

Review article

NANOPURIFICATION: AN EMERGING TECHNIQUE TO PURIFY SEMEN

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Abstract: Scientific procedures nowadays are trying to utilize possible benefits of nanotechnology, which may play a substantial role in nanopurification of semen. Conventional methods of semen purification have yielded good results, but more efforts are to be incorporated so as to get better conception rates in animals after artificial insemination with cryopreserved semen. Recent progress in nanotechnology has provided an alternative for selective removal of dead and defective spermatozoa from an ejaculate. This review is an attempt to summarize information regarding the potential benefits of nanoparticles on reproductive outcomes.

Keywords: Nanotechnology, Nanopurification, Semen, Ubiquitin, Lectin.

Introduction

Production of high quality insemination dose to yield maximum conception rate via artificial insemination is crucial for the success of frozen semen technology (Feugang *et al.*, 2015). The sperm quality parameters in an ejaculate vary between high fertile and low fertile male individuals (Guzick *et al.*, 2001; Lee *et al.*, 2014). An ejaculate from high fertile bull may contain 10-20% dead and defective spermatozoa which may be even higher (40-50%) in low fertile bulls. Since cryopreservation itself results in 40-50% damage to the spermatozoa (Kumaresan *et al.*, 2009), hence an ejaculate with 70-80% progressive motility at pre-freeze will have round about 40-50% progressive motility at post thaw. The corresponding figure in low fertile males will exist between 20-30%, which is nadir for obtaining optimum conception rate after artificial insemination with frozen thawed semen. The reason for decrease in sperm quality parameters during cryopreservation has been attributed largely to sperm cryodamage and oxidative stress (Maia *et al.*, 2010). Oxidative stress mainly results from the dead or defective spermatozoa which act as a source of reactive oxygen species (ROS) during cryopreservation resulting in the damage to normal spermatozoa (Aitken and

Baker, 2013). Therefore removal of such sperms prior to freezing shall result in higher post thaw sperm quality attributes (Feugang *et al.*, 2015). Presence of high concentration of polyunsaturated fatty acids in the plasma membrane of spermatozoa is also considered as one of the key factors contributing to oxidative stress (Hammerstedt *et al.*, 1993) which subsequently lead to lipid peroxidation of sperm plasma membrane resulting in loss of structural and functional integrity of membranes, increasing membrane permeability, DNA damage and cell death (Heish *et al.*, 2006). The standard insemination dose in cattle is 20 million, but only 10 million motile spermatozoa are sufficient for optimum fertility. Post thaw progressive sperm motility of 60-70% will certainly decrease the insemination dose, may be around 15 million and leading to higher population of motile spermatozoa at the site of fertilization (Feugang *et al.*, 2015).

For removal of dead/defective spermatozoa so called semen purification, various methods have been tried with inconsistent results. Among these methods, most recent is the nanotechnology based semen purification which offers an excellent procedure for removal of dead/defective and/or prematurely capacitated spermatozoa (Odhiambo *et al.*, 2014; Feugang *et al.*, 2015) from the ejaculates. Nanoparticles can be produced in different sizes with different composition and based on their biocompatibility with biological fluids. This makes them an excellent device for cell targeting, for which lectin and carbohydrate receptors found on the sperm can serve as great candidates for labelling/binding (Feugang *et al.*, 2015).

Semen Nanopurification

Numerous efforts are going on to develop a nanotechnology based sperm purification method wherein surface determinants on the defective/dead or pre-maturely activated spermatozoa are being exploited. More attention has been focused on ubiquitin, a proteolytic chaperon protein, which is added to the defective or dead spermatozoa during its passage via epididymis through the process of protein ubiquitination (Sutovasky and Kennedy, 2013). Some of the ubiquitin tagged spermatozoa disintegrate within the epididymis, but most of them appear in an ejaculate indicating poor semen quality (Sutovasky *et al.*, 2002; Sutovasky *et al.*, 2003). Additionally, during the process of cryopreservation or freeze-thaw process, the integrity of the sperm acrosome sometimes gets compromised or pre-maturely activated. With the result, the glucosidic residues become exposed which otherwise remain intact within the sperm acrosomal membrane. Such glucosidic residues thus become available for lectin binding (Sutovasky and Kennedy 2013). For this purpose, magnetic nanoparticles are being exploited by coating them with either antibodies against ubiquitin or lectin labels such as

Pisum Sativum Agglutinin (PSA) or Peanut agglutinin (PNA) which can bind to glucosidic residues that become exposed on the outer surface of a sperm with either compromising or prematurely activated acrosome (Baska *et al.*, 2008). Pisum sativum agglutinin (PSA) derived from the pea plant, and Arachis hypogaea agglutinin (PNA, or peanut agglutinin) derived from the peanut plant, are the most commonly used lectins because of their specificity (Graham J.K, 2001). These labelled nanoparticles are mixed with the semen sample and a strong magnet is then applied to concentrate the dead and defective spermatozoa at the bottom, that either bind to lectin coated or antibody coated nanoparticles. In this way dead or defective sperm are removed from the sample and a high quality semen sample is obtained. Compared to filtration and centrifugation-based techniques, semen nanopurification can be used on a whole ejaculate volume, requires no equipment other than a simple magnet bar and tubes. Nanoparticle based semen purification also possesses an advantage of eliminating sperm damage caused by centrifugal force, and can be incorporated entirely in the workflow of semen collection and cryopreservation (Petruska, *et al.*, 2014).

Field insemination trials with nanopurified semen have resulted in increase conception rates in bull and boar. Along with increase conception rate no detrimental effect on dams health or any fetal anomaly has been reported till date. Further studies need to be done on this aspect so to make it regular practice in andrological procedures.

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