CONTENTS

SUMMARY AND CONCLUSION

The current study was conducted at the International Live Stock Mangement Training Center (ILMTC), Sakha, belonging to the Animal Production Research Institute, Agricultural Research center, Ministry of Agriculture in participation with the dept. of Animal Production, Faculty of Agriculture, Mansoura University. The study aimed to compare different methods of evaluation of Holstein and buffalo semen through assessment of conventional methods for evaluation physical, chemical and morphological semen characteristics, along with Sphadex column filtration technique and osmotic shock at hypo and hyper- osmotic levels.

Semen was collected twice weekly from each of five healthy mature Holstein and five buffalo bulls as a rotein work of ILMTC. Immediately, after semen collection, one ejaculate was taken from each bull for 10 weeks (50 ejaculates for each species). The collected semen was transferred to a water bath at 37C°. Semen was evaluated for physical semen characteristics including, ejaculate volume, sperm cell concentration and percentages of gross motility, progressive motility, live, abnormal and intact acrosome spermatozoa. Grade of motility and sperm morphometric characteristics and biochemical characteristics of the seminal plasma were also determined. Sephadex filteration technique and osmotic test was also conducted on raw semen. Osmotic test designed to establish various levels of osmolarity and incubation times that would give the maximum percentage of curled spermatozoa at different hypo-osmotic levels, (0, 50,

,100, 150, 200 and 300 mOsm) or to establish percentage of shrunk spermatozoa at hyper-osmotic levels(400 and 600 mOsm) at 0, 15, 30, 45 and 60 minutes incubation times.

The following results were obtained:

1. Physical characteristics of raw semen:

(1).The present results indicated insignificant differences in physical characteristics of raw semen between Holstein and bufflo bulls including percentages of gross and progressive motility, grade of motility and percentages of live spermatozoa. However, ejaculate volume and percentage of sperm abnormality were significantly ($P>0.001$) higher by about 48% and 31.77%, respectively, in Holstein than buffalo bulls and average sperm cell concentration and spermatozoa with intact acrosome was significantly higher in buffalo than Holstein semen by about 24% and 4.48%, respectively.

(2).Total count of spermatozoa and total out put of motile and live spermatozoa were significantly) higher by about 17.86, 19.3 and 17.4% respectively in Holstein than in buffalo buls. However, total out put of normal and intact acrosome spermatozoa did not differ significantly between both species.

(3).In raw semen, the correllation coefficient was significantly positive between percentages of gross motility and livability, being highly significant ($p<0.001$) in buffalo ($r=0.865$) and significant ($p<0.05$) in Holstein semen ($r=0.854$). Percentage of progressive motility correlated negtively with sperm abnormality percentage in both species, although

strong and highly significant ($p<0.01$) in buffalo ($r=-0.718$), and insignificantly in Holstein ($r=-0.017$) semen. Sperm cell concentration correlated negatively with the ejaculate volume in both species, being strong and significant ($p<0.05$) in Holstein ($r= -0.610$) and insignificantly poor in buffalo semen ($r= -0.196$). Out of motility estimates, only gross motility percentage had positive and significant ($p<0.05$) correlation with sperm cell concentration in buffalo semen ($r= 0.599$). Such correlation was insignificantly negative and poor in Holstein semen. The opposite was found for the correlation of gross motility percentage with intact acrosome spermatozoa, being significant ($p<0.01$) and strong in Holstein ($r=0.731$) and insignificantly poor in buffalo semen ($r=0.371$).

(4).All morphometric characteristics of spermatozoa including total length (67.35 vs. 65.17μm) length (8.6 vs. 7.7 μm), breadth (4.3 vs. 3.9 μm) of head as well as length of middle (13.1 vs 12.0 μm) main (40.8 vs. 38.9 μm) and terminal (3.4 vs. 3.1 μm) pieces and head area (32.9 vs. 26.3 μm)were significantly higher in Holstein than buffalo spermatozoa. However, neck length and breadth/length of head did not differ significantly between both species (1.4 μm and 0.50 μm^2, respectively).

