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Breeding soundness evaluation in crossbred bulls: Can testicular measurements be used as a tool to predict ejaculate quality?

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ABSTRACT

The present study analyzed the relationship between testicular biometry and initial semen quality in Karan Fries (KF) crossbred bulls. Data on semen production traits of KF bulls (n=32) spread over a period of 3 years were analyzed to find out the ejaculate rejection rate (ERR). The scrotal circumference (SC), testicular length (TL) and testicular width (TW) were measured once in a month and 16 ejaculates were collected from bulls (n=24). The relationship between different testicular measurements and the ejaculate quality was studied. To study if the testicular biometry parameters differ between good and poor semen producing bulls, based on the semen evaluation, the bulls were ranked into good and poor. Our study revealed that ERR in KF bulls varied from 23.02 to 100%, with the average of 52.46%. The SC was significantly negatively correlated (–0.459) with ERR. Between good and poor bulls, there was no difference in SC, TL and TW, however, testicular mass (TM) and testicular volume (TV) differed significantly. From the results of the study, it may be inferred that that SC had a positive relationship with initial semen quality, but combination of SC, TL, TW, TV and TM may yield superior results for the selection of crossbred bulls.

Key words: Crossbred bulls, Ejaculate rejection rate, Scrotal circumference, Semen quality

The major aim of selecting a bull for artificial breeding purpose is to propagate the superior germplasm in a wider female population. Although this can be achieved to its maximum extent in purebred and some indigenous bulls, it is the biggest problem in crossbred bulls since the ejaculate quality is poor in these bulls (Kumaresan *et al.* 2012). Although the crossbred bulls are selected for breeding purpose after breeding soundness examination (BSE), the post-selection performance is not comparable with either purebred or indigenous bulls. It was reported that 54.91% of Frieswal crossbred bulls produced poor quality semen (Tyagi *et al.* 2000); 50–55% of the ejaculates obtained from crossbred bulls were of poor quality (Sudheer and Xavier 2000, Mathur *et al.* 2002, Mukhopadhyay *et al.* 2010) and could not be used for cryopreservation thereby hindering the crossbreeding programme.

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The production of spermatozoa/sperm count is related to the scrotal circumference (SC), testicular volume (TV), testicular weight (TM) and shape of the testis (Bailey *et al.* 1996). The relationship between the testicular parameters of animals with semen quality and freezability are documented for pure bred bull (Mohanty *et al.* 1991, Devkota *et al.* 2007) and zebu bulls like Sahiwal (Ahmad *et al.* 2011). Very less information is available relating the age, scrotal morphology and semen quality of crossbred bulls. Generally, testicular measurements are used as an aid to select breeding bulls, however, the standards developed elsewhere for purebreds are directly applied in the selection of crossbred bulls, and the effectiveness of such tools was not assessed in detail. Moreover, some authors reported that scrotal circumference is unlikely to be an accurate predictor of sperm output in AI bulls. Therefore, the present study was an attempt to evaluate the relationship between testicular biometry with semen quality and sperm characteristics in different age groups of Karan Fries crossbred (Holstein Friesian × Tharparkar) bulls so that the same can be used as a tool for selecting quality breeding bulls.

MATERIALS AND METHODS

The present investigation was carried out in two parts. In part 1, a retrospective study was conducted to assess the

semen production ability and quality of ejaculates in crossbred bulls. For this purpose, data on semen production traits of 32 Karan Fries (KF; Holstein Friesian × Tharparkar, exotic inheritance between 50 and 75%) bulls spread over a period of 3 years were collected from the records maintained at Artificial Breeding Research Centre (ABRC), National Dairy Research Institute, Karnal, India. The data pertaining to the total number of ejaculates obtained from a single bull during its productive life time, initial quality of ejaculates, the number of ejaculates found suitable for freezing and the number of ejaculates rejected for freezing was calculated. As per the routine practice of the semen freezing laboratory, ejaculates with mass activity less than 3 and individual motility less than 60% were considered as unsuitable for cryopreservation as these ejaculates do not yield desirable post-thaw sperm quality. Form these data, the ejaculate rejection rate (ERR) for individual bulls were calculated using the following formula.

$$\text{ERR (\%)} = \frac{\text{No. of ejaculates found suitable for freezing}}{\text{Total number of ejaculates obtained from the bull}} \times 100$$

In part 2, an experiment was conducted, over a period of 6 months, to study the relationship between testicular parameters and ejaculate quality in 24 crossbred bulls. The management of experimental animals and the procedures utilized for experimentation are detailed below.

