# COMPARATIVE EVALUATION OF PHYSICO-MORPHOLOGICAL ATTRIBUTES OF SEMEN OF JAFARABADI, MEHSANA (Bubalus bubalis) AND CROSSBRED (HF X KANKREJ) (Bos indicus) BULLS

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## ABSTRACT

Semen ejaculates (90) of 15 mature bulls, 5 each of Jafarabadi, Mehsana and Crossbred (HF x Kankrej, F1) breeds collected at weekly interval during autumn season were studied for HOS test and physico-morphological attributes of spermatozoa and their interrelationships. The mean ejaculate volume (ml), mass activity (score 0-5), individual sperm motility (%), sperm concentration (million/ml), live sperm (%), abnormal sperm (%) and HOS reactive sperm (%) in Jafarabadi buffalo bulls semen were 4.81 ± 0.21, 3.30 ± 0.08, 74.33 ± 0.75, 1466.80 ± 72.43, 84.93 ± 0.59, 10.67 ± 0.43 and 70.47 ± 0.54, respectively. The corresponding values for Mehsana buffalo bulls semen were 5.01 ± 0.23, 3.23 ± 0.12, 73.83 ± 0.67, 1307.43 ± 94.27, 82.47 ± 0.67, 10.83 ± 0.38 and 71.53 ± 0.89; and in HF x K Crossbred bulls semen 6.68 ± 0.31, 2.83 ± 0.09, 72.00 ± 0.51, 1094.80 ± 78.85, 79.60 ± 0.65, 12.87 ± 0.45 and 61.37 ± 0.69, respectively. The three breeds varied significantly ($P < 0.01$) in their seminal attributes. The ejaculate volume and abnormal sperm per cent were significantly ($P<0.05$) higher in crossbreds as compared to buffalo bulls, whereas the values of all other traits were significantly ($P<0.01$) higher in buffalo bulls, particularly in Jafarabadi breed, as compared to crossbreds. The ejaculate volume had significant negative correlations ($P < 0.05$) with most traits in all the three breeds; while mass activity showed significant positive correlations with initial motility, sperm concentration and live sperm percentage. The motility and sperm concentration were positively ($P<0.01$) associated, while live sperm and abnormal sperm were negatively ($P<0.05$) correlated in all three breeds. HOS reactive sperms, however, did not reveal high significant correlations with other traits in any of the breeds studied. Semen quality of Jafarabadi bulls was optimum, followed by Mehsana buffalo and that of crossbred bulls was relatively inferior.

**KEYWORDS:** Seminal attributes, HOS test, Comparative evaluation, Jafarabadi buffalo, Mehsana buffalo, HF x K crossbred bulls

## INTRODUCTION

Male fertility is an important factor in bovine reproduction since a single bull is generally bred to numerous cows. Semen analysis is the most commonly used procedure to evaluate male fertility potential (Rijsselaere *et al.*, 2002). Accurate prediction of fertility of each male or an accurate method for estimating fertilizing potential of semen are extremely useful means for successful exploitation of production potential of sires. However, no single test or combinations of tests have been proved to be totally reliable for accurate prediction of semen quality in relation to fertility. Most of the tests that are used for evaluation of semen are based on physical characters of spermatozoa. The correlations of these physical characters with fertility are highly variable and relatively poor (Belorkar *et al.*, 1990; Shelke and Dhami, 2001; Tiwari *et al.*, 2009). Moreover, crossbred bulls are reported to donate poor quality semen with high discard rate (Rao *et al.*, 1996). Therefore, this study was planned to carry out comparative evaluation of physico-morphological attributes and HOS reactive sperms of buffalo and crossbred bulls semen.

## MATERIALS AND METHODS

The study was carried out at the State Frozen Semen Production and Training Institute, Patan, Gujarat during March - April 2011. Fifteen sexually mature healthy breeding bulls, five each of Jafarabadi, Mehsana and HF x Kankrej (F1) breeds, aged 3-8 years, were included in the study. All these bulls were in good health and under optimal veterinary care. They were maintained in nearly identical nutritional and managerial conditions throughout the period of study and were under regular weekly semen collection schedule using AV. Immediately after collection, the ejaculates (6/ bull) were evaluated for routine physico-morphological attributes, including hypo-osmotic swelling (HOS) test. In all, 90 ejaculates from 15 bulls (6/bull) were studied.

