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Mini Review

Bovine Serum Albumin (BSA) and Foetal Bovine Serum (FBS) for Cryopreservation of Semen in Domestic Animals: Mini Review

Arjuma Khatun and Nafis I Assad*

Division of Animal Reproduction Gynaecology and Obstetrics, FVSc & AH, Shuhama, SKUAST-Kashmir-190006 (J&K), India

***Corresponding Author:** Nafis I Assad, Division of Animal Reproduction Gynaecology and Obstetrics, FVSc & AH, Shuhama, SKUAST-Kashmir-190006 (J&K), India.

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Abstract

Bovine serum Albumin (BSA) is the most abundant plasma protein found in animals that is primarily associated with transport of biologically active components via systemic circulation to the target cells. Foetal Bovine Serum (FBS) is a combination product of various sugars, lipids, amino acids, vitamins, growth factors, trace elements and hormones. It is used for growth and maintenance of most types of cell and tissue cultures. Both BSA and FBS are more advantageous in terms of affordability, availability, ease of handling as well as comprehensive quality control checks and assurance testing than the existing extender additives like egg-yolk, coconut milk or skimmed milk. In order to tap the advantages of these proprietary preparations both BSA and FBS have been used in semen preservation. It was found that BSA can inhibit lipid peroxidation in sperm membrane besides improving motility and viability post freezing. It was also found that BSA can substitute egg yolk in the semen extenders of some animals. FBS has been used in semen cryopreservation to take advantage of its buffering properties and benefit from its proteins that confer mechanical protection to plasma membrane. It was further documented that addition of FBS reduced malondialdehyde production (MDA) and raised glutathione peroxidase activity (GPx) in the extended Semen.

Keywords: Cryopreservation, Semen, FBS, BSA, Domestic animals

Introduction

Artificial insemination (AI) is the most popular assisted reproductive technique used to improve the productivity in animal husbandry practices world over. In large animals like Cattle, Buffalo and Equine, AI has now assumed a routine character given its wide acceptability. Of late it is picking up in small ruminants like sheep and goat as well. Success of any AI program, however, critically depends upon efficient preservation of spermatozoa. Semen preservation at room temperature, refrigeration temperature as well as cryopreservation using liquid Nitrogen has been tried with varied success across domestic animals. Many extenders have been used in this process. Simple extenders like egg yolk-phosphate, skim milk, coconut milk, egg yolk citrate, Illinois Variable temperature extender etc. have been used for room temperature and refrigeration temperature storage of spermatozoa. For cryopreservation extenders like glycerolated Tris-egg yolk extender, soya lecithin, etc. have been used. Diluents used for cryopreservation ensure the supply of energy sources to the sperm cells along with protection from temperature related stress. Generally, the cryopreservation media used include an energy source (fructose, glucose, lactose, trehalose,

etc.) a buffer (Tris), a permeating cryoprotectant (glycerol, ethyleneglycol or dimethyl sulfoxide), a non-permeating cryoprotectant (milk, egg yolk, FBS, BSA), some salts (citric acid, sodium citrate) and antibiotics (penicillin, streptomycin, gentamicin, lincospectin, etc.) [1]. It is only logical that separate studies are undertaken on different components present in these extenders in the pursuit of improvement in the post-thaw sperm viability and fertility of semen.

Studies although limited in number that have been conducted to investigate the effects of non-permeating cryoprotectants like FBS and BSA on spermatozoa during freeze-thaw cycle. Ram and Buck semen received a heightened attention in these studies as many researchers toiled to develop species specific semen extenders in order to leverage AI in these species. It is in this backdrop that the authors carried out a review of literature to compile and consolidate the relevant research works that have been carried out regarding use of FBS and BSA in cryopreservation of semen. The wisdom of the review is to aid and accentuate the efforts being done by various researchers to find alternatives that can be used in semen cryopreservation.

Use of BSA and FBS in semen preservation/cryopreservation

Serum albumin is the protein found in great abundance in the circulatory system of higher animals including mammals [2]. Bovine Serum Albumin-BSA is a low-density water-soluble lipoprotein consisting of 583 amino acids, with a molecular weight of 69000 Daltons [3]. This protein plays a key role in transport of a number of metabolites to their specific target organs, tissues and cells and also removes the free radicals formed during oxidative stress [4]. BSA stabilises the cell membranes and thus protects sperm membrane from rupture and cracking during the freeze-thaw cycle of the semen [5].

Foetal Bovine Serum-FBS is a peculiar combination of components like sugars, lipids, amino acids, vitamins, growth factors, trace elements and hormones, which is used for growth and maintenance of most types of cell and tissue cultures [6,7]. Serum can be frozen and offers myriad advantages in terms of affordability, availability and ease of handling. The comprehensive quality control checks and assurance testing that FBS and BSA undergo are not observed in other extender additives like egg-yolk or skimmed milk [8].

