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An Overview of Cryopreservation of Cattle and Buffalo Bull Semen

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ABSTRACT

Cattle and Buffalo husbandry practice have undergone progressive improvement over the years. Artificial insemination (AI) has become the most important single technique for the improvement of the livestock resources in the tropical developing countries for the development of highbred cattle. Frozen semen (-196ºC) from selected exotic bulls is used for insemination purposes in Africa and Asia with variable results. Some variations in number of services per conception in different lactations have been reported to be due to difference in fertility of exotic frozen semen. In this paper, an overview of the cryopreservation of cattle and buffalo bull semen is discussed with the view of improving the current AI practices in the developing world.

Key Words: Cryopreservation; Cattle; Buffalo; Semen

INTRODUCTION

Artificial insemination (AI) is the most important single technique devised for genetic improvement of animals. Cattle and Buffalo husbandry practices have undergone progressive improvement over the years in tropical developing countries like Nigeria and Pakistan (Samad, 1985; Pathiraja *et al.*, 1988; Sekoni & Oyedipe, 1995). AI gained wide acceptance in the development of the livestock resources in the developing countries (Cheema & Samad, 1986).

The development and application of AI in the last four to five decades especially in cattle have been most striking. Much research has been stimulated by the rapid expansion of this practice. Although the storage of semen for 3 to 4 days may be satisfactory for day-to-day requirements of an AI centre, however, difficulty in maintaining its fertility for a long time causes a serious wastage of semen form valuable sires. With the growing realization of the importance of progeny testing, it became evident that the development of new methods of semen preservation could benefit the existing AI semens. In this paper, an overview of cryopreservation of cattle and buffalo bull semen is discussed.

Extenders and dilution of semen. Considerable work have been done in this area (Polge & Rowson, 1952; Dunn & Haffs, 1953; O'Dele & Almquist, 1954; Emmens & Martin, 1957; Lin *et al.*, 1965; Nagose *et al.*, 1968; Habibullin, 1969; Saiko, 1969; Chaudhari, 1971a; Argunde, 1971). Semen extenders containing sodium citrate, egg yolk, glycerol, sugar etc in different proportions have been reported (Arthur *et al.*, 1983; Merck, 1983; Samad, 1985).

The qualities of a good semen extender outlined by Samad (1985) have been presented in Table I. Extenders differ in composition depending on the species, use, temperature at which the diluted semen is to be stored; and the duration of storage desired. All extenders are based on a particular buffer, which has provided the best results for a given species (Hopkins & Evans, 1989). Buffers play an important role in modulating changes in the pH of extenders due to the products of metabolism in stored sperm.

Table I. Quality of a good semen extender

1	Osmotic pressure and electrolyte balance	Equipment of seminal plasma (285 millimoles)
2	Energy Source	Glucose, fructose and Lactose in appreciable quantities.
3	Buffering Capacity	Should maintain pH of diluted semen.
4	Protection against cold shock.	Lecitten, lipoprotein as casine in mild and egg-yolk protect against cold shock.
5	Cryoprotectant	Glycerol
6	Antibiotics	To get bacteria free semen, use penicillin and streptomycin.

Carbohydrates (sugars) are necessary to provide the energy required for sperm glycolysis, egg-yolk based fractions provide phospholipids necessary to promote membrane stabilization at lower temperature and limit premature acrosomal membrane activation. The commonest extender used for frozen semen is skimmed mild or homogenized mild to which 10% glycerol is added. Tris-citric acid fructose-glycerol yolk extender with 5% glycerol has been used with success (Merck, 1983). The composition of some extenders is given in Table II.