Experimental animals and their management: KF adult bulls (24) maintained at ABRC were utilized for part 2 of the study. The bulls were maintained as per the standard management practices and fed concentrate ration (21% CP and 70% TDN @ 2.5 kg / bull daily) and *ad lib.* seasonal green fodders. Vaccination, deworming, regular checkup for communicable diseases and other herd health programmes were followed as per the standard farm schedule. The bulls were exercised once in a week, the day prior to semen collection using rotary exerciser, so as to maintain the sexual behavior of bulls and ensure quality semen production.

Age, body weight and testicular measurements: Date of birth of the bulls was obtained from records available at the station. Body weights of all the experimental bulls were measured once in a month using digital weighing balance. The scrotal circumference (SC), testicular length (TL) and testicular width (TW) were measured using digital calipers, following the method described by the Society of Theriogenology (Ball *et al.* 1983) once in a month during the entire period of the study. Average values of length and width of both right and left testis for each bull were taken as the measurements for that particular bull. Testicular length (TL) and width (TW) were used to calculate testicular mass (TM) and volume (TV), considering the testicle as a prolate spheroid, using the mathematical formulae, where $TM = 0.5533 \times (TL) \times (TW)^2$, and $TV = 0.5236 \times (TL) \times (TW)^2$, as described by Bailey *et al.* (1996).

Semen collection and initial evaluation: Ejaculates (16) from each of the 24 bulls were collected using artificial vagina method and evaluated for mass activity, individual motility, viability, sperm concentration and membrane integrity of spermatozoa.

The mass motility was measured in 0-5 scale on the basis of swirls and eddies activity as per the standard procedure using 200 × phase contrast microscope provided with a warm stage. The sperm concentration was estimated using the haemocytometer (Salisbury *et al.* 1985) and expressed as million spermatozoa per ml. Sperm viability was determined by Eosin-Nigrosin stain (Campbell *et al.* 1956). Total or partly stained spermatozoa were considered as dead. The membrane integrity of spermatozoa was evaluated using hypo-osmotic swelling test method as described by Jeyandran *et al*. (1984). For sperm viability, and membrane integrity, at least 200 sperms per smear were counted using tally counter. All the semen evaluation was performed by same person throughout the study.

Ranking of bulls as good and poor: The ERR for these bulls were calculated from the existing data (with a minimum of 100 ejaculates/bull) as per the formula cited above. To study the relationship between various testicular and ejaculate quality parameters, based on ERR, the bulls were ranked from 1 to 24 in ascending order of ERR. The top ranked 8 bulls were considered as good bulls and the bottom ranked 8 as poor bulls, and statistical analysis was performed to find the relationship between various parameters.

*Statistical analysis:*Descriptive statistics were calculated for body weight, testicular parameters, seminal attributes and ERR in different experimental groups and the results are represented as mean±SEM. One way ANOVA was performed to compare the means in different categories of bulls. For one-way ANOVA means were separated on the basis of the actual mean pair wise multiple comparisons was performed using Tukey test as post hoc test. When the P value < 0.05, the means were considered to be significantly differing. Pearson product moment correlation was calculated between SC, TM, TV and ERR. Pearson product moment correlation was also calculated between SC, TL, TV, TM and sperm quality parameters. All the analyses were performed using SigmaPlot 11® software.

