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Developments in Goat Semen Cryopreservation

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ABSTRACT

Sperm cryopreservation simplifies its storage for longer time for use in artificial insemination and assisted reproductive technologies. This technique is also important for breed conservation process and has paved the way for other reproductive biotechnologies. Despite the significant progress in the field, frozen-thawed sperm have enormous inconsistency in fertility rates. It is well known fact that semen cryopreservation exhibited detrimental effects on post-thaw semen motility, plasma membrane, acrosomal status and DNA integrity which ultimately effect the fertility outcome. In addition, several attributes are responsible for low quality of goat cryopreserved semen such as breeds, seasons, management practices and cryopreservation protocols. The aim of the review article is to give an insight of distant features of goat semen cryopreservation as well as recent development in goat semen cryopreservation. It also provides concise information on progress made in the advancement in the semen extender development and cryopreservation of goat sperm.

Introduction

Cryopreservation of semen plays a crucial role in the longstanding ex-situ in vitro conservation of endangered breeds viz. Jamunapari, Jakhrana, Surti, Beetal, and Sangamneri. The efficiency and performance of non-descript (>100 million) could be upgraded and improved their genetics using quality frozen semen in a short span of time (Gama and Bressan, 2011). The selection of elite bucks and maximum utilization of their semen for breeding is a familiar method for raising goat production. Artificial insemination with freshly diluted buck semen has inherent limitations that semen of existing live goats can be used for breed improvement. Obviously, large-scale propagation of proven buck semen through AI with frozen semen is the only alternative means for increasing goat productivity (Gangwar et al., 2016). Large scale propagation of buck semen on national as well as the international levels is not possible unless a suitable technology for freezing buck semen is developed. The post-thaw sperm motility and fertility of cryopreserved sperm are found to be low in goats due to several factors such as age, breeds, seasons, management practices, cryopreservation protocols, and non-availability of goat specific semen extender (Bailey et al., 2000; Al-Ghalban et al., 2004; Arrebola et al., 2017). The detailed molecular mechanism and extent of cryopreservation-associated structural damages to buck sperm have not been explored systematically. In the review paper, we will discuss the current cryopreservation technique with distant features of goat semen cryopreservation

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and its limitation. It also provides concise information on progress made in the advancement in the semen extender development as well as alternative cryopreservation technique to overcome the limitation of the current cryopreservation technique.

Semen extenders for goat semen cryopreservation

The fundamental goal of a semen extender is to provide energy to sperm cells, shield them from cold shock, and provide a favorable atmosphere for sperm during cryopreservation process. Goat semen cryopreservation remains a challenge for breeding program of goat (Purdy, 2006). Tris and citric acid is suitable buffer for goat spermatozoa like bull sperm (Mishra et al., 2010). The most commonly used dilutors for goat semen is either egg-yolk or non-fat dried skim milk based semen extenders. Goats are exceptional among livestock because they releases a specific lipase enzymes from their bulbourethral gland during semen donation that interact with semen extender constituents (egg yolk lipids and skim milk triglycerides) and produce a compound that are toxic to sperm (Sias et al., 2005). Therefore, the lipase enzyme in the seminal plasma is called egg-yolk coagulating enzyme (EYCE). The harmful interaction between egg yolk and the EYCE found only for goat semen but does not found in bull, boar, or even in ram (Roy, 1957; Iritani and Nishikawa, 1963; Iritani et al., 1964). The reduced motilty of sperm with milk-based semen extender is due to a protein fraction from the goat bulbourethral gland secretion called SBUIII. The protein fraction has been purified, characterized and identified as a tricylglycerol lipase (Pellicer-Rubio et al., 1997).

The detrimental interactions of goat seminal plasma with egg yolk and milk were first reported by Roy (1957) and Nunes et al. (1982) respectively. Iritani and Nishikawa, 1963 and Iritani et al., 1964 identified EYCE as phospholipase A. Whereas, Pellicer-Rubio et al. (1997) identified SBUIII as a 55–60 kDa glycoprotein lipase (BUSgp60). It is possible that EYCE and SBUIII is the same enzyme (Leboeuf et al., 2000). Iritani and Nishikawa, 1963, reported that EYCE hydrolyses egg yolk lecithin into fatty acids and lysolecithin inducing the acrosome reaction (Upreti et al., 1999), and chromatin decondensation (Sawyer and Brown, 1995) of goat sperm during equilibration or storage period (Aamdal et al., 1965). Similarly, Pellicer-Rubio and Combarnous, (1998) reported BUSgp60 is also responsible for fatty acid production (oleic acid) which is toxic to sperm. Thus, irrespective of exact mechanism of action, EYCE and BUSgp60 in goat semen extenders is harmful to the sperm during cryopreservation process (Pellicer-Rubio et al., 1997; Pellicer-Rubio and Combarnous, 1998). Therefore, alternative strategies have been adopted like addition of BUSgp60 lipase inhibitors in milk-based semen extender, use of cow milk that are lipid free, or using milk of other species in which fatty acid and triacylglycerol structure are different (Pellicer-Rubio and Combarnous, 1998). Kundu et al. (2000; 2001; 2002) reported cryopreservation of goat sperm in egg yolk and milk-free semen extender, but this work was conducted on epididymal sperm.

Recent studies indicate that reducing the concentration of egg yolk to 2.5% cannot damage post-thaw viability of goat spermatozoa. Therefore, freezing extenders containing low concentrations of egg yolk may be used for goat semen cryopreservation (Shamsuddin et al., 2000, Bispo et al., 2011, Ranjan et al., 2015; Sharma and Sood, 2020). However, the controversial results still exist. According to the report of Anand et al. 3% egg yolk presence in the freezing extender cannot improve the quality of frozen goat spermatozoa in comparison with 20% egg yolk (Anand et al., 2017). Therefore, whether this method is valuable or not still needs further research.

