frozen semen:**

For maximum reproductive efficiency, the temperature of semen should be maintained at  $-130\text{ C}$  or lower at all times

If the temperature has risen above  $-80\text{ C}$  the semen should not be used to breed cows because the fertility is greatly reduced.

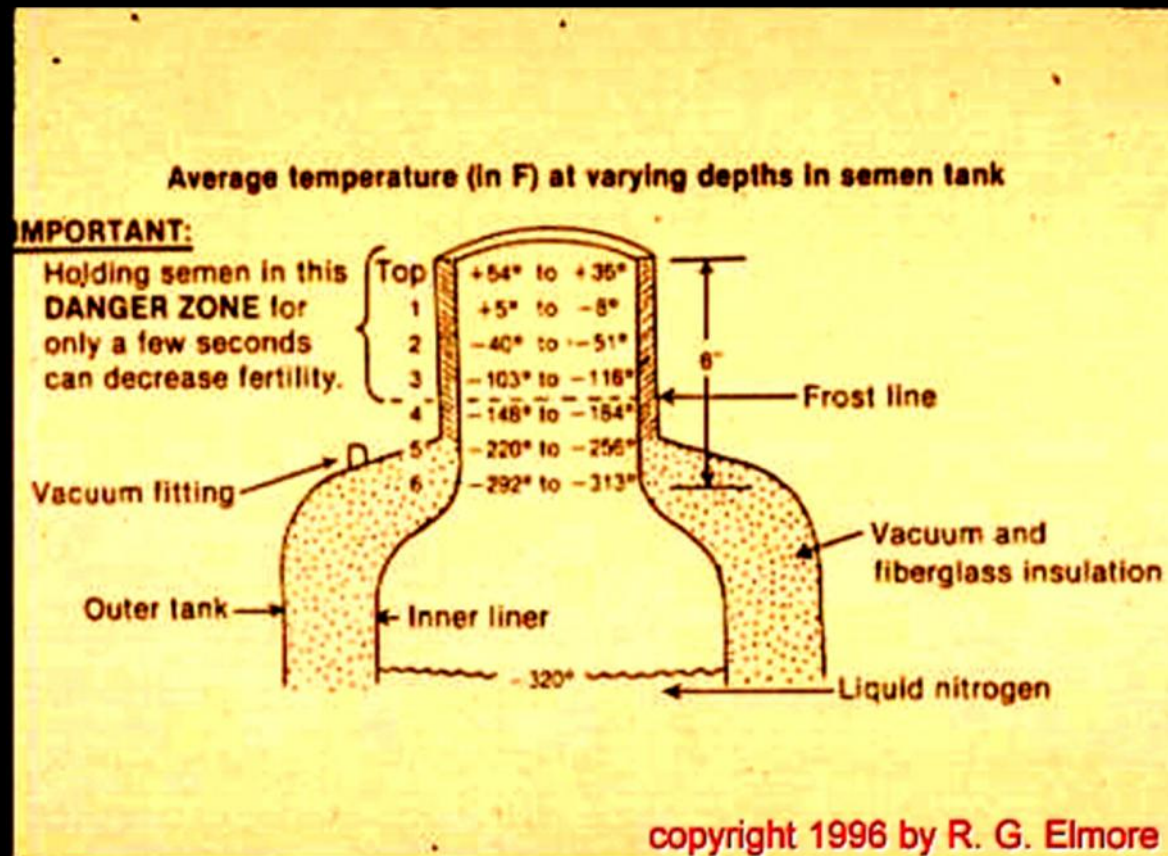
## Handling Frozen Bovine Semen in the Field

### 2-Transfer of semen:

- The first opportunity for improper handling of frozen semen comes during delivery or picking up. Semen can be exposed to elevated temperatures when it transferred from one tank to another. Seminal temperature rose more quickly within long straw with small diameter than the short one with greater diameter.
- In the same time, semen expose to rise in temperature and immediately plunged back into LN, produce a reduction in post thaw motility which is a reliable measurement of fertility and that occurs more rapidly in small diameter straws.