Frozen semen. The majority of AI activity today is performed with frozen semen. Freezing has permitted semen to be collected, processed and used anywhere for years afterwards. Semen freezing (cryopreservation) is most

Table II. Composition of diluters used for preservation of bull semen

Milk yolk glycerol (MYG)	
Milk	75 mL
Egg yolk	20 mL
Glycerol	5 mL
Penicillin G	1000 I.U. mL^{-1}
Streptomycin	1.00 mg mL^{-1}
Lactose yolk glycerol (LYG)	
11% lactose solution	75 mL
Egg yolk	20 mL
Glycerol	5 mL
Penicillin G	1000 I.U. mL^{-1}
Streptomycin	1.00 mg mL^{-1}
Lactose fructose yolk glycerol (LFGY) 3:1	
11% lactose sol.	56.25 mL
6% fructose sol.	18.75 mL
Egg yolk	20 mL
Glycerol	5 mL
Penicillin G	1000 I.U. mL^{-1}
Streptomycin	1.00 mg mL^{-1}
Glucose yolk citrate glycerol (GYCG)	
Dist. Water	100 mL
Glucose	58 mg
Sod. Citrate	5 gm
Egg yolk	20 mL
Glycerol	7 mL
Penicillin G	1000 I.U. mL^{-1}
Streptomycin	1.00 mg mL^{-1}
Tris citrate fructose	
Tris (hydroxymethyl-amino-methane	3.1 mg 100 mL^{-1}
Citric acid	1.97 mg 100 mL^{-1}
Citric acid	1.55 mg 100 mL^{-1}
D (-) fructose	20 mL
Egg yolk	20 mL
Glycerol	7 mL
Penicillin	1000 I.U. mL^{-1}
Streptomycin	1.00 mg mL^{-1}

advanced in the bovine species and has served as a basic model for other species.

There are variations in extenders, the use of freezing points, depression compounds and freezing rates; but the principles of cryopreservation are similar in all species. The procedure employed in the processing of frozen semen in *NAPRI-SHIKA* Nigeria is outlined below:

The information concerning the optimum parameters for semen cryopreservation are not standardized within species and different sources conflict over the best procedures. Glycerol is a freezing point depressing compound which helps prevent ice and solute damage to the cells during freeze–thaw process. Glycerol at the final recommended level is slightly toxic to the sperm if added in one step, so the glycerolated extender is mixed gradually with the semen over 1 h . After the final addition, the semen is ready for packaging.

Recommended procedure for processing and freezing of bull semen (NAPRI-SHIKA, Nigeria)

1. Semen is collected form each of the AI bulls on regular basis using an AV properly assembled and filled with warm water at body temperature at 37ºC.
2. The collected semen sample is placed in a water bath at 30-34ºC and examined microscopically under low power for wave pattern and gross motility. The volume and color are being recorded. The pH of semen is also being checked using an indicator paper for an immediate result. Stains are made using eosin nigrosin for sperm morphological examination thereafter.
3. The IMV electronic photometer is used to determine the concentration, total volume of diluents and number of straws to be filled with diluted semen. The degree of dilution is aimed at giving an insemination dose rate of 25-30 million sperm cells.
4. At 30ºC, the semen is diluted to 50% of the final volume of diluents using the 3% glycerol diluents half of the final volume without any glycerol. Semen is examined under a microscope for individual motility. Semen is transferred into the cold cabinet at +5ºC and allowed to cool and stabilize for about 30 min. The second part 11% glycerol diluent or half of the final volume with 14 mL (14%) glycerol content is added to the semen to make up the total volume which will yield 7% glycerol content.
5. The diluted semen is held at this temperature +5ºC for at least 2 h equilibration time. Straws are filled and sealed by using an automatic filling and sealing machine capable of filling 2,000 straws per hour. Semen is sucked into the straws and on meeting the polyvinyl alcohol powder plug at one end and impervious seal is made. The other end is pinched together and cold sealed. The semen is being checked for individual motility while the straws are held at 5ºC for another 2 h equilibration. After equilibration, a minimum of 4 h, the straws are then ready for freezing by one of the two methods:

a. Horizontal method. Straws are racked horizontally and are frozen in liquid nitrogen vapour at –120 to 130ºC 4 cm above the liquid nitrogen level in the freezing tank for 9 min. The straws are transferred to the container with liquid nitrogen at –196ºC after which the motility of primary freezing is checked and recovery rate assessed.

b. Vertical method. Straws are racked vertically and frozen in liquid nitrogen vapour at –120 to 130ºC with the bottom and 0.5 cm above the liquid nitrogen level for 18 min. Straws are transferred into a container with liquid nitrogen at –196ºC after which the motility of primary freezing is checked and recovery rate assessed.