The ejaculate volume, mass activity and individual motility were assessed as per Salisbury *et al.* (1978). The concentration of spermatozoa (million/ml) in the neat semen was determined by the digital-photometer (Accucell Photometer, IMV Technology, France) at 530 nm wavelength. The percentages of live as well as abnormal spermatozoa were estimated by differential staining technique using eosin-nigrosin stain (Campbell *et al.*, 1953). The morphological abnormalities were classified as of head, mid-piece and tail region (Blom, 1950). Hypo-osmotic swelling test was carried out using 150 mOsm citrate-fructose solution as per Jayendran *et al.* (1984). The data was analysed statistically using CRD and Duncan's NMRT and the breed-wise interrelationships among various traits studied were worked out (Snedecor and Cochran, 1994).

## RESULTS AND DISCUSSION

Breed- and species-wise means (±SE) of various physico-morphological attributes studied in freshly ejaculated semen of Jafarabadi, Mehsana and HF x K crossbred bulls are presented in Tables 1 and 2. All the bulls of Jafarabadi and Mehsana buffalo breeds and HF x K crossbreds consistently donated normal thick milky white and thick creamy yellow semen, respectively. The creamy yellow colouration of crossbred bull’s semen is due to presence of riboflavin pigment secreted from the seminal vesicles.

### Ejaculate Volume and Mass Activity

The ejaculate volume depends upon the body/scrotal size and the weight of the sire. The mean ejaculate volume of semen was significantly ($P<0.01$) higher (6.68 ± 0.31 ml) and mass activity score lower (2.83 ± 0.09) in HF x K bulls than in Jafarabadi and Mehsana buffalo bulls (4.81 ± 0.21 and 5.01 ± 0.23 ml, 3.30 ± 0.08 and 3.23 ± 0.12), however, the latter two breeds did not differ significantly in their mean values for these traits (Table 1). Dhami and Sahni (1994) and Dhami *et al.* (1998) reported significantly higher ejaculate volume and lower mass activity of semen of HF bulls than the Murrah bulls, while Shelke and Dhami (2001) and Rana and Dhami (2003) found non-significant variations for these traits between Gir and Jafarabadi bulls. Further, the mean values obtained in buffalo bulls under study were in line with the reports of Merja (1997), Tiwari *et al.* (2010) and Rao *et al.* (2011), and those in crossbred bulls corroborated with the reports of Belorkar *et al.* (1990), Perumal *et al.* (2009).

### Initial Motility and Viability of Sperms

The mean initial motility of sperms of Jafarabadi and Mehsana bulls was significantly ($P<0.05$) higher (74.33 ± 0.75 and 73.83 ± 0.67 %) than in HF x K bulls (72.00 ± 0.51 %), while the mean live sperm per cent in the semen of Jafarabadi (84.93 ± 0.59), Mehsana (82.47 ± 0.67) and HF x K crossbred (79.60 ± 0.65) bulls differed highly significantly ($P<0.01$) from each other, the value being highest in Jafarabadi bulls and lowest in crossbreds (Table 1). These findings on motility are in accordance with those of Dhami *et al.* (1998) in HF vs Murrah, but are in contrast to those of Shelke and Dhami (2001) in Gir vs Jafarabadi bulls. Shelke and Dhami (2001) and Rana and Dhami (2003), however, found significantly higher mean live sperm per cent in Jafarabadi than in Gir bulls. Further,

the present findings in either of the species were comparable with the reported values of Rana and Dhami (2003), Veerabramhaiah *et al.* (2010) and Rao *et al.* (2011) either in cattle or buffalo semen. Initial sperm motility / livability is an important attribute for acceptance or rejection of the ejaculate for further processing and use in AI, and it is positively correlated with keeping quality, freezability and fertility of that sample (Belorkar *et al.*, 1990; Shelke and Dhami, 2001; Tiwari *et al.*, 2011). All the bulls under study donated acceptable ejaculates in terms of initial motility and viability.

