Cryopreservation of Buffalo Bull Semen- Restriction and Expectation: A Review

Pavan Kumar Mittal1*, A.K. Madan3, Vijay Sharma2, G.S.Gottam1 and Barkha Gupta1

1Department of Veterinary Physiology and Biochemistry,  
2Department of Veterinary Public Health and Epidemiology, Post Graduate Institute of Veterinary Education and Research, Jaipur, Rajasthan-302031, India  
3Department of Veterinary Physiology, College of Veterinary and Animal Sciences, DUVASU, Mathura-281001, India

*Corresponding author

ABSTRACT

Semen cryopreservation is a widely used technique across the world for conservation and proliferation of genetically superior germplasm through artificial insemination. Artificial insemination (AI) is the most conclusive tool to increase genetic improvement, control of venereal diseases and improve herd performance and productivity. For achieving the mission, successful and effective cryopreservation of semen is essential. In recent years, increased demand of animal products due to very rapid growth of human population inspired the investigators to increase per animal milk production using various techniques of Biotechnology. The process of semen cryopreservation is stressful and around 40%-50% of sperm lose their motility and viability due to loss of structural and functional capabilities. One of the factors which affect post thaw survival of sperm is the freezing rate. In buffalo, poor fertility rate with cryopreserved semen is main obstacle in its proliferation. It is need of the time to spread the knowledge of various factors and components such as use of suitable extenders, additives, cryoprotectants, freezing and thawing protocols contributing in cryopreservation of semen to improve the fertility rate of buffalo.

Keywords
Buffalo, Buffer, Cryopreservation, Cryoprotectant, Extender, Semen Processing, Semen

Introduction

The world buffalo population is appraised at 185.29 million, spread in some 42 countries, of which 179.75 million (97%) are in Asia (Buffalopedia, 2015, www.buffalopedia.cirb.res.in). India has 108.7 millions and they comprise approximately 56.7% of the total world buffalo population (DAHDF, Govt. of India, 2015). Buffalo, a triple purpose animal, provides milk, meat and mechanical power to mankind. Due to its highly nutritious milk, leaner meat and best draught power for wet environments, buffalo offers immense potential for improvement of livelihood. Buffalo can efficiently convert low quality feed stuffs like straws and agro-industrial waste into human food, improve soil structure
through bio fertilizer and a financial asset which can be sold when needs arise (Pasha et al., 2012).

The genetic improvement and disease control in domestic animals have primary importance in the success of agriculture and food industry. In this contribution, artificial insemination (AI) is perhaps the most conclusive tool for modern ways of breed improvement. Moreover, the quality of frozen–thawed semen is one of the most influential factors affecting the likelihood of conception (Saacke, 1984). Application of AI with frozen–thawed semen has been reported on a limited scale in buffalo, because of poor freezability and fertility of buffalo spermatozoa when compared with cattle spermatozoa (Kakar and Anand, 1981; Muer et al., 1988; Raizada et al., 1990; Singh and Pant 2000; Andrabi et al., 2001, 2008; Ahmad et al., 2003; Senatore et al., 2004; Kumaresan et al., 2005). In this process, each ejaculate collected from genetically superior male is used to inseminate females at a large scale and also controls sexually transmitted diseases.

**Cryopreservation of spermatozoa**

Cryopreservation is a non-physiological and complex method that involves a high level of adaptation of biological cells to the thermal and osmotic shocks that occur both during the dilution, cooling–freezing and during the thawing procedures (Watson et al., 1992; Holt 2000a, b). Damage occurring during the freezing–thawing procedures affects mainly cellular membranes and nucleus (Blesbois 2007) and affects finally viability and fertility. The cryopreservation development protocols in dairy industry started in 1950s. Semen cryopreservation is a composite process which involves many stages: extension (dilution), cooling, freezing, storage and thawing. During each stages, sperm structure and function are affected (Bailey et al., 2003) resulting in reduced sperm motility (Tuli et al., 1981), acrosomal damage and alteration in sperm membrane integrity (Rasul et al., 2001). Sperm Cryopreservation exposes sperm to mechanical and anisomotic stresses (Hammerstedt et al., 1990) that reduces cell survival and surviving sperm, thereby reducing cell longevity and fertility compared with fresh semen.

Cryopreservation generates sublethal sperm injury due to chemical, osmotic, thermal and mechanical stresses, which may result in loss of viability, motility, damage of deoxyribonucleic acid (DNA), destruction of acrosomal and plasma membrane (Numan Bucak et al., 2007; Rasul et al., 2001). Furthermore, changes in biochemical factors have been recognized during cryopreservation, including depletion of amino acids and lipoproteins, release of glutamic-oxaloacetic transaminase (GOT), decrease in phosphatase activity, decrease in loosely bound cholesterol, protein, inactivation of acrosin enzyme and hyaluronidase, prostaglandins diminution, increase in sodium, decrease in potassium content, reduction of ATP and ADP synthesis and decrease in acrosomal proteolytic activity (Barbas et al., 2009). In fact sperm plasma membrane is a primary target for freezing or cold shock injury (Numan Bucak et al., 2009). The extender used is an important factor in cryopreservation process. These mediums must have adequate pH and buffering capacity, appropriate osmolality and should protect spermatozoa from cryogenic lesion. Tris extender is an important medium that is often used during freezing of bulls, rams, bucks and buffaloes semen (Barbas et al., 2009; Rasul et al., 2001).

The production of Reactive Oxygen Species (ROS) is a normal physiological event in various organs. However, the over-production
of ROS can cause structural damage of sperm membranes (De Lamirande et al., 1997). Reactive oxygen is responsible for sperm dysfunction due to the lipid peroxidation of membranes (Arabi et al., 2008). It has been documented that vitamin E is major antioxidant agent of sperm cells which is a potent scavenger of free radicals and is able to protect plasma membrane from damages mediated by ROS (Reactive Oxygen Species) and LPO (Lipid Peroxidation) (Yousef et al., 2003; Gurel et al., 2005 and Sinclair, 2000). It has also been established that presence of vitamin E is necessary for normal function of male reproductive system.

Sperm viability is depressed by as much as 50% during freezing (Watson 1995). To counteract destructive effects of ROS, seminal plasma has an antioxidant system that seems to be very relevant to protection of sperm (Alvarez and Storey, 1982). Furthermore, due to dilution of semen, antioxidant reserves of seminal plasma are depressed making spermatozoa more vulnerable to cryo insults. Therefore, it becomes all the more essential to incorporate anti-oxidants to semen extenders as protective agents. Assessing the structural and functional integrity of sperm membrane is of paramount importance in order to maintain optimum fertility potential of spermatozoa.

Cryodamage during freeze-thawing process to buffalo semen is higher than cattle spermatozoa due to unique physiology of buffalo spermatozoa and higher polyunsaturated phospholipids levels in plasma membrane (Nair et al., 2006; Sreekjith et al., 2006; Andrabi, 2009). The fertility with frozen semen in buffaloes under field conditions is very poor and has been considered as 30% (Abhi, 1982; Chohan et al., 1992; Anzar et al., 2003; Andrabi, 2009). The reason for poor fertility rate in buffaloes under field conditions is poor post-thaw characteristics of buffalo semen. As a matter of fact, the unique physiology of buffalo sperm requires buffalo specific semen extender to reduce the cryodamages. Improving the semen extender for the cryopreservation that ensures no or little damage to sperm motility, plasmalemma, acrosomal and chromatin integrity might enable to achieve a comparatively higher fertility rate with artificial insemination in buffalo under field conditions. It is well documented that motility, plasma membrane, acrosome and DNA integrity of buffalo bull spermatozoa significantly reduced after process of cryopreservation (Rasul et al., 2001; Kadirvel et al., 2009; Anzar et al., 2010; Kumar et al., 2011).

