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Comparison of Conventional Semen Parameters and Hypo-osmotic Swelling Test between Karan Fries and Sahiwal Bulls under Heat Stress

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ABSTRACT

The present study aimed to determine the differences in semen parameters between Karan Fries (KF) and Sahiwal bulls and the correlation of hypo-osmotic swelling test with conventional semen parameters. A total of 24 ejaculates were collected from each breed and subsequently classified according to their mass activity. The semen samples were further examined for progressive motility, sperm viability, sperm concentration and hypo-osmotic swelling test (HOST). The percentage of hypo-osmotically swelled spermatozoa was higher ($P<0.01$) in ejaculates of Sahiwal bulls than in KF bulls. However, the mass activity, percent progressive motility, viability and concentration of sperms did not differ ($P>0.05$) between the breeds. With respect to association between HOST and conventional semen parameters, HOST showed significant ($P<0.01$) positive correlation with progressive motility (%), sperm viability (%) and sperm concentration in both breeds. This study indicated the HOST as an effective method to test the fertility potential of semen samples for artificial insemination and zebu breeds ejaculates have more fertility potential as compared to crossbred ejaculates under heat stress.

Keywords: Fertility, artificial insemination, bull, spermatozoa

In the present scenario of livestock farming, Artificial Insemination (AI) is the most commonly used assisted reproduction technology that allows an effective use of semen from bulls of high genetic merit so that the rate of genetic development of animals and their production gain can be increased. For successful AI, availability of good quality semen throughout the year is the foremost requirement (Holt *et al.,* 2007). Proper screening of semen ensures selection of good quality semen for AI. Routine screening tests of semen provide important information regarding the functional competence of spermatozoa and the bull fertility (Moazzam *et al.,* 2015). The semen is evaluated conventionally on the basis of motility, morphology, sperm count and viability, which provides limited information regarding the potential fertility and functional integrity of the spermatozoa (Jeyendran *et al.,* 1984). The viability test, which is based on the ability of eosin-Y dye to pass through sperm membrane, indicates only the structural integrity of the sperm plasma membrane. Certain physiological events that occur during the process of fertilization (e.g.: union of sperm with egg, acrosome reaction and capacitation) emphasizes the importance of functionally active sperm plasma membrane and therefore, occurrence of fertilization is impossible with inactive plasma membrane. In order to evaluate the functional and biochemical integrity of sperm plasma membrane, the hypo-osmotic swelling test (HOST) was developed initially in humans (Jeyendran *et al.,* 1984), and later in bovines (Rota *et al.,* 2000), equine (Neild *et al.,* 2000), canine (Rodriguez-Gil *et al.,* 1994), porcine (Perez-Llano *et al.,* 2001) and rainbow trout (Cabrita *et al.,* 1999).

The semen quality is affected by several factors, among which photoperiod, environmental variables, body condition, and feed quality plays a crucial role (Barth and Waldner, 2002). Although the effects of most of these factors could be minimized by better management

practices, the adversity caused due to extreme climatic conditions still remains as a persistent threat to productivity, semen quality and libido of breeding bulls in the tropical conditions. The severity of heat stress on semen quality was comparatively lower in zebu bulls than in European (Brito *et al.*, 2004) and crossbred bulls (Silva *et al.*, 1991). Therefore, the present study was designed to investigate the differences in semen parameters between Sahiwal (zebu) and Karan Fries (crossbred) bulls during summer and to determine their association with HOST.

MATERIALS AND METHODS

Selection of bulls, environmental variables and procurement of semen

The study was conducted at Artificial Breeding Research Centre, National Dairy Research Institute, Karnal and the experimental animals included Karan Fries (n=6) and Sahiwal (n=6) bulls aged between 23 and 37 months. The body weight of these bulls ranged between 205.5 and 310.8 kg. During the experimental period, the average minimum and maximum temperatures were 24.1 and 42.8ºC, respectively, and the relative humidity varied between 7 and 16.7%. Prior to semen collection, the preputial region of all the bulls were cleaned and ejaculates were collected on weekly basis for a period of 4 weeks by artificial vagina method. Immediately after collection, the semen samples were classified based on mass activity and were subsequently examined for progressive motility, sperm concentration, hypo-osmotic swelling test and sperm viability. Semen samples were maintained at 37°C during all the routine seminal tests.

