Title of thesis : Comparative study on certain functional and biochemical parameters in fresh and frozen semen of buffalo bulls with varying reproductive performance

Thesis by : Dr. Yogita Dhaka (V-2010-30-08)

Major Advisor : Dr. Madhumeet Singh

SUMMARY

The study was conducted on 12 apparently healthy Murrah buffalo breeding bulls, maintained at Frozen Semen Bank, RCDF Ltd., Bassi, Jaipur. The selected bulls were divided into 2 groups, each comprising of 6 bulls, according to their known ejaculate quality (donating good or poor quality semen, respectively) to compare their functional and biochemical attributes. Group 1 comprised those bulls, which were donating semen of excellent quality with good freezability and fertility parameters whereas group 2 included those bulls which were frequently donating either initial poor quality semen or higher degree of damage occurred during processing (during equilibration or cryopreservation) but were otherwise healthy.

After collection, initial parameters of ejaculate were recorded. Then the semen was diluted and further processing and freezing was done as per laboratory schedule. Thawing of semen straws was done at 37°C for 30 seconds. Semen evaluation was done at 4 different stages i.e. at fresh diluted, post-equilibration, 0 hr and 1 hr post-thaw. At all these stages the semen samples were evaluated for motility, live and dead count, reaction to hypo-osmotic solution and acrosomal integrity. Additionally, 2 ml diluted semen at all the four stages was centrifuged @ 268 g (2000 rpm) for 25 minutes to separate seminal plasma for biochemical estimation (enzymes and minerals) and this was stored at -20°C pending analysis.

Enzymes (AKP, AST, ALT and hyaluronidase) and minerals (Calcium, phosphorus, magnesium, sodium, potassium and chloride) were estimated in the seminal plasma separated at all the four stages.

5.1 Initial parameters of good and poor quality semen of Murrah buffalo bulls

In present study, the mean ejaculate volume was 3.92±0.08 and 3.38±0.13 ml for good and poor quality ejaculates, respectively. The overall mean sperm concentration in good quality ejaculates was 1593.88±24.22 whereas it was 1503.10±24.60 million per ml in poor quality ejaculates. The mean mass activity of good quality ejaculates was 4.30±0.08 where as in case of poor quality ejaculates it was 1.66±0.08. The mean pH of good quality ejaculates was 6.90±0.003 where as it was 7.35±0.027 in poor quality ejaculates. The differences in mass activity and pH were highly significant (p<0.01).
5.2 Functional parameters of good and poor quality semen of Murrah buffalo bulls

The mean live per cent was significantly higher (p<0.01) in good than poor quality ejaculates at all the four stages. At fresh diluted stage the values were 90.88±0.26 and 64.42±0.91 per cent which declined to 78.29±0.31 and 29.42±1.10 at post thaw stage in good and poor quality ejaculates, respectively. The mean progressive motility was significantly higher (p<0.01) in good quality ejaculates at all the four stages of semen evaluation. A significant decline (p<0.01) was observed in overall mean progressive motility with semen processing stages. The overall mean progressive motility recorded at fresh diluted stage was 86.15±0.34 and 44.48±0.75 per cent which declined to 63.50±0.29 per cent and 18.15±0.69 per cent immediately after post-thaw in good and poor quality ejaculates, respectively.

Significantly higher (p<0.01) HOS reactivity was observed in good quality ejaculates at all the stages of semen processing. Evaluation of HOS reactivity at various stages of processing showed that the HOS reactive spermatozoa percentage declined significantly (p<0.01) from fresh diluted (89.19±0.26 and 63.17±0.93%) to post-thaw stages (77.05±0.31 and 28.44±1.09% immediately after thawing) in good and poor quality ejaculates, respectively. The intact acrosome percentage was higher (p<0.01) in good quality ejaculates at all the four stages of semen evaluation. The overall mean intact acrosomes recorded at fresh diluted stage was 91.21±0.30 and 65.33±0.88 per cent which declined to 70.79±0.32 and 15.88±0.64 per cent at 1 hr incubation stage in good and poor quality ejaculates, respectively. The decline in percent intact acrosomes through various stages of evaluation within good and poor quality ejaculates was significant (p<0.01).

5.3 Enzymatic profile of good and poor quality semen of Murrah buffalo bulls

Concentration of alkaline phosphatase (AKP) recorded in good quality ejaculates was significantly higher (p<0.01) in comparison to poor quality ejaculates at all the four stages of evaluation. The concentrations of AKP increased significantly (p<0.01) as the processing stages advanced in both good and poor quality semen. At fresh diluted stage it was 147.75±1.63 and 113.40±0.74 KAU/dL and this concentration increased to 155.94±1.69 and 121.13±0.79 KAU/dL at post thaw stage in good and poor quality ejaculates, respectively. Similarly, significantly higher AST (p<0.01) concentrations were recorded in poor quality ejaculates at all the four stages of semen processing. The AST concentration increased significantly (p<0.01) from fresh diluted to 1 hr post-thaw incubation in both good and poor quality ejaculates.

