CONTENTS

Page

LIST OF TABLES……. **IV**

LIST OF FIGURES…… **VII**

LIST OF ABBRIVIATION ……..…….. **IX**

I. INTRODUCTION……..…… **1**

II. REVIEW OF LITERATURE……. **4**

1. Cryoperservation of semen……..……. **4**

1.1- Effect of Freezing on semen quality…….…...……. **5**

2. Extenders for freezing semen…….…….…. **6**

2.1. Egg yolk in extenders…….…… **10**

3. Cryoprotectant agents in extenders for frozen semen……..… **11**

3.1. Glycerol…….…….…….. **14**

3.2. Dimethyl sulphoxide (DMSO)…….……... **16**

3.3. Ethylene glycol (EC)…….…….…..…… **17**

3.4. Acetamide and lactamide…….…….….. **19**

4. Antioxidants and lipid peroxidation …….……... **19**

4.1. Glutathione (GSH)…….…….…… **23**

4.2. Effect of GSH on sperm function…….…… **24**

5. Freezing semen process…….…….…… **27**

5.1. Cooling rate and equilibration period.…….…… **27**

6. Enzymatic activity in semen…….…….……... **29**

7. Acrosomal damage…….…….…….. **31**

8. Conception rate…….…….……... **33**

III. MATERIALS AND METHODS……...….…….…. **39**

1. Animals…….…….……..…… **40**

2. Feeding system and management .…….…… **40**

3 . Measurements……...……... **41**

3.1. Semen collection……...……... **41**

Page

3.2. Freezing procedures of semen…… **42**

3.2.1. Semen extension……..…..……... **42**

3.2.2. Equilibration period ……..……...……... **43**

3.2.3. Packing in straws ……..……..…… **44**

3.3. Evaluation of post-thawed semen ……..…… **45**

3.3.1. Percentage of post-thawing sperm motility (%)…….…… **45**

3.3.2. Percentage of freezability of spermatozoa (Recovery rate %) **46**

3.3.3. Acrosomal damage (%)……...…….…… **46**

3.3.4. Enzymatic activities (U/10^9 sperm) ..…….…… **47**

3.4. Conception rate ……..…….……... **48**

4. Statistical analysis ……..……...…. **49**

IV. RESULTS AND DISCUSSION..…….…….…….. **50**

I. The first experiment: "Effect of cryoprotectant type on frozen-thawed semen quality" …….…….…… **50**

I.1. Frozen-thawing sperm motility(%)……...…… **50**

I.2. Freezability of spermatozoa (recovery rate)…..……..……. **56**

I.3. Acrosomal damage…….…….. **61**

I.4. Enzymatic activity……...…… **66**

I.4.1. Activity of aspartate-amino transferase enzyme (U/10^9spermatozoa) …… **66**

I.4.2. Activity of alanine-aminotransferase enzyme (U/10^9spermatozoa)……...…… **71**

I.4.3. Activity of acid phosphatase enzyme (U/10^9 spermatozoa) …….. **76**

I.4.4. Activity of alkaline phosphatase enzyme (U/10^9 spermatozoa) **81**

I.4.5. Activity of lactic dehydrogenase (U/10^9 spermatozoa)……...…... **84**

II. The second experiment "Effect of glutathione (GSH) addition to the extender on frozen-thawed bull semen quality" ……....… **90**

II.1. Percentage of post-thawed sperm motility ……. **90**

Page

II.1.2. Freezability of spermatozoa (Recovery rate)……. **93**

II.1.3. Acrosomal damage……..…….. **98**

II.1.4. Enzymatic activities…… **101**

II.1.4.1. Activity of aspartate-aminotransferase (U/109 spermatozoa) **101**

II. 1.4.2. Activity of alanine-aminotransferase(U/109 spermatozoa).. **104**

II.1.4.3. Activity of acid phosphatase enzyme (U/109 spermatozoa) **108**

II.1.4.4. Activity of alkaline phosphatase enzyme(U/109 spermatozoa) **111**

II.1.4.5. Activity of lactic dehydrogenase enzyme(U/109 spermatozoa) **114**

III . Conception rate……... **117**

V. SUMMARY AND CONCLUSION……..…… **122**

VI. REFERENCES…….… **129**

ARABIC SUMMARY……...……...……..