RESULTS AND DISCUSSION

Breeding soundness evaluation along with pedigree analysis and progeny testing is used to identify the best quality bulls, for propagating the superior germplasm. There is no single factor judging criteria to evaluate the breeding soundness of breeding males. The Society for Theriogenology has set up certain standards for the scrotal circumference of *Bos taurus* animals for selecting as breeding bulls (Chenoweth *et al.* 1992), but this has not been standardized so far with respect to zebu and crossbred bulls, making difficult to follow this in our country. In the present study we attempted to find

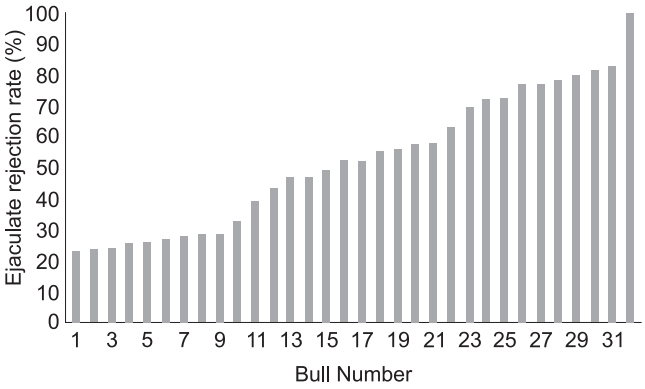

Fig. 1. Ejaculate rejection rate in crossbred bulls.

out the association between testicular biometry and seminal characteristics in Karan Fries crossbred bulls.

Ejaculate rejection rate (ERR) was studied from the records of 32 crossbred bulls spread over 3 years and found that the ERR of the bulls varied from 23.02 to 100%, with the average of 52.46% (Fig. 1). Among the 32 bulls, 8 bulls had ERR of less than 40% while 1 bull had 100% ERR. ERR in remaining bulls ranged from 47 to 83%. The average ERR observed in the present study is in agreement with those reported by Tyagi *et al.* (2000). Similar ERR were also reported earlier. Our findings also supported findings of Mukhopadhyay *et al.* (2010) who reported that at least 50% of the crossbred bulls produce semen which was not fit for cryopreservation. These reports, along with the results of the present study, clearly indicated that there are some inherent problems with the crossbred bulls leading to poor quality semen production. Although the exact reasons for production of poor quality ejaculates in crossbred bulls have not been ascertained, several hypothesis were putforth including the genotype of individual bulls (levels of exotic inheritance), variations in the spermatozoa characteristics like sperm membrane composition, resistance to changing environments during extension and cryopreservation.

The age, body weight (bw), testicular parameters and ejaculate quality are given in Table 1. There was no significant difference in SC, TL, TW and ejaculate volume between bulls of the four age groups (2–3, 3–4, 4–5 and 5–6 years) studied. But, the TM and TV were significantly (P<0.05) lower in bulls belonging to 4–5 years of age than 3–4 years age group. Similarly the TV/kg bw and TM/kg bw were also lower in the bulls belonging to 4–5 years age group compared to bulls in 2–3 or 3–4 years age group although the difference were not enough to be statistically significant. The decrease in TV/kg bw and TM/kg bw in bulls belonging to 4–5 years age group compared to 3–4 years age group might be attributed to the increased bw of bull in 4–5 year age group while maintaining the same level of testicular growth as that of bulls in 3–4 year age group. Our observations on SC, TL and TW are in agreement with Mohanty *et al.* (1991). They reported that the age was significantly correlated with TV and SC. Our results were within the range of SC reported by

Table 1. Age, testicular parameters and ejaculate characteristics in crossbred bulls (n=24)