The ALT concentrations in seminal plasma increased significantly (p<0.01) with advancement in the semen processing stages both in good and poor quality ejaculates. At fresh diluted stage, the mean ALT concentration was 14.75±0.12 and 25.43±0.47 U/L which increased to 17.73±0.11 and 44.87±0.43 U/L after 1 hr incubation in good and poor quality
ejaculates, respectively. Higher (p<0.01) concentrations of hyaluronidase were recorded in poor quality ejaculates and the concentration increased significantly (p<0.01) with semen processing in good as well as poor quality ejaculates. It was 216.54±1.36 and 451.54±2.12 U/ml in freshly diluted semen which increased to 237.56±1.22 and 492.23±2.38 U/ml at 0 hr post thaw stage in good and poor quality ejaculates respectively.

5.4 Mineral profile of good and poor quality semen of Murrah buffalo bulls

The calcium concentrations were significantly higher (p<0.01) in good quality seminal plasma at all the stages of evaluation. There was a significant decline (p<0.01) from fresh diluted (45.18±0.29 and 41.95±0.18 mg/dl) to post-thaw (44.23±0.36 and 40.90±0.18 mg/dl) stage in good and poor quality ejaculates, respectively. Similarly, significantly higher (p<0.01) phosphorus concentrations were recorded in good quality ejaculates. A significant decline (p<0.01) was observed in phosphorus concentration as the semen processing stages advanced in both good and poor quality ejaculates. At fresh diluted stage the mean value was 9.98±0.11 and 6.70±0.10 mg/dl which decreased to 8.78±0.11 and 5.75±0.08 mg/dl at 1 hr incubation post thaw in good and poor quality ejaculates, respectively.

The mean magnesium levels were significantly higher (p<0.01) in good quality ejaculates at all the four stages of semen evaluation. In case of good quality ejaculates, there was a non-significant decline from fresh diluted (7.21±0.11 mg/dl) to 1 hr incubation post-thaw (7.05±0.12 mg/dl), where as in poor quality ejaculates, this decline was significant (p<0.01) from 6.25±0.05 fresh diluted to 6.06±0.04 mg/dl 1 hr incubation post-thaw.

A significant decline was observed in sodium concentration (p<0.01) with processing stages in good as well as poor quality ejaculates. There was a non-significant difference in fresh diluted stage in good and poor quality ejaculates. Significantly higher (p<0.01) potassium concentration was observed in good quality ejaculates. The mean chloride concentration was significantly higher (p<0.01) in good quality ejaculates and there was a significant decline in the mean concentration values with advancing semen processing stages. At fresh diluted stage the mean chloride concentration in good quality ejaculate was 369.40±0.81 and in poor quality ejaculate it was 274.42±0.98 mg/dl which declined significantly (p<0.01) to 355.52±1.02 and 260.99±0.90 mg/dl at immediately post-thaw stage in good and poor quality ejaculates, respectively.

5.5 Correlation between functional and biochemical parameters

The live sperm percentage was significantly (P<0.01) correlated with progressively motile spermatozoa (r=0.92738 and 0.99934, n=192) in good and poor quality ejaculates. Similarly, sperm livability percentage was significantly (P<0.01) correlated with HOS responsive spermatozoa (r=0.98934 and 0.99934, n=192) in good and poor quality semen, respectively. The correlation between live sperm percentage and acrosomal integrity
(r=0.92725 and 0.98273) was also significant (P<0.01) in good and poor quality ejaculates, respectively.

A significant (P<0.01) negative relationship was observed between livability and AKP (r=-0.47586 and -0.59034) and AST (r=-0.9416 and -0.8358) leakage in good and poor quality semen, respectively. Highly negative significant (P<0.01) correlation was observed between per cent livability and leakage of ALT (r= -0.7907 and -0.9165) and hyaluronidase (r= -0.78965 and -0.74037) in good and poor quality ejaculates, respectively.

Live sperm percentage was significantly (P<0.01) correlated with calcium (r=0.21353 and 0.35028) and phosphorus (r=0.43048 and 0.43474) in good and poor quality ejaculates, respectively. Similarly a significant (P<0.05) relationship was observed between sperm livability and magnesium concentration (r=0.18175 and 0.14500. Live sperm percentage was significantly (P<0.01) correlated with sodium (r=0.69600 and 0.84324), potassium (r=0.76092 and 0.72068) and chloride (r=0.77688 and 0.70555) in good and poor quality ejaculates, respectively.