- Finally, We concluded that, straws should be transferred while in goblets containing LN, and the transfers should be completed as quickly as possible, away from wind and sunlight.

## Exposure of semen during removal:



- Semen may also be exposed to damaging temperature each time it is raised into the neck of tank to remove an individual straw for insemination
- The temperature within the neck of tank is much higher toward the top of the tank. Since spermatozoa will be damaged at these temperatures, the canister containing the straws should be raised no higher than absolutely necessary, and the individual straw should be removed for thawing as quickly as possible. The damage for the rest of straws from exposure to elevated temperature is additive.

**So** seminal temperature reached during exposure to the neck of the tank depends upon **the height** to which semen is raised, **length** of exposure, **level** of LN in the tank and **the interval** between exposures.

**Therefore,** semen should be raised no higher than necessary to facilitate removal of straw for thawing and removal should be quickly as possible.

# Handling Semen Below Frost Line of Nitrogen Tank

**Frost line**



Picture from Select Sires  
Courtesy of Dr. Earl Aalseth



## Thawing of Frozen Straw

- **Prepare the thawing bath containing water at 35 C**
- **Remove the lid from the LN tank, identify the canister holding the straws that are to be used, lift the canister no higher than the frost line in the neck of the LN tank**
- **With the thumb and forefinger, quickly grasp the tip of the goblet holding the straws and lower the canister back into the tank**
- **With a long forceps grasp an individual straw from the goblet and lower the goblet to the bottom of the canister within the tank.**
- **Immediately transfer the straw from the storage tank to the thawing bath. Where French straws should be held in the water for 6 seconds.**

## Thawing of Frozen Straw

**straw with taolit paper and inspect it for any cracks or defective seals.**

- **Carefully clip one end of the straw with especial sharp cutter or scissors. The clip line must be straight**
- **Place the straws into the funneled end of the plastic insemination sheath**
- **Complete the parts of inseminating device**
- **Inseminate the cow as will mentioned after.**

**The temperature of the semen must be prevented from reaching excessive levels and the thawing time must be controlled. In the same time be careful and avoid condition that will reduce the temperature of the thawed semen.**

## Insemination of Cow

### **Insemination by Recto-vaginal techniques:**

**Although it is somewhat difficult for the beginner but it is the method of choice for its high conception rate. The technique is done as follow:**

- 1-introduce the left hand through the rectum and make pack racking, after that make dry cleaning of the vulva with taollit paper.**
- 2-Grasp the cervix with lift hand**
- 3-Insert inseminating instrument through vagina.**
- 4-Hold cervix by its posterior end with index and middle finger and thumb, leaving the other two fingers free to help guide the inseminating instrument.**

**5-Guide the instrument into the opening of cervix and manipulate the cervix in all directions to pass the instrument through the cervix.**

**6-Move the fingers and thumb forward so that the manipulation is taking place just forward to the end of instrument.**

**7-Stop the instrument as soon as it reaches the anterior end of the cervix, and do not withdraw the instrument, especially when the cow urinates.**

placement of semen cranial  
to internal cervical os



(R. G. Elmore)

## **Some Problems during insemination:**

- **Insert the instrument into vagina not to enter sub-urethral diverticulum's or external urethral orifice**
- **When muscular contraction force the reproductive tract toward anus and cause vagina to become folded, grasp the cervix with left hand and push it forward to straighten the vagina.**
- **When the cow attempt to expel left hand from rectum with peristaltic muscular contractions, wait until the rectum will be relaxed.**
- **When the cervix can not be grasped or manipulated through contractions of rectal muscles, cup the fingers over the handed rectal wall and pull the hand toward the anus to cause the contracted rectal muscle to be relaxed and softened.**
- **When the vagina fills with air, dispel the air by firm pressure with the hand toward the vulva.**
- **When the bladder is extremely full, manipulate the clitoris to cause urination.**