The traditional operation is to completely remove seminal plasma through centrifugation before dilution using freezing extenders (Naing et al., 2011). In general, goat spermatozoa are washed by centrifuging at 550–950 *g* for 10–15 minutes (Ritar and Salamon, 1982, Singh et al., 1995). However, although removal of seminal plasma enhances the cryosurvival of goat spermatozoa, but some components which naturally present in the seminal plasma are also been lost. It is well known that the biochemical composition of seminal plasma is complex. Some studies have demonstrated a nutritive/protective function of seminal plasma for spermatozoa (Juyena and Stelletta, 2012).

The alternative of animal's origin component like egg-yolk and milk may be replaced by soybean lecithin. The fatty acids present in soybean lecithin are common with cell membrane and can give structural stability to cells (Oke et al., 2010). The use of soy lecithin as a source of lipoproteins in place of egg-yolk has been reported in many studies (Papa et al., 2010; Kakati et al., 2019). The soy lecithin-based semen extender protected drastic reduction of sperm motility and membrane and acrosome integrity durin the cryopreservation process in human (Reed et al., 2009), ram (Forouzanfar et al., 2010), cat (Vick et al., 2010), dog (Kmenta et al., 2011), and goat (Jiménez-Rabadán et al., 2012; Salmani et al., 2014). The exact lipid

composition of soy lecithin and egg-yolk differ (Palacios and Wang, 2005) and hence they interact differently with the lipase enzymes present in seminal plasma of in goat. Therefore, use of soy lecithin in the goat semen extender would be useful and simplified the cryopreservation steps because it does not require washing step of semen before dilution of semen (Dorado et al., 2007). There are few reports related to the negative effects caused by soybean lecithin at present. According to the report of Valle et al. (2012), soybean lecithin may interfere with mitochondrial function in post-thaw spermatozoa. In addition, although soybean lecithin resolves the problems caused by egg yolk, such as contamination, standardization, and agglutination, it can act as the substrate for lipid peroxidation because soybean lecithin contains higher proportions of arachidonic, docosahexaenoic acids and unsaturated fatty acids (Aires et al., 2003).

Goat sperm vitrification

Although traditional cryopreservation techniques have been extensively applied for storage of small ruminant semen, they cannot completely prohibit ice crystal formation, which leads to extensive cell shrinkage and structural damage. To avoid the negative effects induced by ice crystal formation, vitrification can be recommended as an alternative method. Different from traditional freezing processes, vitrification involves a direct phase transition of aqueous solutions from the liquid state to the glassy state, not experiencing the stage of ice crystal formation. Moreover, vitrification of mammalian spermatozoa may not require the addition of egg yolk. It is well known that the main limitation of egg yolk is its undefined components, which may be the primary reason leading to variable results among different research groups. Egg yolk may also bring potential bacterial contamination and disease transmission. Removing egg yolk is more meaningful for goat semen, due to the toxic interaction between phospholipase A in seminal plasma and egg yolk. In addition, glycerol may be unnecessary when vitrification is used for spermatozoa storage. Although glycerol enhances the cryotolerance of spermatozoa, it also produces potential toxic and osmotic stress on spermatozoa. Generally, vitrification is defined as a process in which a liquid turns into a solid without the formation of ice crystals. Vitrification is a simple and cost-effective technique for cryopreservation of sperm and may be implemented for commercial semen stations and semen collection of endangered animals in field conditions (Pradiee et al., 2018). The kinetic sperm vitrification is different from conventional vitrification of oocytes and embryos (Katkov et al., 2006), in which the intracellular and the extracellular environment must become vitrified (Pradiee et al., 2018). Truly, it is the experience Isachenko group in Cologne, Germany, who first reported the successful cryopreservation of human sperm without cryoprotectants in 2002.

The first report of goat sperm vitrification is in the Iberian ibex (*Capra pyrenaica*), also known as Spanish wild goat (Pradiee et al., 2018). A cryoprotectant-free semen extender based on a very high cooling rate was tested for goat sperm vitrification. The sucrose at concentration 100 mM in vitrification medium was found suitable for sperm vitrification. The semen ejaculates from three males were vitrified in pellets. Inseminations in domestic goats using the vitrified spermatozoa resulted in three pregnancies. However, the result was similar to that obtained with slowly frozen ibex sperm. The study permitted the choice of a cryoprotectant free vitrification of goat sperm. Further, the study showed fast warming in compared to recommend thawing temperature (37°C) is important in preventing damage to ibex vitrified sperm. The authors found that the idea of the high warming rate of vitrified sperm is more critical than the cooling rate. Thus, vitrification and a fast warming rate allow for the successful cryopreservation of ibex sperm. Due to the simplicity of the sperm cryopreservation technique, its use under field conditions can be recommended for this type of species. Improvement in the vitrification technique may provide better outcomes in future work.

Conclusion

There is a need to develop a more comprehensive methodology for semen cryopreservation and novel techniques for assessment of the quality and viability of sperm. The current techniques should be combining with new techniques including rapid-freezing or vitrification for increasing post-thaw viability and fertility of the spermatozoa. Vitrification may be better option because it avoids ice crystal formation. Nevertheless, the current protocol of vitrification induces damage to cells such as reduced viability, apoptosis, loss of integrity of DNA and breakdown of cell membrane. The recent advancement in the semen cryopreservation will develop a deeper understanding of goat sperm cryopreservation and enable more valid comparisons of research.

Conflict of interest

None

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