Mass activity

Mass activity was graded on the basis of wave movement under a warmed stage (37°C) of a phase contrast microscope (200 X, Nikon eclipse E600, Tokyo, Japan) (Table 1).

Table 1: Grading of mass activity based on wave movement

Grade	Observation
0	Immotile spermatozoa and absence of waves
+	Sperm cells motile but waves absent
++	Waves in poorly distinguishable motion
+++	Presence of waves in moderate motion
++++	Dark intense waves in rapid motion waking eddies

Progressive motility

It was assessed by observing a drop of diluted semen sample (1:100 in egg yolk medium) on a preheated glass slide (37°C), after applying a cover slip over it. The drop of semen was examined at 400X magnification under a light microscope (Labomad, California, USA).

Sperm concentration

The sperm concentration of each ejaculate was determined using Neubauer's hemocytometer.

Hypo-osmotic swelling test

To carry out the HOS test, a hypo-osmotic solution (150 mosm/L) was prepared by dissolving 0.735g of sodium citrate and 1.35g of fructose in 100ml of distilled water. Thereafter, 100µl of semen was added to 1ml of hypo-osmotic solution and incubated for 1 h at 37ºC in a CO_2 incubator. After incubating, the sperm suspension was mixed well and a small drop was placed on a sterilized glass slide under phase contrast microscope (400X). A total of 100 spermatozoa were counted in different fields and the percentage of spermatozoa positive to HOS test (having coiled tails) was determined.

Sperm viability

Two microliter of neat semen and 20 µL of eosin-nigrosine stain were mixed properly on a clean grease free pre warmed (37ºC) glass slide. Thereafter, 5 µL of the mixture was drawn on another pre warmed (37ºC) glass slide and was further observed for viable colorless cells. On an average a total of 200 spermatozoa were counted under oil immersion objective (1000X) fields and the percentage of live spermatozoa was determined.

Statistical analysis

Analysis of differences between the breeds and mass activity were determined by two way ANOVA with Bonferroni post-hoc-test using GraphPad Prism version 5.01. Correlation analysis was done by SPSS 16.0 package.

RESULTS AND DISCUSSION

Mass activity is the first criteria used to judge the quality of semen (Tomar *et al.*, 1996). Mass activity of a semen

Table 2: Comparison of different semen parameter values (Mean ± SE) of semen samples having similar mass activity collected from Karan Fries (KF) and Sahiwal bulls during hot dry season

MA	N		PM (%)		SV (%)		SC (×10^6)		HOSS (%)	
	KF	SW	KF	SW	KF	SW	KF	SW	KF	SW
+	5	3	42.6±3.3^a	46±3.2^a	41.2±3.2^a	46.3±0.9^a	448±26.3^a	492±56.4^a	32.2±2.5^a	41±0.6^b
++	5	4	59.8±4.0^a	66±3.2^a	56.8±1.2^a	61±1.3^a	707±28.9^a	774±34.4^a	36.6±2.9^a	49.5±0.9^b
+++	8	10	73.3±1.4^a	76.9±1.1^a	78±2.8^a	82.3±0.9^a	1126±39.2^a	1178±32.7^a	57±2.3^a	69.6±0.8^b
++++	6	7	84.9±1.4^a	93.6±0.6^b	92.3±1.8^a	91.7±0.9^a	1410±50.6^a	1546±42.3^a	70±1.5^a	83.1±0.8^b

MA=Mass activity, KF=Karan Fries, SW=Sahiwal, N=No. of ejaculates, PM=Progressive motility, SV=Sperm viability, SC=Sperm concentration and HOSS=Hypo-osmotically swelled spermatozoa. Means with different superscripts between breeds differ significantly (P<0.05)

sample depends on its sperm concentration and individual progressive motility. Motility is one of the most important requirements of fertile semen. Sperm concentration is an important indicator of semen quality in semen used for cryopreservation (Shelke and Dhami, 2001). Conception rate of the female is highly correlated with the number of viable spermatozoa deposited in the female reproductive tract (Gerard and Humblot, 1991). As conventional semen evaluation parameters help to assess only the structural integrity of the sperm cells, they are of limited use in predicting the fertility potential of a bull. So, HOST, a test of biochemical and functional integrity of spermatozoa, can be done for better estimation of fertility potential of bull (Bacinoglu *et al.,* 2008).