Cryopreservation of buffalo semen increases level of reactive oxygen species molecules that caused lipid peroxidation of bio-membrane system by reducing antioxidant potential of cryopreserved semen (Kadirvel et al., 2009; Kumar et al., 2011).

To control level of ROS and promote motility and survival of sperm, numerous antioxidants have proven beneficial in treating male infertility (Sinclair, 2000). Ascorbic acid and vitamin E is naturally occurring free radical scavenger and their presence also assist various other mechanisms in decreasing numerous disruptive free radical processes, including LPO (Bansal and Bilaspuri, 2009). Vitamin C and E are major antioxidants naturally present in mammalian semen against ROS to protect sperm from lipid peroxidation and to maintain its integrity (Bilodeau et al., 2001; Gadea et al., 2004; Bucak et al., 2008; Andrabi, 2009; Akhter et al., 2011). Concentration of antioxidant decreased during freeze-thawing process by dilution of semen with extender and excessive generation of ROS molecules (Andrabi, 2009; Kumar et al., 2011).
Factors affecting freezability

It is found that buffalo spermatozoa are more susceptible to risks during freezing and thawing procedures than cattle spermatozoa, thus resulting in lower fertilizing capacity (Raizada et al., 1990; Andrabi et al., 2008). Moreover, there are specific biochemical factors that affect the capability of spermatozoa to prevent hazards caused by the cryogenic procedures. One of the many possible causes of lower freezability of buffalo bull semen compared to cattle bull can be due to the differences in the lipid ratio of the spermatozoa (Jain and Anand 1976; Tatham 2000; European Regional Focal Point on Animal Genetic Resources, 2003) due to presence of high phospholipids content found in buffalo sperm plasma membrane (Cheshmedjieva and Dimov, 1994). Therefore, there is a requirement to develop biochemically defined extenders and cryogenic protocols that are species specific, and may result in the improvement of viability and fertility of frozen thawed buffalo spermatozoa.

Diluter components

Buffer

A buffer solution is an aqueous solution consisting of a mixture of a weak acid and its conjugate base, or vice versa. Its pH changes very little when a small amount of strong acid or base is added to it. In various species considerable work has already been done on this aspect for diluter preparation. The composition of the diluter (extender) is decided on the basis of storage temperature of the semen, duration and need a suitable buffer for sperm survival during cryopreservation (Rasul et al., 2000). Ideally, a buffer should have following features (i) Minimum salt effects/ buffer concentration (ii) pH between 6 to 8, favorably 7 (iii) Maximum water solubility (iv) Minimum solubility in all other solvents (v) Minimum temperature effects (vi) Well behaved cations interaction (vii) Better ionic strengths and chemical stability (Bates 1961; Good et al., 1966; Good and Izawa 1972; Keith and Morrison, 1981; Andrabi, 2009).

A most suitable buffering system for buffalo bull semen cryopreservation which should possess composition close to natural medium and help in maintaining fertility of the frozen semen. Beginning from the use of various organic buffers for the cryopreservation of the bull semen (Mughal et al., 2017., Foote, 1970). In this consider, Matharoo and Singh (1980) evaluated citrate, Tris or citric acid as buffers for deep-freezing of buffalo spermatozoa. They found during freezing a minimal loss of post-thawed motility with Tris-based extender. However, Chinnaiya and Ganguli (1980) establish better post-thaw sperm motility with Tris-based extender than citrate or citric acid-based extenders and reported spermatozoa chilled (frozen) in citrate and Tris-based extender showed alike degree of acrosomal damage and similar recovery rates and Ahmad et al., (1986) reported that Tris–citric acid based extender is fit for the freezing of buffalo bull spermatozoa regarding post-thaw motility and survivability while Dhami and Kodagali (1990) found that Tris-based extender improved the freezability of buffalo bull spermatozoa.

Identically, in buffalo bull spermatozoa, Singh et al., (1990, 1991) found that minimal release of lactic dehydrogenase and sorbitol dehydrogenase during semen cryopreservation using Tris-based extender followed by citrate and citric acid extenders. Dhami et al., (1994) reported that Tris-based extender provided higher post-thaw spermatozoa motility while Singh et al., (2000) evaluated superior results with Tris-
based buffer as compared to Laiciphos and Biociphos. Rasul et al., (2000) also found ameliorated semen motility rate using Tris citrate as compared to tri-sodium citrate, Tris-Tes or Tris-Hepes and also found that Tris citrate was suitable buffer for semen cryopreservation. Under other conditions, Oba et al., (1994) and Chachur et al., (1997) found nearest effects on semen motility rate, acrosomal integrity and post thaw motility using Tes which has equal value to Tris-based extender.

From these above mentioned results and studies, it is recommended that zwitterion buffers as a superior option for buffalo bull semen dilution or extension, particularly, Tris–citric acid may issue the most satisfactory buffering system to improve the post-thaw freezability and also fertility of buffalo spermatozoa. Graham et al., (1972) reported that the pH of zwitterions buffers is nearest to the pKa (acid dissociation constant) and it is minimal affected by temperature.

**Antibiotics**

The viability or fertility of cryopreserved bovine spermatozoa is extremely affected either directly or indirectly by presence of bacteria in semen (Thibier and Guerin 2000; Morrell 2006). So, control of these bacteria by the addition of various antibiotics in the semen diluters to achieve maximum conception rate through artificial insemination (AI).

Sansone et al., (2000) and Akhter et al., (2008) were generally added benzyl penicillin 1000 IU / ml and streptomycin sulphate 1000 lg / ml alone or in combination as a freezing diluents in semen extender of buffalo bull. However, an inefficient combination of streptomycin and penicillin (SP) in buffalo bull semen to control of bacteriospermia by reason of SP was deleterious to post-thaw quality of spermatozoa (Gangadhar et al., 1986; Aleem et al., 1990; Ali et al., 1994; Amin et al., 1999). Ahmed and Greesh (2001) reported that bacteria separated from buffalo bull semen were resistant to penicillin. However, the antibiotics of better choice to be added in semen extender for efficient cryopreservation of buffalo spermatozoa are gentamicin (500 lg / ml) or amikacin (500 lg / ml) or norfloxacin (200 lg / ml). Recently, a newly combination of gentamicin tylosin and lincomycin, GTLS has manifest extremely capacity to control bacteria and spermatozoal quality present in buffalo bull semen (Hasan et al., 2001; Akhtar et al., 2008). The evaluating of a broad range of antibiotics alone and in combinations is recommended to increase the quality of cryopreserved buffalo bull semen.

**Cryoprotectant**

Cryodamage has been attributed to ice crystal formation, membrane alteration, oxidative stress, osmotic changes, cryoprotectant toxicity and cold shock during cryopreservation (Watson and Martin, 1975). So, Optimum freezing and thawing rates, suitable cryoprotectant and composition of diluter (extender) are key factors for successful semen cryopreservation (Hammerstedt et al., 1990; Curry et al., 1994).

