Chapter 15
Cryopreservation of Spermatozoa:
Recent Biotechnological Advancement in Gamete Preservation Technology

Chanakya Nath Kundu
KIIT University, India

Gopal C Majumder
Jadavpur University, India & Indian Institute of Chemical Biology, India

Ranjan Preet
KIIT University, India

ABSTRACT

Cryopreservation of sperm has many applications in agriculture, biotechnology, and clinical medicine. The spermatozoon is a very specialized cell that loses the ability of biosynthesis, repair, growth and cell division during the final phase of spermatogenesis. Cryopreservation of sperm generally requires a reduction or arrest of the metabolism of cells, thereby prolonging their life. Semen samples of mammalian species are diluted with a suitable diluent [containing a complex extender (e.g., egg-yolk, milk, milk-whey), cryoprotectant (e.g., glycerol)] and processed through different freezing protocol prior to storage in liquid nitrogen (-196°C). Despite the use of complex media and cryoprotectants, a substantial portion of the cells die during freezing and thawing (recovery rate do not exceeds more than 50%). As the complex media contain large numbers of undefined biomolecules (proteins, lipids, carbohydrates), it is difficult to analyze the beneficial/detrimental effects of a particular compound on sperm cryopreservation. In the present book chapter we have briefly discussed the knowledge and limitations of the current semen cryopreservation technology and mainly focused on the development of a simple cryopreservation model using chemically-defined medium and goat cauda-epididymal sperm. Using this model system, several novel cryoprotectants have been identified and biochemical basis of sperm membrane damage during cryopreservation has been investigated.

DOI: 10.4018/978-1-61692-004-3.ch015

INTRODUCTION

Reproductive medicine is now drastically transforming our lives. Couples with impaired reproductive abilities are increasingly availing its benefits to conceive and realize their dreams of having a family. A major force in making this widely available is the ability to preserve fertility (sperms, eggs and embryos) using cryopreservation (storing cells in liquid nitrogen) (Bagchi *et al.,* 2008). Many cancer patients are also electing to preserve their fertility prior to undergoing chemotherapy or radiation (which can destroy reproductive abilities) to have the option of conceiving post-treatment. Cryopreservation of sperm has been highly successful in establishing pregnancies through artificial insemination (AI) and in vitro fertilization (IVF). Semen preservation is a subject of paramount interest because of the extensive use of frozen semen for artificial AI in cattle breeding to improve the production of milk, wool and meat. It is also a useful way of banking cells until needed for experimentation or other applications.

Research on semen preservation has had a long history over two centuries but till date the problem is that even with the best preservation techniques to date post-thaw survival is restricted to about 40-50% of the sperm population. Mammalian spermatozoa have been frozen stored by various methods based on systematic manipulation of cryopreservation protocol (e.g. dilution, cooling, freezing and thawing). Semen samples are generally diluted with a suitable diluent containing a complex extender (*e.g.,* egg-yolk, milk, milk-whey) and a cryoprotectant (*e.g.,* glycerol) prior to storage in liquid nitrogen (-196°C) (Hu *et al*., 2009; Purdy 2006; Witte *et al.,* 2009). During cryopreservation a substantial portion of sperm cells undergo damage there-by reducing greatly motility/fertility potential of spermatozoa (Watson, 2000). But the biochemical basis of cryodamage is largely unknown. Complex media (egg yolk, milk, milk whey *etc.*) have been used as an extender **(cryopreservation medium and cryoprotectant)** for preservation of ejaculated spermatozoa by most of the investigators. Such media were also used for investigating the effect of multiple cryoprotectants (*e.g.* glycerol, sucrose, trehalose *etc.*). As this media contain a large number of undefined biomolecules (proteins, carbohydrate, lipids *etc.*), it is rather difficult to assess the biochemical basis of cryodamage and beneficial or detrimental effects of a particular compound on sperm cryopreservation.

For such an investigation it will be ideal to develop a simple sperm model for cryopreservation in a chemically-defined medium. Mature cauda epididymal sperm is a better model than the ejaculated sperm because the latter cells in view of their exposure to a variety of undefined constituents from the secretions of seminal vesicles, prostate that may complicate the interpretation of the data. An important advantage of the cauda-sperm model is that spermatozoa can be extracted from the epididymides that can be procured rather easily from the slaughter houses. Studies on cryoprotection mechanism using a cell model will require large amount of spermatozoa that will be rather difficult to procure from the ejaculated mammalian semen samples. Recently, we have developed a simple cryopreservation technique using a chemically-defined medium and goat cauda-epididymal sperm as the model system. (Kundu *et al.,* 2000)

In the present chapter we have discussed the existing knowledge and limitations of cryopreservation techniques including extenders, cryoprotectants and freeze-thaw protocol. Emphasis has been given on the development of a novel cauda-epididymal sperm cryopreservation model (using a chemically-defined medium) for screening the better cryoprotectant. Using that model system we have already screened cryoprotecting potentiality of several compounds. Finally, we have provided a novel hypothesis for cryoprotection mechanism offered by cryoprotectant.