LIST OF ABBRIVIATION

AI	**Artificial insemination**
Tris	**(Hydroximethyl) amino methane**
SOD	**Superoxide dismutase**
GL	**Glycerol**
DMSO	**Dimethyl sulphoxide**
EG	**Ethylene glycol**
AC	**Acetamide**
LA	**Lactamide**
CR	**Conception rate**
GSH	**Glutathione**
AST	**Aspartate-aminotransferase enzyme**
ALT	**Alanine- aminotransferase enzyme**
ACP	**Acid phosphatase enzyme**
ALP	**Alkaline phosphatase enzyme**
LDH	**Lactic dehydrogenase enzyme**

SUMMARY AND CONCLUSION

The present study was conducted in the Department of Animal Production, Faculty of Agriculture, Mansoura University, Egypt. The experimental work was carried out in El-Gemmiza Animal Production Research Station, El-Gemmiza Village, Gharbiya Governorate, located in the north eastern part of Nile Delta (31°N), belonging to Animal Production Research Institute, Egypt, during the period from April, 2007 till October, 2008.

Five sexually mature Friesian bulls were used in the present study. The averages of ages and weights of the bulls at the beginning of the experimental work were approximately 3-4 years old and 600-650 kg, respectively .

Two experiments were carried out: The first experiment was carried out to investigate the effect of different types of cryoprotective agents (glycerol, dimethyl sulfoxide: DMSO, ethylene glycol: EG, acetamide: AC or lactamide: LA) and their combinations on post-thawing sperm motility, freezability and acrosomal damage of spermatozoa during thawing-incubation at 37°C for up to 2 hours. Enzymatic activity of aspartate-aminotransferase (AST), alanine- aminotransferase (ALT), acid phosphatase (ACP), alkaline phosphatase (ALP) and lactic dehydrogenase (LDH) in

seminal plasma was also determined during thawing- incubation at 37°C for up to 2 hours.

The second experiment was carried out to study the effects of glycerol with DMSO (the best one of cryoprotectants in the first experiment) supplemented with different levels of Glutathione (0, 0.2 , 0.4 and 0.8 mM/ 100 ml) on post-thawing sperm motility, freezability, acrosomal damage of spermatozoa, and enzymatic activity, during thawing-incubation at 37°C for up to 2 hours. The conception rates of the cows artificially inseminated with the thawed-frozen semen containing 7% glycerol without glutathione, 7% glycerol with 0.4 mM glutathione(the best level), 3.5% glycerol plus 3.5% DMSO without glutathione and 3.5% glycerol plus 3.5% DMSO with 0.4mM glutathione were assessed.

The obtained results could be summarized as the follows:

The first experiment:

Effect of different types of cryoprotective agents on post-thawed frozen semen quality:

1. Frozen-thawed bull semen quality:

1.1 the percentages of post- thawing sperm motility and freezability of spermatozoa added with 3.5% glycerol plus 3.5% DMSO were significantly ($P<0.05$) higher, while the percentage of acrosomal damage of spermatozoa was significantly ($P<0.05$) lower than other cryoprotective agents or their combinations .

1.2. The highest (P<0.05) percentages of the post- thawing sperm motility and freezability of spermatozoa were recorded in the extended bull semen with 3.5% glycerol plus 3.5% DMSO and the lowest (P<0.05) percentage was recorded with the 5% ethylene glycol.

1.3. The lowest (P<0.05) percentage of the acrosomal damage of spermatozoa were recorded in the extended bull semen with 3.5% glycerol plus 3.5% DMSO and the highest (P<0.05) percentage was recorded with the 5% ethylene glycol.

1.4. The advancement of thawing- incubation time at 37^{o}C for up to 2 hours of the thawed – frozen bull semen decreased significantly (P<0.05) the percentages of post- thawing sperm motility and freezability of spermatozoa ,while increased significantly (P<0.05) the percentage of the acrosomal damage of spermatozoa with the different cryoprotective agents.

2. Enzymatic activities:

2.1. The thawed- frozen bull semen extended with 3.5% glycerol plus 3.5% DMSO was significantly (P<0.05) lower the amounts of AST, ALT, ACP, ALP and LDH enzymes into the extracellular medium than other cryoprotective agents or their combinations.