Semen cryopreservation causes deleterious effects regarding biochemical, functional and sperm ultra structural damage (Watson, 2000) causing in a depletion of membrane integrity, acrosome integrity and motility (Woelders et al., 1997 and Celeghini et al., 2007) and fertilizing ability (Purdy, 2006). During the cryopreservation, cryoinjuries are produced and can be lowered and significant enhancement can be obtained through using of cryoprotectants in the semen extender (Holt, 2000 and Johnson et al., 2000). On a large scale, these cryoprotectants are
classified into two categories (permeable and non permeable). Permeable cryoprotectants include methanol, butanediol, glycerol, 1, 2-proparediol, ethylene glycol, propylene glycol and dimethylsulfoxide (DMSO). These cryoprotectants act as intra-cellularly and extra-cellularly have capability to move through the sperm plasma membrane and, reposition the membrane proteins, reduce formation of intracellular ice and thus protect the spermatozoa from damage (Holt, 2000). Non permeable cryoprotectants comprise mannose, raffinose egg yolk, amino acids, sucrose, xylose, fructose, trehalose, dextran, lactose, polyvinyl pyrrollidone (PVP), amides and fatless skimmed milk which do not pierce the sperm membrane (Aisen et al., 2000). Cryoprotectants lower the freezing temperature and finally reduce extracellular ice formation (Kundu et al., 2002). The optimum levels of glycerol and glycerolization are important key factor for the cryopreservation of buffalo semen. They also reported that post-thaw motility of spermatozoa was significantly better in a 5% glycerol extender, whereas the percentage of acrosomal integrity was higher in spermatozoa extended in 3% or 5% glycerol than in spermatozoa extended in 7% glycerol (Jainudeen and Das., 1982). Tris- and milk-based diluents have better level of glycerol as 6% and 9% glycerol for the sodium citrate diluent to acquire better post-thaw motility for buffalo spermatozoa (Kumar et al., 1992). Glycerol is a small, poly-hydroxylated solute with a high solubility in water, and a low toxicity during short-term exposure to living cells. It can interact by hydrogen bonding with water and can permeate across the limiting plasma membrane of many different cell types (Fuller and Paynter, 2004). Ramakrishnan and Ariff, (1994); Nastri et al., (1994) also reported that the reduction in glycerol below 5% decreased the post-thaw motility and acrosome integrity of spermatozoa in the extenders. Holt (2000b); Medeiros et al., (2002) investigated that the physiological role of glycerol as cryoprotectant takes place by lowering the freezing point of water, replacing intracellular water, binding with metallic ions and by reducing the electrolyte profile in the unfrozen portion. So, Glycerol (6 to 7%) is commonly used as cryoprotectant for buffalo bull semen. Abbas and Andrab (2002) studied the effects of different concentrations of glycerol on post-thaw sperm quality and reported that the spermatozoa cryopreserved in 7% were significantly superior to those in other concentrations of glycerol as determined by post-thaw motility, plasma membrane integrity and survivability. Singh et al., (2006) found that single step of glycerolization is more suitable for the cryopreservation of buffalo spermatozoa for post-thaw motility. Dimethyl sulfoxide (DMSO) is a rapid penetrating cryoprotectants having lower molecular weight than glycerol. Yu and Quinn (1994) evaluated that DMSO may inhibit harmful effects of hydroxyl radicals and these radicals appear during cell respiration and are detrimental to cell (Johnson and Nasr-Esfahani 1994). Rasul et al., (2007) investigated the synergistic effects of dimethyl sulfoxide (DMSO) on cryoprotection ability of glycerol during cryopreservation of buffalo sperm in terms of motion characteristics, acrosome morphology and plasma membrane integrity using tris citric acid extender differing in glycerol and DMSO concentrations at various temperatures. DMSO in combination with glycerol is superior in providing cryoprotection in cattle bull (Snedeker and Gaunya 1970) rabbit and buck spermatozoa (Bamba and Adams 1990). During cryopreservation, DMSO has harmful toxic effect rather than the osmotic shock because of the lower molecular weight of DMSO, its penetrating ability into the cell is higher than glycerol (Rasul et al., 2007). Glycerol is
useful for the sperm as its freezing point is much lower than water. Hence, it is suggested that a extender containing glycerol concentration of 5–7% may be suitable for the cryopreservation of buffalo bull spermatozoa. Under other conditions, development of less toxic and more powerful cryoprotectant could make an important contribution in improving post thaw quality of buffalo spermatozoa.

The other important component of semen extenders is egg yolk in the buffalo and and most of the livestock species (Sansone et al., 2000). Egg yolk containing low density lipoproteins (LDL) is highly responsible for sperm protection during cryo-preservation (Pace and Graham 1974; Watson 1976). LDL provides protection to sperm by stabilizing the membrane to adhere with sperm membrane. Sansone et al., (2000); Andrabi et al., (2008) reported that egg yolk which is commonly used at a concentration of 20% in semen extender, is mandatory for freezing of buffalo semen. The higher concentration of egg yolk in extender may have detrimental effects combined with toxicity of dead spermatozoa resulting in lower post-thaw sperm quality (Shannon 1972) probably due to the raised substrate available for hydrogen peroxide formation (Tosic and Walton 1950). Sahni and Mohan (1990) found that egg yolk stimulates enzymes found in the spermatozoa which cause deamination of amino acids causing damage to spermatozoa during storage period. So, Egg yolk is an important and necessary component while diluting semen in the extenders in bovine and many species during cryopreservation protocols (Shannon and Curson, 1983; Priyadarsini et al., 2011). The extender containing duck egg yolk improved freezability of buffalo bull spermatozoa as compared to other avian egg yolks (Andrabi et al., 2008). Polyethylene glycol (PEG) is a non-permeable cryoprotectant and may decrease the process of ice nucleation during cryogenic process and protecting the cellular membrane. Cheshmedjieva et al., (1996) found that the effect of addition of PEG 20 to egg yolk based freezing medium on the lipids in buffalo spermatozoa and concluded that incorporation of PEG 20 in semen extender preserved the lipids of frozen buffalo spermatozoa. In future, further studies on PEG 20 may be a better alternate for the cryopreservation of buffalo spermatozoa.

Sugars like as raffinose, trehalose lactose, sucrose and dextrans are non-permeable cryoprotectant and also added in semen extender (Nagase et al., 1964). Sugars are not capable of diffusing across a plasma membrane and produce an osmotic pressure inducing cell dehydration and lower incidence of intracellular ice formation. These sugars also interact with reorganizing the membrane and phospholipids in the plasma membrane which results in surviving of sperm during cryopreservation process (Molinia et al., 1994; Aisen et al., 2002). Dhami and Sahni (1993) reported that the post-thaw quality of spermatozoa was superior with raffinose (1%) in Tris-based extender compared to other extenders in terms of release of lactate dehydrogenase. The newer international trends in disease control consider the ingredients of animal origin (egg yolk) to be a
source of contamination to the semen (Bousseau et al., 1998), hence emerges the need of using non-animal sources. It is feasible that extenders having ingredients of animal origin (egg yolk) can be the source of pathogens resulting in the contamination of semen (Bousseau et al., 1998; Marco-Jimenez et al., 2004; Ruigh et al., 2006). In this regard, lecithin from non-animal source like soya needs to be trialed as a non-permeable cryoprotectant in extender for cryopreservation of buffalo spermatozoa.

