SPERM MORPHOLOGICAL ABNORMALITIES IN ASTHENO OLIGOSPERMIC FRIESWAL BULLS

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ABSTRACT

Eighteen mature Frieswal (Holstein Friesian X Sahiwal; having 5/8 exotic inheritance) bulls of similar age (29.79±0.90 months) were divided into 2 categories i.e. poor (N=9, sperm motility < 50% and concentration <600 million/ml) and normospermic bulls (N=9). Sperm viability and morphology was evaluated by eosin, nigrosin staining technique. The mean (±SE) number of total viable, morphologically normal and progressively motile sperm (in millions) per ejaculate in poor bulls (1234.53±266.96, 636.21±150.18 and 272.22±68.06, respectively) was significantly (P<0.01) lower than that of normospermic bulls (5248.71±646.02, 5278.11±657.15 and 2476.75±360.93, respectively). Total abnormal head, mid piece and tail (%) in poor bulls (27.25±3.52, 11.08±1.17 and 32.83±3.79, respectively) were significantly (P<0.01) higher than normospermic bulls (7.02±0.96, 5.20±0.75 and 8.64±1.46, respectively). Total sperm abnormality (%) in poor bulls was 3.4 times higher than normospermic bulls and differences were highly significant (P<0.01). Among the major morphological defects, sperm abnormalities such as pyriform/pear shaped head, deformed head, presence of proximal protoplasmic droplet and 'Dag' like defect and among minor defects, abnormalities such as tapered head/narrow head, free head, coiled mid-piece and tail, broken mid piece, bent tail and presence of distal protoplasmic droplets were significantly (P<0.01) higher in poor bulls as compared to normospermic bulls. In this study, it was concluded that poor Frieswal bulls not only have reduced sperm motility and concentration, but also have wide spectrum of spermatogenetic defects with excessive occurrence of various kinds of both major and minor sperm morphological abnormalities to the tune of 3-4 times higher than normal attributes.

Key words: Sperm abnormality, Frieswal Bull, Poor semen quality

More number (nearly 60%) of crossbred bulls are rejected from germplasm production centers due to various reasons such as low sperm concentration, less sperm motility and unacceptable freezability (Tyagi *et al.,* 2006). Poor semen quality and male infertility of various species has been reported to be associated with several genetic and non genetic factors, such as hypogonadism, cryptorchidism, structural abnormalities of the male genital tract, chronic illness, scrotal injury, thermal insult, genital infection, medication, nutritional deficiencies, constitutive chromosomal abnormalities, micro deletion of Y chromosome, gene mutation etc (Dohle *et al.,* 2002). Genetically precious (on production traits) AI bulls born from elite dams mated by proven sire, but unfit for frozen semen production are culled from the germplasm production stations on economic ground. There is lack of literatures on reasons for high rate of reproductive unsoundness in crossbred bulls and detailed studies on qualitative attributes of semen in poor performer bulls are not available. Present study gives detailed accounts on incidence of various kinds of sperm abnormalities in poor Frieswal bulls, which might give an insight to find reasons for disturbed/ defective spermatogenesis in crossbred bulls that are poor in reproduction, otherwise superior in production traits.

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The present study was conducted on 18 Frieswal (Holstein Friesian X Sahiwal; having 5/8 exotic inheritance) bulls (no. of ejaculates= 56) maintained at Bull Rearing Unit (BRU), Project Directorate on Cattle, Meerut. Semen was collected twice a week using an artificial vagina (42-45 °C) at early morning by the same semen collector. Bulls were divided into 2 categories i.e. (a) poor bulls (sperm motility < 50% and concentration <600 million/ml) and (b) normospermic bulls. Age of the bulls of 2 categories was kept almost similar (28.83± 1.51 and 30.50±1.10 months, respectively) to nullify age related changes in semen quality between the groups. All the bulls were kept under similar management, feeding and housing conditions. Immediately after semen collection, ejaculates were placed in a water bath at 37°C and evaluated for volume, color, consistency, sperm concentration and progressive motility per cent. Sperm concentration was estimated by using a photometer (Accucell, IMV- France). Semen was diluted (approx. 1: 10) in Tris - egg yolk - citric acid – fructose- glycerol (6.4%) extender and progressive motility was determined on a subjective scale of 0-100 % with nearest 5% intervals, viewing at least 5 microscopic fields at 200 x magnification of a phase-contrast microscope fitted with stage warmer (37°C).

Sperm viability and morphology was evaluated by eosin nigrosin staining technique (Hancock, 1952) by counting 100 spermatozoa per ejaculate and observed (100x) under oil immersion. When multiple abnormalities were observed in the same spermatozoa, only one abnormality was recorded by giving first priority to abnormal head, followed by mid piece and tail, proximal droplet with third and distal droplet as the last priority. Data were subjected to analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS 7.5).