SEMEN ATTRIBUTES

A total of twenty-four ejaculates were collected for each breed (Sahiwal and KF). Based on the observed mass activity, the ejaculates were classified into different categories (Table 2). Ejaculates were subsequently examined for progressive motility, sperm concentration, hypo-osmotic swelling test and sperm viability. Then, comparative analysis of ejaculates was performed between the breeds and between the mass activities. Significant variations were observed between both breeds as well as between mass activities with regards to percent spermatozoal progressive motility, percent viability, sperm concentration and percent HOST positive sperms (Table 2).

All the semen parameters increased significantly (P< 0.01) with the increase in mass activity of ejaculates in case of both breeds. Similar observations were reported by Bansal and Cheema (2014) in fresh crossbred cattle bull semen in which progressive motility, sperm count, viability and HOST values increased with increase of mass activity. The findings of the present study regarding the semen parameters of KF bulls were in agreement with previous reports on KF semen (Bhakat *et al.,* 2014; Panmei *et al.,* 2015). Semen attributes of Sahiwal bulls found in the present study were in accordance with the findings of Ray and Ghosh (2013). Considering the breed difference, Sahiwal bulls ejaculates showed significantly (P<0.01) higher mean values for percent progressive motility, percent viability, spermatozoal concentration and percent hypo-osmotically positive sperms than in KF bulls ejaculates. When analyzed for different mass activities, percent progressive motility was significantly (P< 0.05) higher for + 4 mass activity in the Sahiwal bulls ejaculates than in KF bulls ejaculates. However, the higher values in Sahiwal ejaculates for +1, +2, and +3 mass activities did not differ significantly. Though Sahiwal bulls' ejaculates showed higher values for percent viability (except for mass activity +4) and spermatozoal concentration than KF bulls, they were not significant for all the mass activities. It was noteworthy that Sahiwal bulls stamped higher values for HOST positive sperms that were highly significant (P< 0.01) for mass activities +2, +3, and +4 but significant (P< 0.05) for mass activity +1. The findings were in corroboration with the observations of Hossain *et al.* (2012) who reported that the sperm concentration and motility were higher in semen samples of Sahiwal bulls as compared to Sahiwal × Friesian and local zebu × Friesian bulls; Zubair *et al.* (2013) who found that the mean values of HOST, motility, normal morphology and viability of semen samples of Sahiwal bulls were 47%,

60%, 56% and 70% respectively; whereas in case of semen samples of crossbred bulls, they were 27%, 50%, 40% and 55.8% respectively. The results are also in agreement with previous reports suggesting that heat stress has comparatively more severe effects on semen quality of European breeds (Brito *et al.*, 2004) and crossbred bulls (Silva *et al.*, 1991) than on semen quality of zebu bulls.

Present investigation showed highly significant positive correlation between HOST positive sperms (%) and progressive motility (%), sperm viability (%) and sperm concentration in case of ejaculates of both KF crossbred bulls and Sahiwal bulls. In semen samples of KF crossbred bulls, HOST showed highly significant ($P<0.01$) positive correlation with progressive motility ($r=0.840$), sperm viability ($r=0.942$) and sperm concentration ($r=0.911$). In semen samples of Sahiwal bulls, HOST showed highly significant ($P<0.01$) positive correlation with progressive motility ($r=0.941$), sperm viability ($r=0.975$) and sperm concentration ($r=0.946$). This was in agreement with previous reports on semen of crossbred cattle bull (Bansal and Cheema, 2014), Sahiwal, Friesian and crossbred (Sahiwal X Frisian) cattle bull (Zubair *et al.*, 2013), Nili-Ravi buffalo, Sahiwal bull (Lodhi *et al.*, 2008) and Sahiwal bulls (Singh *et al.*, 2015). However, Ray and Ghosh (2013) reported weak correlation between HOST and progressive motility/ viability and no correlation between HOST and sperm concentration. Martins *et al*., (2013) found no correlation between HOST and other semen attributes.

CONCLUSION

The semen parameters of KF bulls were more vulnerable to heat stress than the Sahiwal. Zebu cattle are more tolerant to heat stress and its fertility is less affected as compared to crossbred cattle. The HOST test was found to be the best method to predict the fertility potential of bulls as compared to other conventional semen evaluation parameters viz. mass activity, percent progressive motility, percent viability and sperm concentration.

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