**Other additives**

Attempts have been made to enhance the freezability of bubaline semen by adding different substance such as metabolic stimulants, detergents, antioxidants and chelating agents due to decrease the harmful effects of cryogenic procedures. Bhosrekar et al., (1990) found that the effect of addition of caffeine or triethanolamine lauryl sulphate to Tris citric acid based extender and evaluated that the incorporation of the detergent upgraded the post thaw spermatozoa motility. Detergents have protective effects and may be exerted directly on the sperm membrane or emulsifying the egg yolk lipids which becomes immediately available to the plasma lemma during cryopreservation (Graham et al., 1971; Arriola and Foote 1987; Buhr and Pettitt 1996). Singh et al., (1996) found that incorporation of ascorbic acid (2.5 mM) in the buffalo bull semen extender yielded a significantly enhance the post-thaw motility and longevity. Although, Kolev (1997) reported that incorporation of 0.3 mg/ml of vitamin E in extender had exhibit better effects on acrosomal integrity, survivability and motility of cryopreserved buffalo bull spermatozoa and tocopherol (vitamin E) obstructs lipid peroxidation (LPO) in membranes, acting as a scavenger of lipid peroxyl and alkoxy radicals, thus preventing oxidative damage in cryopreserved bovine semen (Beconi et al., 1991). Fabbrocini et al., (2000) recommended that addition of 1.25 mM sodium pyruvate into the extender resulting in significantly improved post-thaw progressive motility and viability due to its antioxidant property during cryopreservation of buffalo semen. It is also reported that addition of oviductal proteins obtained from various stages of the oestrous cycle into extender affected the post-thaw semen characteristics due to lowered the LPO levels in buffalo spermatozoa during cryopreservation (Kumaresan et al., 2006). Recently, Shukla and Misra (2007) studied that addition of Bradykinin (2 ng/ml) in Tris-based extender to improve post thaw buffalo semen characteristics during cryopreservation. Ijaz et al., (2009) found that the Butylatedhydroxytoluene (BHT) at concentration 1.0 and 2.0 mM in tris citrate egg yolk extender provided better results for cryopreservation of buffalo bull semen. From the above cited studies, the incorporation of some additives in buffalo semen extender in terms of improvement in the quality of frozen–thawed buffalo spermatozoa.

**Osmotic pressures**

Semen dilution is a prime importance to maintain fertilizing potential of cryopreserved buffalo semen (Rasul et al., 2000). In the extender the concentration of the solutes become high, the osmotic pressure is elevated. So, number of solute particles affects the osmotic pressure as well as freezing point of the solvents in a solution. When spermatozoa are exposed to hyperosmolal solution, more extra cellular ice crystals are formed (Watson, 1995) and vice versa. Polyhydric alcohols and water have high osmotic permeability coefficient which increases the shifting of these substance across the palsa membrane of the spermatozoa during cryopreservation (Noiles et al., 1993). Spermatozoa either swell or
shrink under hypotonic/ hypertonic solution (DU et al., 1994; Gilmore et al., 1996). Any change in the osmotic pressure during cryopreservation causing damage of plasma membrane of spermatozoa. Hence, osmotic pressure plays a crucial role in semen cryopreservation and affects the frozen semen quality (Khan and Ijaz, 2008). The buffalo bull semen have been found different osmotic pressures, which include 293.33 mOsm/kg (Sansone et al., 2000), 268.81 mOsm/L (Khan and Ijaz, 2008) and 289.4 mOsm/kg (Mughal et al., 2013). From above cited studies, it is concluded that buffalo bull semen has osmotic pressure lower than cattle semen.

Semen processing

Preliminary processing

The semen collection station is commonly located near the semen laboratory and it is commonly suggested that semen should be processed as early as possible after collection. No changes have been recognized in morphology, motility and survivability of spermatozoa, if the semen was processed within 1 h of collection (Fabbrocini et al., 1995). Generally semen dilution, having all compulsory constituents, is done at 30°C to 37°C. Vale et al., (1991) suggested the ejaculates should be kept for 10 to 15 minutes in its own plasma, after sometimes semen of buffaloes. To prevent agglutination immediately added the diluents very soon after collection may and also maintain the motility of spermatozoa. On the other words, there is delay in semen processing immediately after semen collection, it should be diluted with suitable extender and stored at 5°C (Televi et al., 1994).

Semen dilution

In case of buffalo semen freezing, one step or two steps dilution methods are being used. Del Sorbo et al., (1994) correlated both methods (one and two steps) using tris-egg yolk-based extenders and reported that the two-step method provide superior results with long 6 h equilibration, while one-step dilution method required shorter period 2 to 4 h equilibration time before freezing. Fabbrocini et al., (1995) reported that in two step dilutions method, addition of glycerol showed better sperm motility when glycerol was added 1 h before freezing. Del Sorbo et al., (1995) also found two step semen dilution methods using sodium pyruvate (1.25 mM) with second dilution 1h prior to freezing the semen.

Cooling rates, equilibration time and freezing

After semen dilution, the semen is cooled at a temperature of 4 °C or 5 °C. Cooling is a necessary to spermatozoa to lowered metabolism. Diluted semen is cooled slowly to avoid cold shock. Cold shock is produced to impair function of membrane proteins that are necessary for structural integrity or ion metabolism of spermatozoa (Watson 2000). During freezing, buffalo spermatozoa are more susceptible to cryoinjury than cattle spermatozoa (Raizada et al., 1990). These cryoinjury can be lowered by optimizing the cooling and freezing rates and using suitable diluting media (Kumar et al., 1992). Talevi et al., (1994) studied both slow and fast cooling protocols in buffalo bull semen and found the slow cooling (28°C to 5°C in 1 hour) and rapid cooling (28°C to 5°C in 15 minutes) protocols had no significant differences in post-thaw motility of spermatozoa. Mazur et al., (1972); and Mazur, (1977) found that spermatozoa are exposed to high salt concentration and osmolality with changes in PH during slow cooling process. While during fast cooling, intracellular water may not pass out of membrane, resulting in intracellular ice crystals formation. Sahni and Mohan (1990)
reported that no significant difference on post-thaw sperm motility between these two cooling rates. In terms of other study, Sukhato et al., (2001) found that cooling rates of 20 or 30°C / min yielded better progressive post thaw motility and fertility using the effects of three cooling rates (10, 20 or 30°C / min) from 4°C to each of either -40°C, - 80°C or -120°C before plunging into liquid nitrogen. Anzar et al., (2010) worked at different cooling rates for buffalo bull spermatozoa and reported higher motility, plasma membrane integrity and acrosomal morphology at high freezing rates of -30°C/ minute. The beneficial effects of slow cooling rate of diluted semen are considered at 5°C due to rapid penetration of glycerol in the cell membrane (Ennen et al., 1976; Gilbert and Almquist, 1978). However investigators prefer slow cooling rate of 0.2 to 0.4°C/minute for the pre-freezing processing of the buffalo bull semen.

Researchers found different equilibration durations during cryopreservation. Regarding equilibration, it is generally called the ‘glycerol equilibration’ period, during this stage; glycerol rapidly penetrates into the spermatozoa to establish equilibrium between its intracellular and extracellular concentration and other osmotically active extender components (Salamon and Maxwell, 2000). Singh et al., (1989); and Del Sorbo et al., (1995) recommended short equilibration periods of 2 to 4 h while another investigators favored longer duration of about 6 h (Haranath et al., 1990; Televi et al., 1994). It is commonly trusted that semen should be kept at 5 °C for not less than 2 h and not more than 6 h before freezing for cryopreservation of buffalo bull semen.

Cassou (1964) reported that semen is filled and sealed commonly at 5 °C in the straws of 0.25 ml or 0.5 ml capacity. Straws of 0.25 ml are generally used because of their cost and storage space. Filled and sealed straws are placed in horizontal position 1 to 4 cm above liquid nitrogen gas for 10 to 20 minutes before plunging into liquid nitrogen gas at -196°C.

**Thawing rates and temperature**

Different thawing temperature and time were used by the researchers. Mazur, (1984) found that fast thawing of semen prevents formation of recrystallization of water. Rao et al., (1986) compared two thawing rates (37°C for 30 s and 75°C for 9 s) and found that the best value for post-thaw motility was observed for semen thawed at 37°C for 30 s. Dhami et al., (1996) reported different thawing rates at 4°C for 5 min, 40 °C for 1 min or 60 °C for 15 s and found that thawing of semen at 60 °C for 15 s yielded high post-thawing spermatozoal recovery and longevity. El-Amrawi (1997) worked at different thawing procedure and got best fertility rates when semen was thawed at 35°C for 60 seconds.