**Sperm Concentration**

Among the two species, Jafarabadi buffalo bull's semen had significantly (P<0.01) higher mean sperm concentration per ml than HF x K bull's semen (1466.80 ± 72.43 vs 1094.80 ± 78.85 million), however, the value for Mehsana bulls was intermediate (1307.43 ± 94.27 million) (Table 1). Dhami *et al.* (1998) reported similar findings between HF and Murrah bulls from Tarai region. Rana and Dhami (2003) also reported lower mean sperm concentration/ ml in Gir than Jafarabadi buffalo semen. Moreover, the present values of sperm concentration are well comparable with the reports of Singh *et al.* (2000) and Rao *et al.* (2011) in cattle, and of Dhami *et al.* (2001), Selvaraju *et al.* (2008) and Tiwari *et al.* (2010) in buffalo semen. Sperm concentration per ml is one of the most important traits of semen quality for acceptance in frozen semen production and in determining the number of doses that could be produced per ejaculate.

**Sperm Abnormalities**

The motility, velocity and penetrability into the vestment membranes of ova reside in normal morphology of spermatozoa. Greater the number of normal sperm better is the mobility and fertility of that semen. In the present study, the mean values of sperm head and mid-piece abnormalities did not differ significantly between three breeds, but the sperm tail abnormalities were significantly (P<0.01) higher in the semen of HF x K crossbred bulls than in Jafarabadi and Mehsana buffalo semen (Table 2). Similar values of sperm head abnormalities were reported by different workers either in cattle or buffalo bulls (Rana and Dhami, 2003; Mandal *et al.*, 2009; Veerabramhaiah *et al.*, 2010). Some workers, however, reported much higher values in either buffalo or cattle (Baburao *et al.*, 2000 and Mandal *et al.*, 2010), while others found lower values (Rao *et al.*, 1996; Tiwari *et al.*, 2009). Comparable values of sperm mid-piece abnormalities were also reported by Veerabramhaiah *et al.* (2010) in Murrah buffalo bulls, but Mandal *et al.* (2010) reported much higher value. Baburao et al. (2000) observed comparable sperm tail abnormalities in different breeds of cattle, whereas, Singh *et al.* (2000), Rana and Dhami (2003) and Mandal *et al.* (2010) reported higher sperm tail abnormalities in different cattle breeds.

No significant difference was observed between Jafarabadi and Mehsana bulls for total sperm abnormalities, however, HF x K crossbred bulls had significantly (P<0.01) higher values of mean total sperm abnormalities than buffalo bull's semen. The present values of total sperm abnormalities compared favourably with the reports of Veeraiah *et al.* (1999) in cattle and of Bhavsar *et al.* (1988) in Mehsana buffalo semen. In contrast, Shelke and Dhami (2001) found greater total sperm abnormalities in Jafarabadi bulls than in Gir bulls. Some workers reported much higher mean values of total sperm abnormalities in buffalo (Dhami *et al.*, 2001) and in cattle semen (Rana and Dhami, 2003; Mandal *et al.*, 2009), while others found much lower values either in buffalo (Tiwari *et al.*, 2009) or cattle semen (Singh *et al.*, 2000; Perumal *et al.*, 2009).

**Hypo-Osmotic Swelling Test**

Assessment of sperm membrane function appears to be a significant marker for the fertilizing capacity of spermatozoa, since it is involved in metabolic changes with the surrounding medium and in the process of capacitation, acrosome reaction and fusion with the oocyte membrane. The HOS test evaluates whether an intact membrane is biochemically active, thus, it is more conclusive

in evaluating biochemical function of sperm membrane and thereby fertilizing ability of spermatozoa (Jayendran and Zaneveld, 1992).

**Table 1. Mean (±SE) initial semen quality of Jafarabadi, Mehsana and HF x K crossbred bulls**

| Breed | Ejaculate volume (ml) | Mass activity (score 0-5) | Initial motility (%) | Sperm conc. (million/ml) | Live sperm (%) |
|---|---|---|---|---|---|
| Jafara-badi | 4.81±0.21$^{b}$ | 3.30±0.08$^{a}$ | 74.33±0.75$^{a}$ | 1466.80±72.43$^{a}$ | 84.93±0.59$^{a}$ |
| Mehsana | 5.01±0.23$^{b}$ | 3.23 ±0.12$^{a}$ | 73.83 ±0.67$^{a}$ | 1307.43±94.27$^{ab}$ | 82.47±0.67$^{b}$ |
| HF x K (F1) | 6.68±0.31$^{a}$ | 2.83 ±0.09$^{b}$ | 72.00 ±0.51$^{b}$ | 1094.80±78.85$^{b}$ | 79.60±0.65$^{c}$ |

Means bearing common superscript within the column do not differ significantly (P>0.05).

