学 位 論 文 の 要 旨

論文題目　Study of Lipid Components in Bovine Frozen Sperm
（ウシ凍結精子に含有する脂質成分に関する研究）

広島大学大学院生物圏科学研究科
Bioresource Science 専攻
学生番号　D180963
氏　　名　Md. Mazharul Islam

Mammalian sperm migrate through uterus to oviduct by maintaining linear motility pattern during in vivo fertilization process. For the migration process sperm require adequate energy to reach the oocyte for fertilization. Sperm gets maximum energy from mitochondria ATP production. The quality of sperm deteriorates in the process of cryopreservation. Sperm membrane integrity, acrosomal integrity and mitochondrial abnormality are the main physical negative effect of sperm cryopreservation. Therefore, the present study was aimed to investigate the recovery of bovine frozen sperm quality after thawing and washing. To achieve the aim, firstly effect of lipid mixture in thawing media of bovine frozen sperm were studied. After that, molecular mechanism of mitochondrial ATP production by using fatty acids were investigated. Finally, the sperm membrane potentiality with mitochondrial membrane potentials of sperm with cholesterol were studied.

1. Lipid Mixture (LM) in thawing media is beneficial to keep sperm motility with other quality parameters in healthy status of bovine frozen sperm

Cryodamage affect sperm motility and membrane structure highly. In Experiment 1, it was hypothesized that LM in thawing media maybe beneficial for bovine frozen sperm to keep good motility with sound membrane structure by maintaining their membrane lipid composition healthy and there may be a correlation between lipid components and sperm motility. To clear the hypothesis CASA, GC-MS and FCM with different stains were performed using bovine frozen sperm. It was observed that fatty acids from lipid mixture was up taken by LM thawed

sperm compare to control and there was a positive correlation between fatty acids amount and total sperm motility. It was also observed that LM thawed bovine frozen sperm showed significantly good motility parameters compare to control group. Moreover, it was also uncovered that membrane integrity, mitochondrial activity and live sperm percentage was significantly higher in LM thawed sperm compare to without LM thawed sperm used as control. Although, it is known that lipid components can improve sperm motility, but this is the maiden report considering the variability in the amount of lipid components in sperm membrane of bovine frozen sperm and their correlation to sperm motility according to author best of knowledge.

2. Sperm linear motility is induced by saturated fatty acid being used as energy substrate in the mitochondria of bovine frozen sperm

Successful fertilization depends on sperm linear motility and motility patterns are dependent on energy substrate and their effective utilization in energy producing segments of sperm. In Experiment 2, it was hypothesized that bovine sperm could uptake fatty acids (FAs) in thawing media and produce ATP in mitochondria for maintaining linear motility. To clear this hypothesis; bovine frozen sperm was thawed in FAs containing thawing media and the sperm motility was analyzed by commuter assisted sperm analysis (CASA). The kinetic changes of FAs level in sperm were detected by GC-MS. The mitochondrial activity of sperm treated with FAs was analyzed as fluorescence intensity of JC-1 staining and oxygen consumption rate (OCR). FA transporter (CD36 and GOT2) was observed by whole-mounted immunofluorescence. It was observed that sperm linear motility was significantly increased by the thawing with FA. Interestingly, it was revealed that long chain saturated fatty acids (LCSFA) (C14, C16, C18) were decreased during 30 min incubation, except very long chain saturated fatty acid (C22). Moreover, another crucial factor was observed that the bovine frozen sperm possessed FA transporter in midpiece where the fluorescence signals were detected after the treatment with fluorescence-tagged FA. Furthermore, it was discovered that the treatment with

FA activated electron transportation in mitochondria through β-oxidation. These findings highlight the molecular mechanism of fatty acid uptake from sperm membrane to mitochondria and ATP production through β-oxidation process.

3. Cholesterol in thawing media regain mitochondrial potential which accelerates ATP production in mitochondria that induces linear motility in bovine frozen sperm

For successful fertilization sperm linear motility is very important and this is induced by mitochondrial ATP production. In Experiment 3 it was supposed that cholesterol may be up taken by sperm and used to remake the mitochondrial membrane potential along with sperm membrane potential which was damaged during cryopreservation process. To clarify the hypothesis bovine frozen sperm was thawed in CHL contained thawing media and the sperm motility was analyzed by commuter assisted sperm analysis (CASA). The cholesterol uptake signal was observed by fluorescence intensity in the mid piece and head region of sperm after the treatment with fluorescence-tagged CHL. The mitochondrial activity of sperm treated with CHL was analyzed as fluorescence intensity of JC-1 staining and oxygen consumption rate (OCR). OCR was higher in CHL treated group due to up taken CHL was used to renovate the altered mitochondrial structure. ATP production rate was also significantly higher in CHL thawed group compare to control. Sperm linear motility was significantly ($P<0.05$) increased by the thawing with CHL. Sperm linear motility is prompted by CHL in thawing media of bovine frozen sperm might provide a new vision for modernizing the artificial insemination technique of both livestock animals and human infertility care.

In conclusion, this study unveiled the mechanism of lipid mixture to improve the linear motility pattern and membrane integrity of bovine frozen sperm. Two part in lipid mixture fatty acids and cholesterol contribute separately to rescue the quality of bovine frozen sperm after thawing in a lipid mixture containing media. Interestingly, this study uncovers the energy metabolism of bovine frozen sperm thawed in lipid mixture or fatty acid containing thawing media with the molecular strategy of fatty acid uptake to the sperm mitochondria from the membrane.

Moreover, cholesterol helps to recover the membrane integrity and mitochondrial membrane potentials declined during cryopreservation process and contribute to regain the linear motility pattern through accelerate ATP production rate. This study invented new information for reproductive science to maintain linear motility of sperm after thawing and washing which is prerequisite for fertilization. Therefore, the new story developed from the present study is strongly believed to be fruitful to the future research in AI technology for livestock and human infertility care as inexpensive technique. New approach of thawing frozen sperm from the present study will increase the conception rate definitely and ultimately the production of livestock will increase which may be beneficial to overcome poverty, increase income and attain the SDGs of the united nations (UN).

Keywords: Frozen sperm, Linear motility, ATP Production, Mitochondrial Potential.