**Future directions**

Future research should highlight on freezing protocols improvement for lower spermatozoa damage during buffalo bull semen cryopreservation. To achieve this goal, diluents composition, freezing protocols along with the improvement of presently used extenders must be focused for buffalo semen cryopreservation. Commercially available extenders i.e. Triladyl (Minitub Germany), Biociphos (IMV, France) and Laciphos (IMV, France), etc. should also be tried for cryopreservation of buffalo bull semen. Therefore, a better understanding of the fundamental principle of cryopreservation of buffalo spermatozoa is necessary according to the specific requirements. Moreover, there is a need to develop biochemically defined extenders and cryogenic procedures that may result in improvement in viability and fertility of frozen thawed buffalo spermatozoa.
References

Abbas A, Andrabi SMH, (2002). Effect of different glycerol concentrations on motility before and after freezing, recovery rate, longevity and plasma membrane integrity of Nili-Ravi buffalo bull spermatozoa. Pak Vet J 22, 1–4.

Abhi, H. L. (1982). Note on the freezing of buffalo semen by land shut method and fertility of frozen semen in comparison to liquid semen under field conditions. Indian J. Anim. Sci. 52: 809-811.

Ahmad Z, Anzar M, Shahab M, Ahmad N, Andrabi SMH, (2003). Sephadex and sephadex ion-exchange filtration improves the quality and freezability of low grade buffalo semen ejaculates. Theriogenology 59, 1189–1202.

Ahmad, K. and Foote, R.H. (1986). Post thaw survival and fertility of frozen bull spermatozoa treated with antibiotics and detergent. J. Dairy Sci., 69: 535.

Ahmed, K. and M. Greesh. (2001). Effect of antibiotics on the bacterial load and quality of semen of Murrah buffalo bulls during preservation. Indian J. Anim. Reprod., 22: 79-80.

Aisen EG, Medina VH, Venturino A, (2002). Cryopreservation and post-thawed fertility of ram semen frozen in different trehalose concentrations. Theriogenology 57, 1801–1808.

Aisen, E.G., H.L. Alvarez, A. Venturino and J.J. Garde. (2000). Effect of trehalose and EDTA on cryoprotective action of ram semen diluents. Theriogenology, 53: 1053-1061.

Akhter S, Ansari MS, Andrabi SMH, Ullah N, Qayyum M, (2008). Effect of antibiotics in extender on bacterial and spermatozoal quality of cooled buffalo (Bubalus bubalis) bull semen. Reprod Domest Anim 43, 272–278.

Akther, S., Rakha, B. A., Ansari, M. S., Andrabi, S. M. H. and Ullah, N. (2011). Storage of Nili-Ravi buffalo (Bubalus bubalis) semen in skim milk extender supplemented with ascorbic acid and α tocopherol. Pak. J. Zool. 43: 273-277.

Aleem M, Chaudhry RA, Khan NU, Rizvi AR, Ahmed R, (1990). Occurrence of pathogenic bacteria in buffalo semen. Buffalo J 6, 93–98.

Ali H, Din A, Samad HA, Ali S, Sabri MA, (1994). Comparative effects of combiotic, ampicillin and gentamycin sulphate on motility percentage, liveability and absolute index of liveability in the buffalo bull semen. Pak Vet J 14, 223–227.

Alvarez, J. G. and Storey, B. T. (1982). Spontaneous lipid peroxidation in rabbit epididymal spermatozoa, its effect on sperm motility. Biol. Reprod. 27: 1102–1108.

Amin AS, Darwish GM, Maha SZ, Hassan HM, (1999). Trial to control Chlamydia psittaci in processed buffalo semen. Assi Vet Med J 40, 319–332.

Andrabi SMH, (2009). Factors Affecting the Quality of Cryopreserved Buffalo (Bubalus bubalis) Bull Spermatozoa. Reprod Dom Anim 44, 552–569.

Andrabi SMH, Ahmad N, Abbas A, Anzar M, (2001). Effect of two different antibiotic combinations on fertility of frozen buffalo and Sahiwal bull semen. Pak Vet J 21, 166–169.

Andrabi SMH, Ansari MS, Ullah N, Anwar M, Mehmood A, Akhter S, (2008). Duck egg yolk in extender improves the freezability of buffalo bull spermatozoa. Anim Reprod Sci 104, 427–433.

Anzar, M., Z. Rasul, T.A. Ahmed and N. Ahmad. (2010). Response of buffalo spermatozoa to low temperatures during cryopreservation. Reprod. Fert. Develop., 22: 871-880.

Anzar, M., Farooq, U., Mirza, M. A., Shahab, M. and Ahmed, N. (2003). Factors affecting the efficiency of artificial insemination in cattle and buffalo in Punjab, Pakistan. Pak. Vet. J. 23: 106-113.

Arabi, M. and Seidaie, S. R. (2008). Assessment of motility and membrane peroxidation of bull spermatozoa in the presence of different concentration of vitamin C. Vet. Med. J. Shahrekord. 2: 39-46.

Arriola J, Foote RH, (1987). Glycerolation and thawing effects on bull spermatozoa frozen in detergent-treated egg yolk and whole egg extenders. J Dairy Sci 70, 1664–1670.

Bailey JL, A Morrie and N Cormier, (2003).
Semen cryopreservation: success and persistent in farm species. Canadian J. Anim. Sci., 83:393-401.

Bamba K, Adams CE (1990). Freezing rabbit semen by use of BF5 diluent. Lab Anim 24:172–175.

Bansal, A. K. and Bilaspuri, G. S. (2009). Antioxidant effect of vitamin E on motility, viability and lipid peroxidation of cattle spermatozoa under oxidative stress. Anim. Sci. Pap. Rep. 27: 5-14.

Barbas, J. P. and Mascarenhas, R. D. (2009). Cryopreservation of domestic animal sperm cells. Cell Tissue Bank. 10: 49-62.

Bates RG, (1961). Amine buffers for pH control. Ann N Y Acad Sci 92, 341–356.

Beconi M, Affranchino M, Schang LM, Beorlegui NM, (1991). Influence of antioxidants on SOD activity in bovine sperm. Biochem Int 3, 545–553.

Bhosrekar, M.R., J.R. Purohit and B.R. Mangurkar. (1990). Studies on the effect of additives to semen diluent. Indian J. Anim. Reprod., 11: 85-88.

Bilodeau, J. F., Blanchette, S., Gagnon, C. and Sirard, M. A. (2001). Thiols prevent H2O2-mediated loss of sperm motility in cryopreserved bull semen. Theriogenology. 56: 275-286.

Cheshmedjieva SB, Dimov VN, (1994). Effect of freezing on phospholipid distribution of buffalo spermatozoa plasma membranes. In: Vale WG, Barnabe VH, Mattos JCA de, Proceedings of 4th World Buffalo Cong., Sao Paulo, Brazil. International Buffalo Federation, Roma, Italy, pp. 519–521.

Chinnaiya, G.P. and N.C. Ganguli. (1980). Freezability of buffalo semen in different extenders. Zbl. Vet. Med. A., 27: 563-568.

Chohan, A. R., Iqbal, J., Asghar A. A. and Chaudhary, M. A. (1992). Fertility of liquid and frozen semen in Nili Ravi buffaloes. Pak. Vet. J. 12: 4-5.

Curry MR and Watson PF. (1994). Osmotic effects on ram and human sperm membranes in relation to thawing injury. Cryobiology 31: 39-46.