Packaging. Semen is packaged in 3 ways viz:

1. Glass ampoules of 0.5 to 1 mL of extended semen.
2. Glyvinyl chloride straws of 0.25 to 0.5 mL of extended semen.
3. Pelleted semen of approximately 0.1 mL

The glass ampoules are still generally in use. The straws are gaining wide popularity because they require less storage space, have better freezing characteristics and suffer less sperm loss especially in the USA.

Freezing. Packaged semen has been freezed through the following methods: mechanical freezing, dry ice, liquid air,

liquid oxygen, liquid nitrogen

Although success has been achieved with all these methods, liquid nitrogen is almost exclusively used now in cryopreservation of semen.

Storage. Frozen semen are stored at -196ºC in liquid nitrogen for transportation anywhere AI is desired.

Thawing. Thawing is achieved by placing the straw into a 37ºC water bath for 30 to 60 seconds. All post thawing evaluations are performed at 37ºC. Semen must meet the acceptance minimum criteria before distribution for AI programs.

Some ultra structural changes have been observed following cryopreservation of spermatozoa in a variety of domestic animals. The introduction of modern histopathological procedures have led to the understanding of normal ultra structural of the mammalian spermatozoa, but the understanding of ultra structural changes post-freezing have been countering the difficulties encountered in AI programs. Hancock (1951) observed changes in ejaculated ram and bull spermatozoa on cooling to 0ºC. This was accompanied by decrease in motility and metabolic activity. Later, Lype *et al.* (1963) reported loosening and disintegration of the acrosome on cold shock. Other workers, Nicander and Bane (1968), Healey (1969) and Chaudhari (1971b) reported several other ultra structural changes like segmental columns of the neck region, loose head and tails, folded acrosome margin, etc.

Evaluation of fertility of frozen semen post thawing. A variation in the number of services per conception in different lactations has been reported to be due to difference in fertility of exotic frozen semen (Cheema & Samad, 1986). Many properties of the spermatozoa are important for successful fertilization. Table III shows the methods of checking the quality of fertility of the frozen semen.

Table III. Methods of checking the quality (fertility) of diluted or frozen semen

Methods	Semen characteristics
Laboratory	Motility
	Sperm cell morphology
	Sephadex filter test
	Determination of Enzyme leakage (GOT) dure to acrosomed danage and sperm cell membrane.
	Liveability at 370 C
	Absolute index of liveability
	Amplified restruction fragments length polymorphism (AFPL) technique.
Field	Fertility is the only accurate determinant of the quality of frozen semen.

Motility is universally used for evaluating semen. Sascke and White (1972) correlated motility with fertility of frozen bull semen. The correlation between normal acrosome ridges and fertility was 0.06. In Pakistan, Buffalo motility rates showed a correlation of 0.25–0.48 with fertility.

The "SEPHADEX Filter Test" is an objective essay for more than one of the properties of frozen spermatozoa. Leakage of intracellular enzymes, in particular, glutamic oxaloacetic transaminase (GOT) is used to assay for membrane damage during preservation of semen (Graham *et al.*, 1976). More recently, the amplified restriction fragment length polymorphism (AFLP) techniques that is used to asses semen motility, plasma membrane integrity and acrosome integrity in post thaw semen have been resorted (Thurston *et al.*, 2001).

CONCLUSION

Cryopreservation is the only method through which spermatozoa form genetically superior elite bulls can be preserved at –196ºC in the liquid nitrogen. The frozen semen can safely and easily be transported to the remotest parts of the world. The most important need of the tropical developing countries like Nigeria and Pakistan is a suitable extender and preservation technique in which spermatozoa can be preserved at –196ºC without loosing their fertilizing ability. Extenders containing lactose-egg yolk glycerol showed better performance during laboratory evaluation, but milk-egg yolk glycerol extender gave higher conception rate when frozen semen was inseminated. Thawing at 37ºC for 10 seconds gave higher fertility than 20ºC for one minute or 5ºC for 2 seconds. Further studies in this area study investigate the effects of nutrition and other environmental factors on the fertility of post-thawed semen in the tropical developing countries.

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