The plasma membrane of spermatozoa is fairly abundant in polyunsaturated fatty acids and thus susceptible to damage due to peroxidation. Peroxidation leads to generation of reactive oxygen species-ROS with consequent loss of sperm motility [9]. It has been demonstrated that in an aerobic *in vitro* system, sperm cells undergo spontaneous lipid peroxidation [10]. Sarıozkan., *et al*. observed that lipid peroxidation of the liquid rabbit semen was inhibited when BSA was added to the incubation medium [11]. Nair., *et al*. reported that, albumin effectively maintained sperm motility and offered protection against detrimental effect of dilution shock [9]. Kreider., *et al*. reported that maintenance of motility in equine spermatozoa was enhanced when albumin was added either to seminal plasma or diluents when compared to the same media without albumin [12]. Matsuoka and his co-workers documented that in ram semen diluents, BSA can improve the motility and viability of spermatozoa following freezing and can substitute egg-yolk *in-toto* in case of the ram semen diluents [13]. They reported the highest post-thaw sperm motility when 15% BSA was added to the diluent. BSA has successfully been substituted for egg yolk in rainbow trout and turkey spermatozoa [14,15]. Saleh., *et al*. revealed that BSA when used at a concentration of 5% in the media containing 5% glycerol showed best results in frozen ram semen with respect to motility and plasma membrane integrity [16]. They also evidenced a significant decrease in dead spermatozoa and mitochondrial apoptosis post-thawing. Kaewkesa along with his team documented an improved post-thaw quality of buck semen after adding 4 mg/mL BSA to an egg yolk based freezing medium [17]. Zhang., *et al*. investigated the effect of different concentrations of BSA on Boar semen quality. They found that BSA improved semen quality in terms of sperm motility, plasma membrane and acrosomal integrity as well as total anti-oxidative capacity [18]. It was evidenced that 4 g/l BSA when added to semen sample diluted with Modena extender showed best results. Sperm motility, plasma membrane integrity, acrosome integrity, T-AOC activity and MDA content after 7 days' preservation were 54%, 49%, 78%, 1.03 U/ml and 17.5 nmol/ml, respectively. Alomar studied the effect of BSA on the motility patterns of fresh Ram semen using hydrogen peroxide as stressor. He also investigated the ability of BSA to replace egg yolk in media used for chilled semen. It was observed that BSA significantly improved the motility parameters in the samples treated with H_2O_2 and replacing a part of egg yolk by BSA enhanced all velocity parameters. However, use of BSA as total substitution

of egg yolk resulted in a significant decrease ($P < 0.05$) in all CASA motility parameters [19]. Elif Gokçe., *et al*. studied the effect of BSA supplementation on Ram semen stored at 5^0C in soya lecithin extender [20]. It was found that 5 mg/ml BSA supplementation had beneficial effects on all evaluation parameters including motility, plasma membrane integrity, acrosomal integrity and MDA concentration.

Although, the protective properties of foetal bovine serum (FBS) in cell culture are well documented and its potential as a cryoprotectant during freezing has largely remained unexplored. It is quite interesting to note that despite its presence in most of the tissue culture media, details regarding its protective mechanism are still lacking [8]. FBS has been used in semen cryopreservation to tap its buffering properties and benefit from its proteins that confer mechanical protection to plasma membrane. It dampens the risk of crystallization and de-crystallization during freeze-thaw cycle [21,22]. Sarıozkan., *et al*. demonstrated its antioxidant properties in rabbit semen stored at 5^0 C [13]. They reported that addition of FBS reduced malondialdehyde production (MDA) and raised glutathione peroxidase activity (GPx). Blank and his co-workers, observed addition of FBS after cryopreservation improved progressive motility, velocity indexes, oxidative status and membrane peroxidation in chicken spermatozoa. Ma Asuncion and others, evaluated the efficiency of Goat serum and BSA on semen quality parameters after being cryopreserved with [8] Tris-Citric Acid-Fructose-Raffinose-Glycerol-based extender. The team reported that when the Tris-Citric Acid-Fructose-Raffinose-Glycerol extender was supplemented 5% egg yolk, 2.5% goat serum or 30 mg BSA, significant improvements in terms of sperm motility, sperm abnormality and acrosome integrity were observed post thawing [23]. While studying the effects of FCS and BSA on semen quality, Lipid peroxidation and DNA fragmentation on rabbit liquid semen stored at 5^0 Alomar found that addition of BSA and FCS showed a significant effect in the maintaining integrity of plasma membrane between 48 and 72 h storage period when compared to the control ($P < 0.01$). Further, supplementation of FCS and BSA reduced the DNA fragmentation and production of malondialdehyde (MDA) at 48 h and 72 h. They also found an increase in the activity of glutathione peroxidase (GPx) antioxidant [19].

Conclusion

In conclusion, this review highlights the efficiency of BSA and FBS in protecting spermatozoa during freeze-thaw cycles. Both BSA and FBS exhibit antioxidant properties, stabilize cell membranes, and enhance sperm motility and viability post-thawing. The review consolidates evidence from diverse studies, affirming the potential of BSA and FBS as valuable additives in semen extenders. Their potential to replace egg yolk completely or partially and reduce the concentration of cytotoxic cryoprotectants like glycerol as well as buffering properties offers new opportunities in semenology. Research pursuits in this area hold a great promise to refine cryopreservation techniques and advancing the success of assisted reproductive technologies in animal husbandry practices worldwide. Ceased of the opportunity one of the authors has already submitted a research proposal for grants so as to investigate the role of FBS and BSA in preservation of Buck Semen.

Conflict of Interest

The authors declare that there are no conflicts of interest associated with this manuscript. The authors undertake that there are no financial or personal relationships that could potentially bias the work within the manuscript.

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