Del Sorbo, C., G. Fasano, A. Fabbrocini, M.J.F. Nastri and G. Sansone. (1994). Studio preliminare su effetti di vari agenti crioprotettivi sulla motilità di spermatozoi bufalini B. bubalis allo scongelamento. In Proceeding of 6th meeting nazionale “Studi della efficienza riproduttiva deli animali di interesse zootecnico.” Bergamo,
Del Sorbo, C., G. Fasano, A. Fabbrocini, S.L. Lavadera and G. Sansone. (1995). Piruvato quale substrato energetico in extenders crioprotettivi. Effetti sulla motilitaallo scongelamento di spermatozoi bufalini Bubalus bubalis. In Proceedings of 7th Meeting Nazionale “Studio Sulla Efficienza Riproduttiva Degli Animali Diinteresse Zootecnico.” Bergamo, Italy. 1: 63-67.

DeLamirande, E., Jiang, H. and Zini, A. (1997). Reactive oxygen species and sperm physiology. Rev. Reprod. 2: 48–54.

Dhami AJ, Sahni KL, (1993). Effect of extenders, additives and deep freezing on the leakage of lactic dehydrogenase from cattle and buffalo spermatozoa. Indian J Anim Sci 63, 251–256.

Dhami AJ, Sahni KL, Mohan G, Jani VR, (1996). Effects of different variables on the freezeability, post-thaw longevity and fertility of buffalo spermatozoa in the tropics. Theriogenology 46, 109–120.

Dhami, A.J. and K.L. Sahni. (1994). Effects of various cooling from 30oC to 5oC, equilibration and diluents treatments on freezeability, post-thaw thermoresistance, enzyme leakage and fertility of bubaline semen. Indian J Anim Sci 63, 251–256.

Foote, R.H. (1970). Fertility of bull semen at high extension rates in Tris buffered extenders. J. Dairy Sci., 53: 1475-1477.

Gadea, J., Selles, E., Marco, M. A., Copy, P., Matas, C., Romar, R. and Ruiz, S. (2004). Decrease in glutathione content in boar sperm cryopreservation, effect of the addition of reduced glutathione to the freezing and thawing extenders. Theriogenology. 62: 690-701.

Gangadhar KS, Rao AR, Subbaiah G, (1986). Effect of antibiotics on bacterial load in frozen semen of buffalo bulls. Indian Vet J 63, 489–493.

Gilmore, J.A., J. Du, J. Tao, A.T. Peter and J.K. Critser. (1996). Osmotic properties of boar spermatozoa and their relevance to cryopreservation. J. Reprod. Fertil., 107: 87-95.

Good NE, Izawa S, (1972). Hydrogen ion buffers. Methods Enzymol 24, 53–68.

Good NE, Winget GD, Winter W, Connolly TN, Izawa S, Singh RMM, (1966). Hydrogen preservation programmes for farm animals. In: Hiemstra SJ (ed.), Publication No. 1 of the European Regional Focal Point on Animal Genetic Resources. Lelystad, Netherlands, p. 30.
ion buffers for biological research. Biochemistry 5, 467–477.

Graham EF, Rajamannan AHJ, Schmehl MKL, Maki-Lanrila M, Bower RE, (1971). Preliminary report on procedure and rationale for freezing boar semen. AI Digest 19, 12.

Graham, E.F., B.G. Crabo and K.I. Brown. (1972). Effect of some zwitterion buffers on the freezing and storage of spermatozoa in bull. J. Dairy Sci., 55: 372–376.

Gurel, A., Coskun, O., Armutcu, F., Kanter, M. and Ozen, O. A. (2005). Vitamin E against oxidative damage caused by formaldehyde in frontal cortex and hippocampus. Biochemical and histological studies. J. Chem. Neuroanat. 29: 173–178.

Hammerstedt RH, Graham JK, Nolan JP (1990). Cryopreservation of mammalian sperm: what we ask them to survive. J Androl; 11:73–88.

Haranath, G.B., T.B. Suryaprakasam, A.V.N. Rao and G. Somasekhararam. (1990). Freezability of semen and fertility of frozen semen packaged in mini and medium French straws: a note. In Acharya, R.M., R.R. Lokeshwara and S. Kumar. (eds). Recent Advances in Buffalo Research, 3: 87–88.

Hasan, S., S.M.H. Andrab, R. Muneer, M. Anzar and N. Ahmad. (2001). Effects of a new antibiotic combination on post-thaw motion characteristics and membrane integrity of buffalo and Sahiwal bull spermatozoa and on the bacteriological quality of their semen. Pak. Vet. J., 21: 6-12.

Holt WV, (2000a). Fundamental aspects of sperm cryobiology: the importance of species and individual differences. Theriogenology 53, 47–58.

Holt WV, (2000b). Basic aspects of frozen storage of semen. Anim Reprod Sci 62, 3–22.

Holt, W.V. (2000). Basic aspects of frozen storage semen. Anim. Reprod. Sci., 62: 3-22.

Ijaz, A., A. Hussain, M. Aleem, M.S. Yousaf and H. Rehman. (2009). Butylatedhydroxytoluene inclusion in semen extender improves the post-thawed semen quality of Nili-Ravi buffalo (Bubalus bubalis). Theriogenology, 71: 1326-1329.

Jain YC, Anand SR, (1976). The lipids of buffalo spermatozoa and seminal plasma. J Reprod Fert 47, 255–260.

Jainudeen MR, Das S, (1982). Effect of level of glycerol, rates of freezing and thawing on the survival of buffalo spermatozoa in straws. In: Jainudeen MR, Nasr AR (eds), Proceedings of Asian-Australian Anim Sci Cong, Serdang, Malaysia. Penerbit University. Kuala Lumpur, Malaysia, pp. 409–411.

Johnson MH, Nasr-Esfahani MH, (1994). Radical solutions and cultural problems: could free oxygen radicals be responsible for the impaired development of preimplantation mammalian embryos in vitro? Bioessays 16, 31–38.

Johnson, L.A., Weitze, K.F., Fiser, P., Maxwell, W.M.C., (2000). Storage of boar semen. Anim. Reprod. Sci. 62, 143–172.

Kadirvel, G., Satish, K. and Kumaresan, A. (2009). Lipid peroxidation, mitochondrial membrane potential and DNA integrity of spermatozoa in relation to intracellular reactive oxygen species in liquid and frozen-thawed buffalo semen. Anim. Reprod. Sci. 114: 125-134.

Kakar SS, Anand SR, (1981). Changes in adenosine 5¢-triphosphate, adenosine energy charge and adenosine 3¢5¢-cyclic monophosphate during the freezing of buffalo semen. J Reprod Fert 62, 543–548.

Keith JE, Morrison JF, (1981). Buffers of constant ionic strength for studying pH-dependent processes. Methods Enzymol 87, 405–426.

Khan, M.I.R. and A. Ijaz. (2008). Effects of osmotic pressure on motility, plasma membrane integrity and viability in fresh and frozen-thawed buffalo spermatozoa. Animal, 2: 548-553.

Kolev SI, (1997). Effect of vitamins A, D, E on the motility and acrosomal integrity of cryopreserved buffalo bulls’ spermatozoa. In: Borghese A, Failla S, Barile VL (eds), Proceedings of 5th World Buffalo Cong., Caserta, Italy. International Buffalo Federation, Roma, Italy, pp. 833–835.

Kumar S, Sahni KL, Mohan G, (1992). Effect of different levels of glycerol and yolk on freezing and storage of buffalo semen in milk, Tris and sodium citrate buffers. Buffalo J 8, 151–156.

Kumar S, Sahni KL, Mohan G, (1994). Effect of
yolk, glycerol and sugars on post-thaw survival of buffalo spermatozoa in Tris dilutor. Indian J Anim Sci 64, 362–364.

