CONTENTS

V. SUMMARY AND CONCLUSIONS

Five sexually mature buffalo bulls (7-10 years) raised at the ILMTC were used in this study. Semen was collected from the experimental bulls for 5 weeks (50 ejaculates for each bull). Only semen with mass motility of 70% or more was pooled and divided into 10 replicates for different treatments. Semen was extended (1:20) using five different extenders including Tris-egg yolk (TEY), buffalo skim milk (BSM), buffalo whole milk (BWM), camel skim milk (CSM) and camel whole milk (CWM). Each extender was divided into two parts; the first was supplemented with 100 IU antioxidant (Superoxide dismutase; SOD) per ml extender and the second part without supplementation (10 extenders). Semen was equilibrated for 4 hours and storage at -196°C for one month. Frozen straws for each extender were thawed at six rates 30°C/30 sec, 30°C/60 sec, 37°C/30 sec, 37°C/60 sec, 45°C/30 sec and 45°C/60 sec. Post-thawing recovery rate of motile and live spermatozoa was calculated. Physical semen characteristics including motility, livability and abnormality of spermatozoa were determined in post- diluted, post-equilibrated and post-thawed semen at different thawing rates. Thereafter, reduction rate (%) in motile and live and rate of increase in abnormal spermatozoa between dilution and equilibrium were calculated. Activity of transaminases including aspartate (AST) and alanine (ALT) as well as activity of lactic dehydrogenase (LDH) were determined in pre- and post-freezing semen.Biometrical characteristics

of sperm head (length, maximal and basal breadth) were estimated and head area of the spermatozoa was calculated post-equilibrium and post-thawing. Acrosome reaction, head to head agglutination (HHA) and hypo-osmotic swelling tests were used to evaluate the effect of different types of extenders and antioxidant supplementation on quality of post-thawed spermatozoa at a rate of 37°C/30 sec. According to the obtained results for semen evaluation after thawing ; the conception rate is examined for buffalo cows artificially inseminated with semen extended with TEY, CWM and CSM with or without antioxidant supplementation was calculated .

The obtained results could be summarized as follows:

1. Effect of type of extender and antioxidant supplementation on:

1.1. Progressive sperm motility percentage:

- Post-dilution progressive motility was the highest ($P<0.05$) with CSM (77.0%). It increased ($P<0.001$)with than without SOD (66.0 *vs*. 71.8%). The highest motility was observed for CSM with SOD (79.0%), while the lowest motility was for BWM without SOD (53.0%).

- Post-equilibrated motility was the highest ($P<0.05$) with CSM (73.0%). It was improved ($P<0.001$) with SOD (59.6 *vs*. 66.4%). It was the highest with CSM with SOD (76.0%) and the lowest with BWM without SOD (44.0%).

- The reduction rate in motility between dilution and equilibrium was higher ($P<0.05$) for extenders without than with SOD (7.5 *vs*. 9.7%).

The highest rate of reduction was recorded for BWM without SOD (17.0%) and the lowest (2.8%) for CSM with SOD.

- Post-thawing motility was the highest (P<0.05) with CSM with SOD (39.3%) and the lowest (14.1%) with BWM without SOD. CSM without SOD resulted in higher (P<0.05) motility than the other types of extenders either with or without SOD, but was significantly (P<0.05) lower only than CSM with SOD. It was the highest (P<0.001) with 37 oC/30 sec. SOD markedly increased post thawing motility. CSM had the lowest rate of increase in post- thawing motility at a rate of 37/30 from 52.0 to 54.0% when it supplemented with SOD compared to without supplementation.

- Recovery rate (RR) of motile spermatozoa was higher for CSM with or without SOD (49.7 and 48.8%, respectively) than the other extenders. While, BWM with or without SOD showed the lowest recovery rate of motile sperm, being 28.3 and 26.7%, respectively. RR was the highest (P<0.001) with 37 oC/30 sec (55.1%). CSM supplemented with or without SOD showed the highest recovery rate of motile sperm at all thawing rates especially in semen thawed at a rate of 37/30 (68.3%) as compared to the other extenders with or without SOD versus the lowest values for BWM with or without SOD at all thawing rates.

1.2. Grade of sperm motility (Score from 1-5):

- Post-dilution, CSM showed the highest (P<0.05) grade of motility (4.8). Grade of motility was higher (P<0.001) with than without

SOD (3.5 *vs*. 4.0). The highest score was recorded for CSM with SOD (5.0), while BWM without SOD showed the lowest values (2.0).

- Post-equilibrium, grade of motility was the highest (P<0.001) with CSM (4.1). Grade of motility showed a tendency of higher scores for extenders with SOD (3.0) than those without supplementation (2.8). Using CSM with or without SOD indicated higher grade of motility as compared to the other extenders.

- Reduction rate in grade of motility between dilution and equilibrium was the highest (P<0.05) for BWM (30.7%). Reduction rate in grade of motility was higher (P<0.05) for extenders with than without SOD (20.7 vs. 24.0%). Rate of reduction in grade of motility was the highest (37.5%) for BWM with SOD, while the lowest reduction (13.0%) was recorded for CSM without SOD.