2.2. The lowest (P<0.05) amounts of the AST , ALT, ACP, ALP and LDH enzymes into the extracellular medium were recorded in The thawed- frozen bull semen extended with 3.5% glycerol plus 3.5% DMSO and the highest (P<0.05) amounts were recorded in the 5% ethylene glycol.

2.3. The advancement of thawing- incubation time at 37^{o}C for up to 2 hours of the thawed – frozen bull semen increased significantly (P<0.05) the leakage of AST, ALT, ACP, ALP and LDH enzymes into the extracellular medium with the different cryoprotective agents or their combinations.

The second experiment:

Effect of different levels of glutathione (GSH) on post-thawed semen quality:

1. Frozen-thawed bull semen quality:

1.1. Supplementation of glutathione at levels of 0.2, 0.4 and 0.8 mM /100 ml to the thawed-frozen bull semen significantly (P<0.05) higher the percentage of post-thawing sperm motility and freezability of spermatozoa, while significantly (P<0.05) lower the percentage of the acrosomal damage than free glutathione medium.

1.2. The highest (P<0.05) percentages of the post- thawing sperm motility and freezability of spermatozoa were recorded with the thawed-frozen

bull semen added with glutathione at a level of 0.4 mM and the lowest (P<0.05) percentage with free glutathione medium.

1.3. The lowest (P<0.05) percentage of the acrosomal damage of spermatozoa were recorded with the thawed-frozen bull semen added with glutathione at a level of 0.4 mM and the highest (P<0.05) percentage with free glutathione medium.

1.4. The advancement of thawing- incubation time at 37^{o}C for up to 2 hours of the thawed – frozen bull semen decreased significantly (P<0.05) the percentages of post- thawing sperm motility and freezability of spermatozoa ,while increased significantly (P<0.05) the percentage of the acrosomal damage of spermatozoa with the different levels of glutathione or free glutathione medium.

2. Enzymatic activities:

2.1. The thawed- frozen bull semen extended with 3.5% glycerol plus 3.5% DMSO added with 0.2, 0.4 or 0.8 mM / 100 ml glutathione was significantly (P<0.05) lower the amounts of AST, ALT, ACP, ALP and LDH enzymes into the extracellular medium than free glutathione medium.

2.2. The lowest (P<0.05) amounts of the AST, ALT, ACP, ALP and LDH enzymes into the extracellular medium were recorded in the thawed- frozen bull

semen extended with 3.5% glycerol plus 3.5% DMSO added with 0.4 mM glutathione and the highest ($P<0.05$) amounts were recorded with free glutathione medium.

2.3. The advancement of thawing- incubation time at 37^{o}C for up to 2 hours of the thawed – frozen bull semen increased significantly ($P<0.05$) the leakage of AST, ALT, ACP, ALP and LDH enzymes into the extracellular medium with the different glutathione levels or free glutathione medium.

3. Conception rate (%):

The highest ($P<0.05$) conception rate (85%) was obtained for cows artificially inseminated with the thawed-frozen semen extended with 3.5% glycerol plus 3.5% DMSO supplemented with 0.4 mM glutathione, followed by 7% glycerol supplemented with 0.4 mM glutathione (80%) as compared to 75% for those inseminated with semen extended with a combination of 3.5% glycerol plus 3.5% DMSO free GSH. While, the lowest ($P<0.05$) conception rate (70%) of the cows artificially inseminated was recorded with the thawed-frozen semen extended with 7% glycerol free glutathione medium (control).

In conclusion, a high proportion of the bull spermatozoa retained active motility after freezing to and thawing from -196^{o}C in a medium containing dimethyl sulfoxide as a cryoprotective agent. The obtained results revealed that dimethyl sulfoxide with glycerol protects bull spermatozoa more effectively than other cryoprotective agent (ethylene glycol, acetamide or lactamide) during freezing and thawing. The thawed – frozen bull semen added with 0.4 mM glutathione / 100ml is more efficient in maintaining post-thawing sperm livability, freezability, acrosomal integrity, enzymatic activities and subsequent fertilizing efficiency of bull spermatozoa than free glutathione medium.

Therefore, it can be recommended to extension and freezing of bull semen with 3.5% glycerol plus 3.5% dimethyl sulfoxide supplemented with glutathione at a level of 0.4 mM / 100ml for artificially insemination programs to enhance of conception rate.