Kumar, R., Jagan, G., Mohanarao Arvind and Atreja, S. K. (2011). Freeze-thaw induced genotoxicity in buffalo (Bubalus bubalis) spermatozoa in relation to total antioxidant status. Mol. Boil. Rep. 38: 1499-1506.

Kumar, S., K.L. Sahni and G. Mohan. (1992). Effect of different levels of glycerol and yolk on freezing and storage of buffalo semen in milk, tris and sodium citrate buffers. Buffalo J., 2: 151-156.

Kumaresan A, Ansari MR, Abhishek G, (2005). Modulation of post-thaw sperm functions with oviductal proteins in bufaloes. Anim Reprod Sci 90, 73-84.

Kumaresan A, Ansari MR, Garg A, Kataria M, (2006). Effect of oviductal proteins on sperm functions and lipid perox-ipation levels during cryopreservation in bufaloes. Anim Reprod Sci 93, 246-257.

Kundu, C.N., J. Chakraborty, P. Dutta, D. Bhattacharyya, A. Ghosh and G.C. Majumder. (2002). Effects of dextrans on cryopreservation of goat caudaepididymal spermatozoa using a chemically defined medium. Reproduction, 123: 907-913.

Marco-Jimenez F, Puchades S, Moce E, Viudes-de-Cartro MP, Vicente JS, Rodriguez M, (2004). Use of powdered egg yolk vs fresh egg yolk for the cryopreservation of ovine semen. Reprod Domest Anim 39, 438–441.

Matharoo, J.S. and M. Singh. (1980). Revivability of buffalo spermatozoa after deep freezing the semen using various extenders. Zbl. Vet. Med. A., 27: 385-391.

Mazur, P. (1977). The role of intracellular freezing in the death of cells cooled at supraoptimal rates. Cryobiology, 14: 251-272.

Mazur, P. (1984). Freezing of living cells: Mechanisms and implications. Am. J. Physiol., 247: C125-142.

Mazur, P., S.P. Liebo and E.H.Y. Chu. (1972). A two-factor hypothesis of freezing injury. Exp. Cell Res., 71: 345-355.

Medeiros CMO, Forell F, Oliveira ATD, Rodrigues JL, (2002). Current status of sperm cryopreservation: why is it better. Theriogenology 57, 327–344.

Molinia FC, Evans G, Casares PI, Maxwell WMC, (1994). Effect of monosaccharides and disaccharides in Tris-based diluents on motility, acrosome integrity and fertility of pellet frozen ram spermatozoa. Anim Reprod Sci 36, 113–122.

Morrell JM, (2006). Update on semen technologies for animal breeding. Reprod Domest Anim 41, 63–67.

Muer SK, Roy SB, Mohan G, Dhoble RL, (1988). Cryogenic changes in seminal protein of cattle and buffalo. Theriogenology 30, 1005–1010.

Mughal, D. W., Ijaz, A., Yousaf, M. S., Wadood, F. & Umer, F. M. (2017). Cryopreservation of buffalo (Bubalus bubalis) semen-limitations and expectations. Buffalo Buletin, 36(1), 1-14.

Mughal, D.H., A. Ijaz, M.S. Yousaf, H. Rehman, M. Aleem, H. Zaneb and F. Wadood. (2013). Assessment of optimal osmotic pressure of citrate egg yolk extender for cryopreservation of buffalo bull (Bubalus bubalis) semen, J. Anim. Plant Sci., 23(4): 964-968.

Nagase, H., T. Niwa, S. Yamashita and S. Irie. (1964). Deep freezing bull semen in concentrated pellet form. II. Protective action of sugars. In Proceedings of 5th International Congress on Animal reproduction and artificial insemination, Trento, Italy. 2: 1111-1113.

Nair, S. J., Brar, A. S., Ahuja, C. S., Sangha, S. P. and Chaudhary, K. C. (2006). A comparative study on lipid peroxidation, activities of antioxidant enzymes and viability of cattle and buffalo bull spermatozoa during storage at refrigeration temperature. Anim. Reprod. Sci. 96: 21-29.

Nastri MJF, Del Sorbo C, Fabbrocini A, Fasano G, Sansone G, (1994). Performances motility in cooled and freeze thawed B. bubalis spermatozoa at different osmotic pressures. In: Bradley M, Cummins JM (eds), Proceeding of 7th Int. Symp. Spermatology, Cairns, Australia. Australian Society of Reproductive Biology, Victoria, Australia, pp. 99–100.

Noiles, E.E., P. Mazur, P.F. Watson, F.W. Kleinhans and J.K. Critser. (1993). Determination of water permeability
coefficient for human spermatozoa and its activation energy. Biol. Reprod., 48: 99-109.

Numanbucak, M., Atessahin, A., Varisli, O., Yuce, A., Tekin, N. and Akcay, A. (2007). Influence of trehalose, taurine, cysteamine and hyaluronan on ram semen. Microscopic and oxidative stress parameters after freeze–thawing process. Theriogenology. 67(5): 1060-1067.

Numanbucak, M., Tuncer, P. B., Sariozkan, S. and Ulutas, P. A. (2009). Comparison of the effects of glutamine and an amino acid solution on post thawed ram sperm parameters, lipid peroxidation and antioxidant activities. Small Ruminant Res. 81: 13-17.

Oba, E., E.J. Fuck, S.D. Bicudo, F.O. Pap and O.M. Ohashi. (1994). Preliminary study on different mediums for deep freezing of buffalo semen, p. 579-581. In Vale, W.G., V.H. Barnabe and J.C.A.de. Mattos (eds.) Proceedings of 4th World Buffalo Congress, Sao Paulo, Brazil. International Buffalo Federation, Roma, Italy.

Pace MM, Graham EF, (1974). Components in egg yolk which protect bovine spermatozoa during freezing. J Anim Sci 39, 1144-1149.

Pasha, T. N. and Hayat, Z. (2012). Present situation and future perspective of buffalo production in Asia. J. Anim. Plant Sci. 22: 250-256.

Priyadharsini, R., Jindal, S.K., Sharma, D., Ramachandran, N., Kharche, S.D. and Goel, A.K. (2011). Effect of different egg yolk level on cryopreservation capabilities of jakhrana goat semen. J. Ani. Sci. Adv. 1: 28-37.

Purdy, P.H. (2006). A review on goat sperm cryopreservation. Small Rum. Res., 6: 215-225.

Raizada BC, Sattar A, Pandey MD, (1990). A comparative study of freezing buffalo semen in two dilutors. In: Acharya RM, Lokeshwar RR, Kumar AT (eds), Proceedings of 2nd World Buffalo Cong, New Delhi, India. International Buffalo Federation, Roma, Italy, pp. 66–74.

Ramakrishnan P, Ariff MO, (1994). Effect of glycerol level and cooling rate on post-thaw semen quality of Malaysian swamp buffalo.

In: Vale WG, Barnabe VH, Mattos JCA de, Proceedings of 4th World Buffalo Cong, Sao Paulo, Brazil. International Buffalo Federation, Roma, Italy, pp. 540–542.

Rao AVN, Haranath GB, Sekharam GS, Rao JR, (1986). Effect of thaw rates on motility, survival and acrosomal integrity of buffalo spermatozoa frozen in medium French straws. Anim Reprod Sci 12, 123–129.

Rasul Z, Ahmed N, Anzar M, (2007). Antagonist effect of DMSO on the cryoprotection ability of glycerol during cryopreservation of buffalo sperm. Theriogenology. 68, 813–819.