Semen quality of poor normospermic Frieswal bulls are given in table. All the semen quality attributes between the two groups differed significantly (P<0.01) except ejaculate volume. Seminal attributes of normospermic Frieswal bulls were almost similar as reported earlier in this and other HF crossbred bulls (Bhoite *et al.*, 2008). In poor bulls sperm concentration/ ml and progressively motile sperm per cent were nearly 1/3rd of normospermic bulls although the ejaculatory volume was almost same. Similarly, total sperm count, live sperm count, morphologically normal sperm count and progressively motile sperm count per ejaculate in poor Frieswal bulls were even less than 1/2nd , 1/4th , 1/ 7th and 1/9th of values obtained in normospermic bulls.

Total sperm abnormality (%) in poor bulls was 3.4 times higher than normospermic bulls and differences were highly significant (P<0.01). Total major sperm abnormality (%) was 4 times and minor abnormality (%) nearly 3 times higher in poor bulls as compared to normal bulls. Among major morphological defects, sperm abnormalities such as pyriform / pear shaped head, deformed head, presence of proximal protoplasmic droplet and 'Dag' like defect were significantly higher (P<0.01) in poor bulls as compared to normal bulls. Similarly, among the minor sperm abnormalities, tapered head/narrow head, free head, coiled mid-piece and tail, broken mid piece, bent tail and presence of distal protoplasmic droplets were significantly (P<0.01) higher in poor bulls as compared to normospermic bulls.

Irrespective of the types of abnormalities, total abnormal head, mid piece and tail (%) were nearly 4, 2 and 4 folds, respectively higher (significant, P<0.01) in poor bulls as compared to normal bulls. Such higher incidences of abnormal sperm in the ejaculate indicated serious disturbance in the process of spermatogenesis in poor bulls. It was not as simple as reduced rate of spermatogenesis and/or only maturation deficiency that had lead to astheno-oligozoospermic conditions. Reasons for such a wide spectrum of spermatogenetic defect along with testicular insufficiency in large number of genetically superior (in production traits) *Bos indicus* x *Bos taurus* crossbrerd bulls have not been properly investigated, unlike human species (Dohle *et al.*, 2002) where infertility of individual person bears tremendous family and social importance. Idiopathic forms of male subfertility / infertility such as OAT (oligo-astheno-teratozoospermia) syndrome may be explained by factor like endocrine disruption, genetic abnormalities and various environmental stressors. Males with extreme oligozoospermia and azoospermia reported to have numerical and structural chromosomal abnormalities (Chandley 1998), sub-microscopic deletion of azoospermia factor region (AZF) of Y chromosome (Vogt *et al.*, 1996) and mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, commonly associated with congenital defect in vas deference (Dohle *et al.*, 1999, 2002). Men with oligo-azoospermia reported to have autosomal abnormalities like Robertsonian and reciprocal translocation and inversions (Nakamura *et al.,* 2001). However, in bovine species sporadic cases have been reported (Patel *et al.,* 2006). In some poor bulls we have also observed occasional non motile ejaculates (cryptozoospermia). Such observations have been reported in men having Klinefelter syndrome (Dohle *et al.,* 2002).

In this study we concluded that Frieswal bulls with astheno-oligozoospermia like conditions, not only have reduced sperm motility and concentration, but have wide spectrum of spermatogenetic defects with excessive occurrence of various kinds of both major and minor sperm morphological abnormalities, however, detailed andrological investigation is warranted through cytogenetic and molecular genetic approaches.

Table : Mean (±S.E.) semen quality parameters of poor and normospermic Frieswal bull

Particulars	Poor bulls (n=9) (No. of ejaculates = 24)	Normospermic bulls (n=9) (No. of ejaculates = 32)	Over all (n=18) (No. of ejaculates = 56)	P values
Age (months) of bulls	28.83 ± 1.51^{a}	30.50 ± 1.10^{a}	29.79 ± 0.90	0.3643
Ejaculate volume (ml)	5.15 ± 0.55^{a}	4.46 ± 0.30^{a}	4.76 ± 0.29	0.2524
Concentration ($\times10^6$ per ml)	523.25 ± 68.71^{A}	1421.09 ± 108.25^{B}	1036.30 ± 90.61	0.0000
Initial progressive motility (%)	21.67 ± 3.03^{A}	54.69 ± 1.99^{B}	40.54 ± 2.79	0.0000
Total number of sperm per ejaculate ($\times10^6$)	2916.64 ± 550.56^{A}	6449.12 ± 718.25^{B}	4935.20 ± 525.40	0.0005
Non-eosinophilic sperm (live) %	43.19 ± 5.34^{A}	77.21 ± 1.53^{B}	62.63 ± 3.43	0.0000
Total number of live sperm per ejaculate ($\times10^6$)	1234.53 ± 266.96^{A}	5248.71 ± 646.02^{B}	3528.35 ± 478.63	0.0000
Total number of normal sperm per ejaculate ($\times10^6$)	636.21 ± 150.18^{A}	5278.11 ± 657.15^{B}	3288.72 ± 488.95	0.0000
Total number of motile sperm per ejaculate ($\times10^6$)	272.22 ± 68.06^{A}	2476.75 ± 36.93^{B}	1531.96 ± 253.82	0.0000

Row wise means with different superscripts differ significantly (small letters $P<0.05$; capital letters $P<0.01$)

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