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Review article

Cryopreservation of bull semen: Evolution from egg yolk based to soybean based extenders

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ABSTRACT

Since the inception of bovine semen cryopreservation, egg yolk and milk based extenders have been used to protect sperm from the detrimental effects of cooling and freezing. In recent years, demand for alternatives to conventional commercial extenders has arisen as the risk of introducing exotic diseases through transporting egg yolk based products has been recognized. Egg yolk can also interfere with sperm evaluation and the presence of particulate material in the extender may reduce fertility. Soybeans contain lecithin, a phospholipid fraction that can substitute for high molecular weight lipoprotein and phospholipids from egg yolk and prevent or ameliorate damage to the sperm plasma membrane that occurs during extension, cooling, and cryopreservation. Soy lecithin based extenders have been evaluated for processing and freezing bovine semen, although extender from soybean milk has not been studied as extensively. Commercially available soy lecithin based extenders are used increasingly but remain under scrutiny and are not universally accepted. With these observations in mind, this review is intended to examine effects of conventional cryopreservation procedures, methods of assessment, and potential for developing soybean extract as an acceptable alternative to traditional egg yolk and milk based extenders for bull sperm cryopreservation.

Contents

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1. Introduction

In modern cattle breeding, where artificial insemination (AI) is the most widely applied tool for facilitating extensive utilization and distribution of semen from genetically superior sires, cryopreservation is a critical part of the process. The potential of artificial insemination can be fully explored and attained only with frozen semen which permits indefinite storage and distribution. With advances in frozen semen technology, post-thaw semen quality has improved significantly. Though the relationship between semen quality and fertility remains difficult to ascertain, lower conception with frozen compared to fresh semen is generally assumed. A popular notion is that approximately 50% of sperm are rendered immotile by cryopreservation and sperm that remain motile are compromised due to cryoinjury, leading to decreased fertilizing capacity (Watson, 1995; Watson, 2000). Advances in cryopreservation techniques for bull semen have progressed slowly over the past several decades and are currently fairly standardized.

Effects of cryopreservation on sperm function and fertility have been studied extensively in many species, particularly in the bull. Sperm ultrastructure, particularly the plasma membrane and outer acrosomal membranes, are compromised during cryopreservation. Induction of premature capacitation (cryo-capacitation) and acrosomal reaction, altered mitochondrial function and thereby reduced motility are among the most significant influences of cryopreservation on the sperm (Parks and Graham, 1992; Hinkovska-Galcheva et al., 1989; Muller et al., 1999; Bailey et al., 2003). Cholesterol efflux from sperm and influx of Na^+ and Ca^{2+} ions are major contributors to cryocapacitation (Breitbart et al., 1984; Langlais and Roberts, 1985; Robertson et al., 1990; Bailey and Buhr, 1994; Bailey et al., 1994; Lin and Kan, 1996; Parrish et al., 1999). Reactive oxygen species (ROS) released from dead or dying sperm are also of concern due to their adverse effects on the live sperm subpopulation in the frozen semen dose (Shannon and Curson, 1972; Kodama et al., 1996; de Lamirande et al., 1998; Upreti et al., 1998).

Numerous approaches have been attempted to reduce sperm damage by modifying extenders and freezing protocols. Addition of cryoprotectant agents such as glycerol, and other components such as egg yolk, milk, bovine serum albumin, polyvinyl alcohol and liposomes to extenders have been reported to reduce the detrimental effects of freezing and thawing sperm (Batellier et al., 2001). Extenders protect sperm from cold shock, osmotic stress, and alterations in membrane fluidity and permeability plus provide energy substrates for sperm metabolism (Batellier et al., 2001; Medeiros et al., 2002). A variety of extenders have been used successfully for bull semen cryopreservation such as citrate-sugar-, lactose-, saccharose-milk-, egg yolk- and some plant base extenders. Variants of Tris-egg yolk- and milk-based extenders have emerged as universal extenders for bull semen cryopreservation (Medeiros et al., 2002; Barbas and Mascarenhas, 2009; Singh et al., 2012).

However, with increasing emphasis on biosecurity issues and controlling disease with international semen shipment, egg yolk extenders have become suspect for facilitating the transmission of diseases. *Escherichia coli, Staphylococcus, Streptococcus, Pseudomonus, Haemophilus, Salmonella*, Avian influenza, *Campylobacter, Listeria* as well as *Mycoplasma* can be transmitted by egg yolk (Thibier and Guerin, 2000). Besides the risk of disease transmission, particulates in yolk and milk pose difficulty in semen evaluation and quality control (Stradaioli et al., 2007).

Growing concern over issues with egg yolk as a semen extender components motivated consideration of alternate plant-based extenders that effectively maintain sperm viability and fertility while minimizing the risk of disease transmission. Among plant based extenders, soybean contains lecithin, a substitute for high molecular weight lipoprotein in egg yolk that can prevent or repair damage to the sperm plasma membrane during cryopreservation. The potential of soybean based extenders for replacing egg yolk extenders has been investigated by several researchers. Most studies have tested the efficacy of commercially available soy lecithin based extenders (Hinsch et al., 1997; Bousseau et al., 1998; Van Wagtendonk-de Leeuw et al., 2000; Aires et al., 2003; Muiño et al., 2007; Arifiantini and Yusuf, 2010; Akhter et al., 2011) but only sporadic studies have been reported so far using total soybean extract as a base medium for bull semen extender (Pankaj, 2006; Kasimanickam et al., 2011; Singh et al., 2012). Further, no standard protocol had been developed to date for extracting soybean milk for use in bull semen extender or other applications.

The present review intends to elaborate upon different facets of cryopreservation including the effects, assessment and control of cryoinjury, and then consider the effectiveness of extenders currently used in commercial semen processing and the potential for soybean milk based extender.

2. Factors affecting fertility of cryopreserved sperm

Successful sperm cryopreservation depends upon several interrelated factors, including initial quality of sperm, extender composition, cryoprotectant, cooling protocol, packaging, thawing rate, and interaction of these components, as well as individual animal variation (Cooter et al., 2005; Andrabi, 2007; Clulow et al., 2008). Some loss in sperm viability is inevitable due to the cumulative effects of semen processing procedures prior to and during the actual freezing process. Moreover, reduced fertility with cryopreserved semen suggests that post-thaw sperm function is compromised even in the viable subpopulation post-thaw (Samper et al., 1991; Watson, 1995; Watson, 2000).

To attain successful fertilization, sperm must remain progressively motile, able to produce energy in the form of ATP for cellular processes, maintain plasma membrane and acrosomal integrity and retain enzymes necessary for oocyte penetration. Disruption of any of these sperm features during semen processing and cryopreservation will likely compromise fertilizing ability. A well-established effect on sperm membrane integrity and function is through the fate of intracellular water during semen freezing. As the temperature of extended sperm is reduced to below freezing, the extender undergoes rapid cooling. As the temperature is reduced further, extracellular ice crystals begin to form from the supercooled water in the