- Post-thawing, grade of motility was the highest (P<0.05) with CSM supplemented with SOD and the lowest for BWM with or without SOD. It was the highest (P<0.001) with 37/30. The highest score of motility was obtained for CSM with SOD at 37/30.

1.3. Live sperm percentage:

- Post-dilution, CSM showed the highest (P<0.05) live sperm percentage (76.6%). SOD increased (P<0.001) live sperm percentage from 66.7 to 72.1%. The highest live sperm percentage was recorded for CSM supplemented with SOD (77.6%).

- Post-equilibrium, live sperm percentage was the highest (P<0.001) with CSM (72.0%). It was higher (P<0.001) with (65.3%) than without SOD (62.1%). It was the highest for CSM with SOD.

- The rate of reduction in live sperm percentage between dilution and equilibrium increased (P<0.05) with SOD. Rate of reduction was the highest (13.3%) for BSM with SOD, while the lowest reduction (5.6%) was recorded for CSM with SOD.

- Post-thawing, live sperm percentage was the highest (P<0.05) for CSM with SOD and the lowest for BWM with or without SOD. CSM without SOD increased (P<0.05) live sperm percentage than the other types of extenders even with SOD, but was significantly (P<0.05) lower only than CSM with SOD. SOD increased (P<0.05) live sperm percentage in all types of extenders used in this study. Live sperm percentage in post thawed semen was the highest (45.06%, P<0.001) at a rate of 37/30. The highest percentage of live sperm was obtained for CSM with SOD and thawed at 37/30 (58.2%)

- Recovery rate of live spermatozoa was (P<0.001) the highest for CSM with or without SOD (56.7 and 56.0%, respectively). RR of live sperm was the highest (P<0.001) at a rate of 37 oC/30 sec (64.4%). CSM with or without SOD showed the highest RR of live sperm at all thawing rates especially in semen thawed at the rate of 37/30 (68.5%).

1.4. Sperm abnormality percentage:

- Post-dilution, CSM showed lower (P<0.05) sperm abnormality (15.9%) than that in the other extenders (22.1-23.5%). SOD decreased (P<0.001) sperm abnormality from 24.2 to 18.8%. The highest percentage of abnormality was recorded for CWM without SOD (26.6%), and the lowest for CSM with SOD.

- Post-equilibrium, sperm abnormality percentage was the lowest (P<0.05) for CSM compared with the other types of extenders. It was significantly (P<0.001) lower for extenders with (24.8%) than without SOD (28.1%). It was the highest for TEY without SOD and the lowest for CSM with SOD. CWM showed lower rate of increase (P<0.05) in sperm abnormality (11.9%) than the other types of extenders (23.8-27.6%). This rate was significantly (P<0.05) higher with than without SOD (16.5 vs. 31.4%). It was the highest (40.6%) for BSM with SOD, and the lowest (11.3%) for CWM without SOD.

- Post-thawing, sperm abnormality percentage was the highest (44.2%, P<0.05) with BWM without SOD and the lowest (30.3%) for CSM with SOD. It was significantly (P<0.001) the highest (54.46%) at a rate of 45/60. It was the highest for BWM supplemented with SOD and thawed at 45/60 (60.6%).

2. Effect of type of extender and antioxidant supplementation on:

2.1. Activity of AST:

- Pre-freezing, activity of AST was the lowest (46.3 U/l, P<0.001) in CSM and the highest (55.9 U/l) in BWM. SOD reduced (P<0.001)

AST activity from 53.0 to 49.7 U/l. The highest AST activity was obtained for BWM without SOD and the lowest for CSM with SOD.

- Post-freezing, AST activity in post-thawed semen was the highest (P<0.001) for BWN (66.6 U/l). SOD reduced (P<0.001) AST activity (56.4 *vs*. 64.8 U/l). The highest AST activity was recorded for BWM without SOD (72.4 U/l), and the lowest values (52.2 U/l) for CSM with SOD.

- Rate of increase in AST activity was the highest (P<0.001) for both BWM and CSM and the lowest for TEY. SOD decreased (P<0.001) the rate of increase in AST activity by about 60% from 22.2 to 13.3%.

2.2. Activity of ALT:

- Pre-freezing, activity of ALT was the highest (P<0.001) for BWM (33.2 U/l). SOD reduced (P<0.001) ALT activity from 31.3 to 27.2 U/l). The highest ALT activity was obtained for BWM without SOD (35.4 U/l), while the lowest values (23.4 U/l) were obtained for CSM with SOD.

- Post-freezing, ALT activity was the highest (P<0.001) for BWM (51.3 U/l). It was lower (P<0.001) for extenders with than without SOD (38.9 *vs*. 45.2 U/l). The highest ALT activity was recorded for BWM without SOD (56.8 U/l), while the lowest values (33.2 U/l) were obtained for CSM with SOD.

- Rate of increase in ALT activity in post-thawed semen was higher (P<0.001) for BWM than the other types of extenders. It was nearly similar in extenders with or without SOD supplementation. Rate of increase in ALT activity was lower for BSM and BWM with than without SOD and an opposite trend was observed for the other types of extenders.