The per cent progressive motility was significantly (P<0.01) correlated with HOS reactive (r=0.92052 and 0.94212) and per cent intact acrosome (r=0.94709 and 0.95266) in good and poor quality ejaculates, respectively. Similarly, significant (P<0.01) negative relationship was observed between progressive motility and AKP (r=-0.45140 and -0.66370) and AST (r=-0.9292 and -0.7337) leakage in good and poor quality ejaculates, respectively. Highly negative significant (P<0.01) correlation was observed between per cent progressive motility and leakage of ALT (r= -0.7787 and -0.8954) and hyaluronidase (r= -0.76605 and -0.68714) in good and poor quality ejaculates, respectively.

Progressive motility was significantly (P<0.01) correlated with calcium (r=0.21883 and 0.33624) and phosphorus (r=0.46707 and 0.51561) in good and poor quality ejaculates, respectively. A non-significant relationship was observed between progressive motility and magnesium (r=0.12246) in good quality ejaculate, where as in poor quality ejaculate, there was a significant (P<0.01) relationship (r=0.20495). The correlations of progressive motility with sodium (r=0.67512 and 0.89136), potassium (r= 0.73808 and 0.61697) and chloride (r=0.78694 and 0.71250) were significant (P<0.01) in good and poor quality ejaculates.

The HOS reactive spermatozoa percentage was significantly (P<0.01) correlated with per cent intact acrosome spermatozoa (r=0.91623 and 0.98272, n=192) in good and poor quality ejaculates, respectively.

The correlation of HOS reactive spermatozoa with AKP (r=-0.47729 and-0.58627) and AST (r=-0.9360 and -0.8376), were significant (P<0.01) in good and poor quality ejaculates, respectively. Highly negative significant (P<0.01) correlation was observed between HOS reactive spermatozoa and leakage of ALT (r= -0.7965 and -0.9177) and hyaluronidase (r= -
0.78737 and -0.74026) in good and poor quality ejaculates, respectively. Mean HOS reactive spermatozoa was significantly (P<0.01) correlated with calcium (r=0.19565 and 0.35496) and phosphorus (r=0.42809 and 0.43346) in good and poor quality ejaculates, respectively. A significant (P<0.01) relationship was recorded between HOS reactive spermatozoa and magnesium (r=0.19163) in good quality ejaculate. Whereas, in poor quality ejaculate, there was a significant (P<0.05) relationship (r=0.14716) of magnesium on HOS reactive spermatozoa percentage. Mean HOS reactive spermatozoa was significantly (P<0.01) correlated with sodium (r=0.70064 and 0.84402), potassium (r=0.75416 and 0.72238) and chloride (r=0.77414 and 0.70352) in good and poor quality ejaculates, respectively.

A significant (P<0.01) negative relationship was recorded between per cent intact acrosome spermatozoa with AKP (r= -0.51428 and -0.60056) and AST (r= -0.9268 and -0.8076) leakage in good and poor quality ejaculates, respectively. Highly significant (P<0.01) negative correlation was observed between intact acrosome spermatozoa with leakage of ALT (r= -0.7724 and -0.9081) and hyaluronidase (r= -0.85093 and -0.76107) in good and poor quality ejaculates, respectively.

Intact acrosome percentage was significantly (P<0.01) correlated with calcium (r=0.18750 and 0.38229) and phosphorus (r=0.47493 and 0.44462) in good and poor quality ejaculates, respectively. Similarly, a significant (P<0.05) relationship was observed between intact acrosome spermatozoa and magnesium (r=0.15049 and 0.16756) in good and poor quality ejaculates, respectively. The correlations of per cent intact acrosome spermatozoa with sodium (r=0.62641 and 0.84825), potassium (r= 0.75116 and 0.69577) and chloride (r=0.74740 and 0.72969) in good and poor ejaculates were significant (P<0.01) in good and poor quality ejaculates, respectively.

Conclusions
1. Significantly higher mass activity and lower pH values were recorded in good quality semen.
2. There was a significantly higher percentage of live, progressively motile, HOS reactive and intact acrosome spermatozoa in good quality semen.
3. Greater loss of plasma membrane integrity and higher leaching of intracellular enzymes is detected in poor quality semen.
4. Lower AST, ALT and Hyaluronidase and higher AKP concentrations were recorded in good quality semen.
5. Relatively lower calcium, phosphorus, magnesium, sodium, potassium and chloride levels were recorded in poor quality semen.
6. There was a strong correlation between functional and biochemical parameters of good as well as poor quality semen of Murrah buffalo bulls.