**Table 2. Mean (±SE) initial sperm abnormalities and HOS reactive spermatozoa of Jafarabadi, Mehsana and HF x K crossbred bulls**

| Breed | Head abnormality % | Mid-piece abnormality % | Tail abnormality % | Total sperm abnormalities % | HOS +Ve sperm % |
|---|---|---|---|---|---|
| **Jafara-badi** | 4.27±0.34 | 1.70±0.15 | 4.70±0.45$^{b}$ | 10.67±0.43$^{b}$ | 70.47±0.54$^{a}$ |
| **Mehsana** | 4.27±0.31 | 1.63±0.17 | 4.93±0.35$^{b}$ | 10.83±0.38$^{b}$ | 71.53±0.89$^{a}$ |
| **HF x K (F1)** | 4.67±0.33 | 1.70±0.14 | 6.50±0.36$^{a}$ | 12.87±0.45$^{a}$ | 61.37±0.69$^{b}$ |

Means bearing common superscript within the column do not differ significantly (P>0.05).

The overall means of hypo-osmotic reactive spermatozoa recorded in the fresh semen of Jafarabadi and Mehsana buffalo bulls were significantly (P<0.01) higher (70.47 ± 0.54 and 71.53 ± 0.89 %) than in HF x K crossbred bulls (61.37 ± 0.69 %), however, no difference in mean values of HOS reactive spermatozoa was found between buffalo breeds (Table 2). Rana and Dhami (2003) reported similar trend for Gir (54.35 ± 3.04) versus Jafarabadi buffalo semen (58.70 ± 2.29), whereas, Lodhi *et al.* (2008) found no significant difference for hypo-osmotic reactive spermatozoa between Sahiwal and Nili-Ravi buffalo bulls. Tiwari *et al.* (2009) found comparable result of hypo-osmotic reactive spermatozoa in Tarai buffalo, whereas Perumal *et al.* (2009) recorded higher HOS reactive sperms in the semen of Jersey breed. The fertilizing ability of bovine spermatozoa bears positive association with this attribute, and hence is an important indirect test to assess fertilizing potential of a bull or an ejaculate.

### Interrelationships among various Spermatozoal Attributes

Among physico-morphological attributes, the ejaculate volume, mass activity and initial motility of Jafarabadi buffalo semen did not reveal any significant interrelationship, although the sperm concentration per ml had significant positive correlations with mass activity (0.865, P<0.01) and initial motility (0.425, P<0.05). The live sperm per cent had highly significant negative correlations with abnormal sperm per cent (-0.843, P<0.01). The HOS reactive sperm and abnormal sperm did not reveal significant correlation with other traits (Table 3).

**Table 3. Interrelationships (r) between various physico-morphological attributes of semen in Jafarabadi, Mehsana buffalo and HF x Kankrej crossbred bulls**