2.3. Activity of LDH:

- Pre-freezing, activity of LDH was lower (P<0.05) for CSM and CWM than TEY, BSM and BWM. SOD reduced (P<0.05) ALT activity by about 3.3% from 369.7 to 357.6 U/l). The highest reduction in LDH activity was obtained for BWM without SOD and the lowest one was for CSM with SOD.

- Post-freezing, LDH activity was lower (P<0.001) for CSM and CWM than the other extenders. It was lower (P<0.001) for extenders with than without SOD (391.6 *vs.* 404.2 U/l). The highest LDH activity was recorded for BWM without SOD (427.0 U/l) and the lowest (364.6 U/l) for CSM with SOD.

- The rate of increase in LDH activity in post-thawed was the highest (11.8%) for CWM without SOD and the lowest (7.7%) for CSM with or without SOD.

3. Effect of type of extender and antioxidant supplementation on:

3.1. Head length of spermatozoa:

- Pre-freezing, sperm head was significantly (P<0.05) the longest for BWM (8.71 µm). While, head length was not affected significantly

by SOD. BWM without SOD showed the longest heads (8.72 µm), while CSM with or without SOD showed the shortest heads (7.88 µm).

- Post-freezing, sperm head was significantly ($P<0.001$) shorter for CSM (7.67 µm) than the other types of extenders (7.81-7.90 µm). While, head length was not affected significantly by SOD. BWM without SOD showed the longest heads (7.90 µm), while CSM with SOD the shortest ones (7.68 µm).
- The rate of reduction in head length after freezing was the highest for TEY, BSM and BWM. The effect of SOD on rate of reduction in head length was not significant. The highest rate of reduction was obtained for BWM without SOD and the lowest for CSM with SOD.

3.2. Head width:

- Pre-freezing sperm head was significantly ($P<0.05$) the widest for TEY, BSM and BWM (4.43-4.50 µm). However, it was nearly similar for extenders with (4.28 µm) or without (4.30 µm) SOD. The widest head was found for BWM and BSM without SOD (4.52 µm) and the shortest ones (3.88 µm) for CSM with or without SOD.
- Post-freezing head width was nearly similar for all types of extenders with or without SOD, showing a tendency to be the narrowest for CSM with or without SOD (3.86 µm) as compared to the other types of extenders (3.90-3.98 µm).
- The rate of reduction in head width was significantly ($P<0.001$) higher for TEY, BSM and BWM than those for CWM and CSM. The effect of SOD on rate of reduction in head width was not

significant. The highest rate of reduction was obtained for BWM without SOD and the shortest ones for CSM with or without SOD.

3.3. Head area

- Pre-freezing head area was significantly (P<0.05) the largest for BSM and BWM (33.41-33.64 μm^2). However, it was not affected by SOD, being nearly similar for extenders with (30.88 μm^2) and without (30.73 μm^2) SOD. The largest head area was obtained for BWM without SOD (33.83 μm^2) and the smallest (26.26 μm) for CSM without SOD.
- Post-freezing head area was significantly (P<0.05) smaller for CSM than those of the other types of extenders. However, it was not affected by SOD, being nearly similar for extenders with (26.44 μm2) and without (26.49 μm2) SOD. The largest head area was obtained for BSM without SOD (27.07 μm2) and the smallest (25.39 μm) for CSM without SOD.
- The rate of reduction in head area was the highest for BSM and BWM. However, it was not affected significantly by SOD supplementation. The highest rate of reduction was found for BWM without SOD and the shortest ones for CSM with SOD.

4. Evaluation of buffalo spermatozoa:

4.1. Hypo-osmotic swollen test (HOS-test)

The response of spermatozoa to curl with HOS-test was significantly (P<0.05) the highest for CSM (56.4%), while BWM showed the lowest response (31.6%). SOD significantly (P<0.001) increased response to HOS-test (41.6 vs. 44.7). The highest response

was obtained for CSM with SOD and the lowest was for BWM without SOD.

4.2. Acrosome reaction:

Percentage of reacted spermatozoa was significantly (P<0.05) the highest for CSM (63.5%), while BWM showed the lowest reaction (47.3%). It was higher for extenders with than without SOD (51.3 *vs*. 56.5). Reaction of spermatozoa was the highest for CSM with SOD (65.6%) and the lowest for BWM without SOD (45.2%).

4.3. Head to head agglutination test:

Percentage of agglutinated spermatozoa was significantly (P<0.05) the highest for CSM (62.1%), while BWM showed the lowest percentages (36.6%). It was higher for extenders with than without SOD supplementation (48.2 *vs*. 52.7).

4.4. Conception rate (CR):

CR was higher for semen extended with CSM with (90%) or without SOD (80%) as compared to that extended with TEY without SOD (70%). However, it was close for semen extended with CSM without SOD and TEY with SOD (80%).

It could be concluded that CSM with or without antioxidant can be used in semen extension during cryo-preservation and supplementation of CWM , TEY and CSM with 100 IU/ml from superoxide dusmitase improved recovery rate of post thawed semen at the rate of 37oC/30 sec in term of the highest motile and live as well as the lowest abnormal sperm percentages.