Parameters	Age of bull (in years)			
	2–3 (n=6)	3–4 (n=8)	4–5 (n=10)	5–6 (n=8)
Body weight (kg)	485.33 ±17.49	577.27 ±11.56	664.6 ±10.52	696.5 ±20.03
SC (cm)	36.50 ±0.71	37.01 ±0.76	38.07 ±0.70	37.97 ±0.87
Testis length (cm)	12.70 ±0.08	14.50 ±0.30	13.52 ±0.41	15.18 ±0.36
Testis width (cm)	6.87 ±0.11	7.05 ±0.25	6.38 ±0.17	6.73 ±0.15
TV (cm^3)	313.97^{ac} ±12.18	379.83^{a} ±24.03	$291.0\ 8^{bc}$ ±19.61	365.31^{ac} ±57.97
TM (g)	331.78^{ac} ±12.87	401.37^{a} ±25.39	307.54^{bc} ±20.72	384.50^{bc} ±37.78
TV/kg BW (cm^3/kg)	0.65 ±0.05	0.66 ±0.04	0.44 ±0.03	0.52 ±0.03
TM/kg BW (g/kg)	0.69 ±0.05	0.70 ±0.04	0.46 ±0.03	0.54 ±0.04
Volume (ml)	2.93 ±0.55	4.64 ±0.34	4.76 ±0.23	4.60 ±0.22
Sperm conc. (10^6/ml)	890.0 ±148.94	950.82 ±58.14	894.82 ±43.42	763.50 ±38.12

Mean with different superscript within a row differ significantly (P<0.05). SC, scrotal circumference; TV, testicular volume; TM, testicular mass.

Table 2. Testicular parameters, ejaculate quality and sperm characteristics

Parameters	Good bulls (n=8)	Poor bulls (n=8)
BW (kg)	612.67±41.49	593.13±21.67
SC (cm)	38.16±1.30	38.26±0.53
Testicular length (cm)	14.26±0.42	13.29±0.78
Testicular width (cm)	7.18±0.43	7.05±0.16
TV (cm^3)	390.65^{a}±45.71	346.16^{b}±23.91
TM (g)	412.81^{a}±48.31	365.80^{b}±25.27
TV/kg bw (cm^3/kg)	0.65±0.09	0.59±0.06
TM/kg bw (g/kg)	0.69±0.09	0.63±0.06
Ejaculate volume (ml)	4.70±0.73	4.92±0.38
Sperm concentration (10^6/ml)	972.40±56.43	967.40±114.15
Motility (%)	68.00^{a}±1.50	38.00^{b}±2.11
Live and dead cells (%)	87.10^{a}±0.85	82.28^{b}±1.63
HOST positive cells (%)	58.70^{a}±1.77	31.38^{b}±1.60
ERR (%)	32.40^{a}±4.59	63.04^{b}±3.21

Mean with different superscript within a row differ significantly (P<0.05);bw, body weight; SC, scrotal circumference; TV, testicular volume; TM, testicular mass; ERR, ejaculate rejection rate.

Castro *et al*. (1991) in Nellore bulls. We recognized that the testicular development in crossbred bulls takes place during early ages and reaches high level of growth during 3 to 4 years age and then remains plateau. This is in agreement with the earlier findings of Coulter (1991) and Ahmad *et al*. (2011). After 3–4 years, the increase in body weight without corresponding change in testicular growth may reduce the TV/Kg bw and TM/kg bw.

Between good and poor bulls, there was no difference in SC, TL and TW (Table 2) however, TV and TM differed significantly ($P<0.05$). It is a well established fact that quantity of sperm production is related to the SC, TV, TM and shape of the testis (Bailey *et al.* 1996). A larger than average SC measurement was found related to greater TM and TV, and to higher sperm production. A small SC often associated with small testis, is found related to infertility. However, measuring the SC only would not give a clear picture about the fertility status of bulls, but TV and testicular weight are highly correlated to sperm production. Bulls with smaller than average SC and longer testis, may have a larger TV or TM than bulls with higher SC and shorter testis (Bailey *et al*. 1996). When the correlation between testicular parameters and ERR was analyzed, it was observed that the SC was significantly negatively correlated (–0.459) with ERR. A positive association of SC, TL, TV and TM with sperm quality was also noticed. Hence, combination of SC with TL and TW would give fairly reliable picture of ejaculate quality, indicating that these parameters could be used to assess the sperm production ability of the CB bulls too.

Based on our study, it can be concluded that that SC had a positive relationship with initial semen quality, but combination of SC, TL, TW, TV and TM may yield superior results for standardizing the breeding soundness evaluation protocol for the selection of crossbred bulls.

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