(5).Concentration of albumin showed the highest difference between both species, being significantly ($p<0.001$) higher in Holstein than buffalo seminal plasma by about 54%, while total proteins, globulin and phospholipids concentrations were significantly ($p<0.001$) higher in Holstein than buffalo seminal plasma by about 34, 20 and 38%, respectively. However, fructose concentration was significantly ($P<0.001$) higher by about 26% in buffalo than Holstein seminal plasma.

(6).Activity of GOT, GPT and LDH was highly significant ($P<0.001$) in buffalo (52.6, 28.6 and 499.7 U/L) than Holstein (44.6, 24.3 and 390.9 U/L) seminal plasma . However, GOT/GPT ratio did not differ significantly between both species (1.87).

2. Sephadex column filter technique:

(1).In post-filtrated semen, significant ($P<0.001$) improvement in motility, livabilty and abnormality of spermatozoa were occurred however, sperm cell concentration was reduced significantly ($P<0.001$) in post-filtrated semen.

(2).Sperm abnormality showed the highest recovery rate, being significantly ($P<0.05$) higher in Holstein (-57.6%) than in buffalo (-52.1%) semen, followed by live spermatozoa (16.7 and 13.7%, respectively). While recovery rate of progressive motility percentage did not differ significantly between Holstein (15.7%) and buffalo (14.4%) semen . The lowest recovery rate was observed in percentage of spermatozoa with intact acrosome, being insignificantly higher in Holstein (10.2%) than in buffalo (7.1%) semen.

3. Osmotic shock:

3.1. Curled spermatozoa percentage:

(1).Percentage of total curled spermatozoa and frequency distribution of type A of curling were significantly ($P<0.001$) higher by about 9 and 31% in buffalo than Holstein bulls. However, frequency distribution of

types B and C of curling was lower by about 17 and 22% in buffalo than Holstein semen.

(2).Percentage of total curled spermatozoa of both species significantly ($P<0.05$) decreased by decreasing the osmotic level from 600 up to 0 mOsm, showing the highest percentage of curling with 0 mOsm solution and the lowest values with 600 mOsm one, being higher in buffalo than Holstein semen at all osmolarity levels. The increase in total curling was associated with a significant ($P<0.05$) increase in the frequency distribution of curling types B and C of curled spermatozoa and significant ($P<0.05$) decrease in the frequency distribution of curled spermatozoa type A. Percentage of total curling and type A of curling was higher in buffalo than Holstein spermatozoa, while those of type B and C were higher in Holstein than buffalo semen.

(3). Percentage of total curled spermatozoa in both species increased by increasing incubation time, being higher in buffalo than Holstein semen at all incubation times. A pronounced increase was observed up to 45 min., thereafter the rate of increase was not significant in both species up to 60 min. incubation time. It was observed a significant ($P<0.05$) decrease in curling type A and increase in type C up to 30 minutes. While a significant ($P<0.05$) increase in curling type B was observed up to 60 minutes. Generally, frequency distribution of curling type A was higher in buffalo than Holstein semen, however, those of types B and C were the opposite.

3.2. Shrunk spermatozoa:

(1) Percentage of shrinkage was consistently higher in buffalo than Holstein spermatozoa (48.96% vs. 55.64%).

(2) Percentage of shrunk spermatozoa was significantly ($P<0.001$) higher at 600 than 400 mOsm (48.0% vs. 56.6%) in both species, being higher in buffalo than Holstein semen at both hyper-osmolarity levels.

(3) Percentage of shrunk spermatozoa significantly ($P<0.05$) increased by increasing incubation time from 0 up to 45 min. with highest rate of increase between 15 and 30 min. in both species, being higher in buffalo than Holstein semen at all incubation times.

The present results indicated pronounced variations in response to the conventional methods of evaluation of different physical, chemical and morphological characteristics between semen of both species. Using Sephadex column filter technique has beneficial effects on improving spermatozoa quality, by increasing motility, livability and reducing abnormality of spermatozoa of both species. In addition, osmotic tests provide a precise technique for measuring alterations in sperm viability and membrane integrity of spermatozoa, in particular at 0 mOsm. Thus water test could be used as an important additional indicator of male fertility.