Rasul Z, Anzar M, Jalali S, Ahmad N, (2000). Effect of buffering systems on post-thaw motion characteristics, plasma membrane integrity, and acrosome morphology of buffalo spermatozoa. Anim Reprod Sci 59, 31–41.

Rasul, Z., Ahmed, N. and Anzar, M. (2001). Changes in motion characteristics, plasma membrane integrity and acrosome morphology during cryopreservation of buffalo spermatozoa. J. Androl. 22(2): 278-283.

Ruigh, L., Bosch, J.C., Brus, M.C., Landman, B., Merton, J.S., (2006). Ways to improve the biosecurity of bovine semen. Reprod. Dom. Anim. 41, 268–274.

Saacke RG, (1984). Semen quality: importance of and influencing factors. In: Proceedings of 10th NAAB Tech. Conf. A. I. Reprod Milwaukee, WI, USA. National Association of Animal Breeders, Columbia, USA, pp. 30–36.

Sahni, K.L. and G. Mohan. (1990). Yolk as a cryoprotectant in deep-freezing of bovine semen. Indian J. Anim. Sci., 60: 828-829.

Salamon S, Maxwell WMC, (2000). Storage of ram semen. Anim Reprod Sci 62, 77-111.

Sansone, G., M.J.F. Nastri and A. Fabbrocini. (2000). Storage of buffalo (Bubalus bubalis) semen. Anim. Reprod. Sci., 62: 55-76.

Senatore EM, Verberckmoes S, Pascale M, Presicce GA, (2004). A deep uterotubal semen deposition in Mediterranean Italian bufaloes using a new artificial insemination device. Reprod Fertil Dev 16, 133.
Shannon P, (1972). The effect of egg yolk level and dose rate of semen diluted in caprogen. Proceedings of 7th Int. Cong. Anim. Reprod. A.I. Munich, Germany. International Congress on Animal Reproduction and Artificial Insemination, Milan, Italy, pp. 1440–1442.

Shannon, P., Curson, B., (1983). Effect of egg yolk levels on the fertility of diluted bovine sperm stored at ambient temperatures. N. Z. J. Agric. Res. 26, 187–189.

Shukla MK, Misra AK, (2007). Effect of Bradykinin on Murrah buffalo (Bubalus bubalis) semen cryopreservation. Anim Reprod Sci 97, 175–179.

Sinclair, S. (2000). Male infertility nutritional and environmental considerations. Altern. Med. Rev. 5: 28–38.

Singh B, Chand D, Singh P, Yadav NK, (1996). Effect of vitamin C addition in the diluent on the quality of deep frozen Murrah buffalo (Bubalus bubalis) semen. Int J Anim Sci 11, 131–132.

Singh J, Pangawkar GR, Biswas RK, Srivastava AK, Sharma RD, (1990). Studies on lactic dehydrogenase and sorbitol dehydrogenase release in relation to deep freezing of bualo semen in certain extenders. Theriogenology 34, 371–378.

Singh M, Pant HC, (2000). Effect of post thaw incubation on semen quality of bualo bulls comparison with cattle. Buffalo Bull 19, 51–54.

Singh P, Singh I, Singh S, Sharma RK, (2006). Initial stage glyceralization prevents the incidence of backward sperm motility during cryopreservation and increases buffalo semen freezability. Indian J Anim Sci 76, 777–779.

Singh P, Singh S, Hooda OK, (1999). Effect of different level of egg yolk on freezability of bualo semen. Haryana Vet 38, 26–28.

Singh, J., G.R. Pangawkar, R.K. Biswas and N. Kumar. (1991). Studies on buffalo sperm morphology during various stages of freezing in certain extenders. Indian J. Anim. Sci., 12: 126-129.

Singh, N.P., R.S. Manik and V.S. Raina, (1989). Effect of cysteine fortification on cryopreservation of buffalo semen in milk whey extenders. Theriogenology, 32: 979-986.

Singh, P., J.K. Jindal, S. Singh and O.K. Hooda. (2000). Freezability of buffalo bull semen using different extenders. Indian J. Anim. Reprod., 21: 41-42.

Snedeker WH, Gaunya WS (1970). Dimethyl sulfoxide as a cryoprotective agent for freezing bovine semen. J Anim Sci 30:953–956.

Sreejith, J. N., Brar, A. S., Ahuja, C. S., Sangha, S. P. S. and Chaudhary, K. C. (2006). A comparative study on lipid peroxidation, activities of antioxidant enzymesand viability of cattle and buffalo bull spermatozoa during storage at refrigeration temperature. Anim. Reprod. Sci. 96: 21-29.

Sukhato P, Thongsodseang S, Utha A, Songsasen N, (2001). Effects of cooling and warming conditions on post-thawed motility and fertility of cryopreserved buffalo spermatozoa. Anim Reprod Sci 67, 69–77.

Talevi, R., S. Pelosi, G. Sansone, F. Grasso and D. Matassino. (1994). Effects of different pre-freezing rates on buffalo sperm motility and ultrastructure preservation. In Proceedings of 4th International Buffalo Congress, São Paulo, Brazil. 3: 537-539.

Tatham B, (2000). Increasing Buffalo Production; Using Reproduction Technology. Report Rur. Indust. Res Corp. Dev., Kingston, ACT, Australia.

Thibier, M. and B. Guerin. (2000). Hygienic aspects of storage and use of semen for artificial insemination. Anim. Reprod. Sci., 62: 233-251.

Tosic J, Walton A, (1950). Metabolism of spermatozoa. The formation and elimination of hydrogen peroxide by spermatozoa and eects on motility and survival. Biochem J 47, 199–212.

Tuli, R.K., Singh, M., Matharoo, J.S., (1981). Effect of different equilibration times and extenders on deep freezing of buffalo semen. Theriogenology 16, 99–104.

Vale, W.G., O.M. Ohashi, H.F.L. Ribeiro and J.S. Sousa. (1991). Semen freezing and artificial insemination in water buffalo in the amazon valley. Buffalo J., 2: 137-144.

Watson PF, (2000). The causes of reduced fertility with cryopreserved semen. Anim Reprod Sci 60 61, 481–482.
Watson PF, Kunze E, Cramer P, Hammerstedt RH, (1992). A comparison of critical osmolality and hydraulic conductivity and its activation energy in fowl and bull spermatozoa.

Watson, P. F. (1995). Recent developments and concepts in the cryopreservation of spermatozoa and the assessment of their post-thawing function. Reprod. Fertil. Dev. 7: 871–91.

Watson, P. F., & Martin, I. C. A. (1975). Effects of egg yolk, glycerol and the freezing rate on the viability and acrosomal structures of frozen ram spermatozoa. Australian journal of biological sciences, 28(2), 153-160.

Woelders, H., A. Mathijs and B. Engel. (1997). Effects of trehalose, and sucrose, osmolality of the freezing medium, and cooling rate on viability and intactness of bull sperm after freezing and thawing. Cryobiology, 35: 93-105.

Yousef, M. I., Abdallah, G. A., and Kcalamel, K. I. (2003). Effect of ascorbic acid and vitamin E supplementation on semen quality and biochemical parameters of male rabbits. Anim. Reprod. Sci. 76: 99-111.

Yu ZW, Quinn PJ, (1994). Dimethyl sulphoxide: a review of its applications in cell biology. Biosci Rep 14, 259–281.

How to cite this article:

Pavan Kumar Mittal, A.K. Madan, Vijay Sharma, G.S.Gottam and Barkha Gupta. 2019. Cryopreservation of Buffalo Bull Semen- Restriction and Expectation: A Review. Int.J.Curr.Microbiol.App.Sci. 8(01): 1351-1368. doi: https://doi.org/10.20546/ijcmas.2019.801.144