SUMMARY AND CONCLUSION

This study was carried out during the period from November 2006 to April 2007 to investigate the effect of adding different types and levels of cryoprotectants on sperm viability during different freezing processes of buffalo semen (1st experiment). Also, the effect of different types and levels of antioxidants on sperm motility and acrosome status in post-thawed semen was studied (2nd experiment).

Five sexually mature buffalo bulls aged 7-10 years were used for semen collection by means of an artificial vagina. Ejaculates were obtained from each buffalo bull twice/week for 10 weeks collection period (100 ejaculates).

In the 1st experiment, the main extender used for semen dilution was Egg Yolk-Citrate-Tris with different types (glycerol, GL; dimethyl sulfoxide, DMSO and ethylene glycol, EG) and levels (5, 7 and 10% for each) of cryoprotectants. Then, semen was frozen in liquid nitrogen (LN) and thawed at 37°C/30 sec. Percentage of progressive motility of spermatozoa was determined pre- and post-dilution, post- equilibration period for 4 or 6 h, post-thawing after 24 h freezing period.

In the 2nd experiment, the main extender used for semen dilution was Tris-egg-yolk-citrate extender containing 7% glycerol. Total of 10 extenders, 9 with

3 types and 3 levels of antioxidant, including catalase (250, 500 and 1000 IU), glutathione (GSH, 0.4, 0.8 and 1.2 mM) and ascorbic acid (AA, 0.5, 1.0 and 1.5 g/l) were compared to unsupplemented extender (control). Semen was extended with different types and levels of antioxidants, frozen in LN (-196oC) and thawed at 15oC/60 sec, 35oC/30 sec and 55oC/15 sec. Percentages of sperm motility and damage acrosome were determined in post-thawed semen. Conception rate was detected in 100 sexually mature buffalo cows (10 animals in each group) artificially inseminated with different types and levels of antioxidants.

The obtained results could be summarized as the following:

The 1st experiment:

1. Sperm motility in post-diluted semen was the highest (72.9%, $P<0.05$) with GL, followed by DMSO (98.8%), while, EG showed the lowest motility (63.6%). The differences between DMSO and each of GL and EG were not significant.

2. Increasing level of cryoprotectant resulted in gradual reduction in sperm motility, being significant ($P<0.05$) only by increasing the level from 7 to 10%, whereas sperm motility slightly decreased from 70.7% at a level of 5% to 69.3% at a level of 7%, while it decreased ($P<0.05$) to 65.2% at a level of 10%.

3. The effect of interaction between type and level of cryoprotectants on sperm motility in post-diluted semen was not significant. In post-equilibrated semen, sperm motility with GL or DMSO was higher (68.7 and 64.8%, respectively, $P<0.05$) than with EG (557%).

4. Sperm motility with GL or DMSO did not differ significantly. Increasing level of cryoprotectant from 5 to 7% resulted in slight and insignificant reduction in sperm motility from 65.1 to 64.9%. This reduction was significant ($P<0.05$) by increasing the level from 7 to 10%, being 59.2% at a level of 10%.

5. Sperm motility was higher ($P<0.05$) with 6 than 4 hours (58.9 vs. 67.2%) as equilibration period. The effect of interaction was significant ($P<0.001$) on sperm motility in post-equilibrated semen only between type and level of cryoprotectants. In post-thawed semen, sperm motility was the highest (36.4%, $P<0.05$) with GL, moderate (29.7%) with DMSO and the lowest (9.5%) with EG.

6. Increasing level of cryoprotectant from 5 to 7% resulted in slight and insignificant increase in sperm motility in post-thawed semen from 27.4 to 28.8%. Sperm motility sharply decreased ($P<0.05$) by increasing the level from 7 to 10%, being 17.6% at a level of 10%. The effect of interaction of type with level of cryoprotectants was not significant on sperm motility in post-thawed semen.

7. Aactivity of AST in post-thawed semen was significantly (P<0.05) lower in post-thawed semen extended with EG (46.4 U/L) than with glycerol or DMSO (48.8 and 51.9 U/L, respectively), being the highest with DMSO (Table 20). Such trend indicated more beneficial effect of EG on membrane integrity of spermatozoa rather than that with glycerol or DMSO.

8. Activity of ALT in post-thawed buffalo semen was the highest (P<0.05) with DMSO (30.7 U/L), moderate with glycerol (26.7 U/L) and the lowest with EG (23.1 U/L). The effect of level of cryoprotectant, activity of ALT was insignificantly lower with cryoprotectants at level of 7% than with 5 or 10%.

9. Activity of ACP in post-thawed semen was lower (P<0.05) in post-thawed semen extended with glycerol (4.2 U/ml) than with DMSO or EG (5.4 and 5.9 U/ml, respectively). Activity of ACP in semen was lower (P<0.05) with cryoprotectants at a level of 7% (4.8 U/ml) than that with 5 or 10% (5.5 and 5.1%, respectively).

10. Activity of ALP in post-thawed semen was the lowest (P<0.05) in semen extended with glycerol (72.5 U/L), moderate with DMSO or EG (88.3 and 132 U/L, respectively).

The 2nd experiment:

1. Adding catalase (500 or 1000 IU), GSH (all levels) and AA (0.5 g/l) increased (P<0.05) sperm motility as compared to the control, being the highest (62.1%) for semen with 1000 IU of catalase.

2. All types and levels of antioxidants decreased (P<0.05) damage acrosome percentages as compared to the control, except for the highest level of AA (1.5 g/l), which did not differ significantly from that of the control.

3. Catalase (1000 IU) showed the lowest (P<0.05) damage acrosome percentage (6%). Increasing level of catalase and decreasing level of GSH or AA markedly decreased damage acrosome percentage.

4. Sperm motility percentage was higher (P<0.05) with a rate of 55°C/15 sec than 35°C/30 sec than 15°C/60 sec (43.0, 32.1 and 27.0, respectively).

5. Adding catalase (1000 IU) or AA (0.5 or 1.0 mM) increased (P<0.05) sperm motility percentage in semen thawed by different rates, being 38.3, 37.4 and 37.1%, respectively.

6. The highest conception rate (80%) was obtained from buffalo cows inseminated with catalase supplementation (1000 IU).

Based on the foregoing results of both experiments, the present study may conclude that:

These results indicated beneficial effects on improving sperm motility when buffalo semen was extended in Tris-based extender containing 7% glycerol as a cryoprotectant agent. In addition, Tris-based extender containing catalase at a level of 1000 IU in frozen semen thawed at a rate of 55^{o}C/15 sec showed the highest post-thawing motility and the best fertilizing capacity of buffalo spermatozoa. Supplementation of semen diluted with Tris-extender with natural antioxidant (ascorbic acid) a level of 0.5 g/l or 0.4 mM of GSH resulted in buffalo bull spermatozoa to maintain their fertilizing capacity at cooling temperature (5^{o}C) for 72 hours.