| Seminal traits | Ejaculate Volume | Mass activity | Initial motility | Sperm count/ml | Live sperm | Abnormal sperm |
|---|---|---|---|---|---|---|
| **Jafarabadi buffalo bulls** | | | | | | |
| Mass activity | -0.031 | - | - | - | - | - |
| Initial motility | 0.104 | 0.348 | - | - | - | - |
| Sperm count/ml | -0.011 | 0.865** | 0.428* | - | - | - |
| Live sperm | 0.316 | -0.081 | 0.126 | -0.052 | - | - |
| Abnormal sperm | -0.158 | -0.099 | -0.060 | -0.084 | -0.843** | - |
| HOS reacted sperm | 0.096 | 0.125 | 0.012 | 0.045 | -0.244 | 0.098 |
| **Mehsana buffalo bulls** | | | | | | |
| Mass activity | -0.436* | - | - | - | - | - |
| Initial motility | -0.128 | 0.554** | - | - | - | - |
| Sperm count/ml | -0.471** | 0.859** | 0.530** | - | - | - |
| Live sperm | 0.576** | 0.491** | -0.138 | -0.469** | - | - |
| Abnormal sperm | -0.308 | 0.094 | -0.096 | 0.190 | -0.455* | - |
| HOS reacted sperm | -0.096 | 0.106 | -0.003 | 0.180 | -0.007 | 0.051 |
| **HF x Kankrej crossbred bulls** | | | | | | |
| Mass activity | -0.370* | - | - | - | - | - |
| Initial motility | -0.261 | 0.493** | - | - | - | - |
| Sperm count/ml | -0.383* | 0.953** | 0.534** | - | - | - |
| Live sperm | 0.341 | -0.353 | -0.263 | -0.425* | - | - |
| Abnormal sperm | -0.238 | 0.052 | 0.090 | 0.124 | -0.525** | - |
| HOS reacted sperm | -0.237 | 0.153 | 0.042 | 0.184 | -0.058 | 0.065 |

* Significant at P<0.05 level; ** Significant at P<0.01 level (2-tailed).

In Mehsana bulls, the ejaculate volume had significant negative correlations with mass activity (-0.436, P<0.05) and sperm concentration (-0.471, P<0.01), and positive correlations with live sperm (0.576, P<0.01). The mass activity had highly significant positive correlations with initial motility (0.554, P<0.01) and sperm concentration (0.859, P<0.01) and significant negative correlations with live sperm per cent (-0.491, P<0.01). Sperm concentration was significantly correlated with initial motility (0.530, P<0.01) and live sperm (-0.459, P<0.01). Live sperms and abnormal sperms did not reveal significant correlation with other traits.

In HF x K (F1) bulls, like Jafarabadi buffalo semen, the ejaculate volume did not reveal significant correlation with any of the assessed traits, except with mass activity (-0.370) and sperm concentration (-0.383). Similarly, mass activity revealed significant (P<0.01) positive correlations only with initial motility (0.493) and sperm concentration (0.953). Sperm concentration revealed significant negative correlations with live sperm per cent (-0.425, P<0.05), and the live sperm and abnormal sperm per cent were negatively correlated (-0.525, P<0.01) (Table 3).

The present findings on interrelationships among physico-morphological attributes of semen in all three breeds also corroborated well with most reports, particularly of Jayendran *et al.* (1984), Bhavsar *et al.* (1988), Belorkar *et al.* (1990), Dhami and Sahni (1994), Raval and Dhami (2006) and Rao *et al.* (2011). Further, Shelke and Dhami (2001) reported significant (P<0.01) positive correlations for mass activity of Gir and Jafarabadi bull semen with initial motility, live spermatozoa and sperm concentration, while initial motility had significant (P<0.01) positive correlation with live sperm % and sperm concentration. Live sperm per cent had significant positive correlation with sperm concentration. Abnormal spermatozoa had significant positive correlation with live sperm and sperm concentration only in Gir bulls. Sajjad *et al.* (2007), however, could not find any correlation of ejaculate volume in Nili-Ravi buffalo with sperm motility, sperm concentration and total sperm abnormalities, although the sperm motility percentage had positive correlation with sperm concentration. Tiwari *et al.* (2009) also found significant positive correlation for progressive motile sperm with sperm concentration, live spermatozoa and HOS reactive spermatozoa, while sperm concentration had significant positive correlations with live sperm and HOS reactive sperm, and live sperm had positive correlation with HOS reactive sperm. Prasad *et al.* (1999) noted similar non-significant correlations for HOS positive sperm with other attributes in crossbred bull's semen. Ramachandaran *et al.* (2006) reported significant positive correlations (P<0.01) of mass activity and progressive motility with per cent live spermatozoa and motile spermatozoa in fresh semen of Sahiwal bulls. The present correlation findings clearly suggested that fertility of semen is governed by its initial quality, especially motility, viability, morphology, sperm concentration and HOS positive sperm. Thus, assessment of semen through routine tests coupled with HOS test can be used as a guide to judge the fertility of semen of bulls, while selecting them for AI programme.

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