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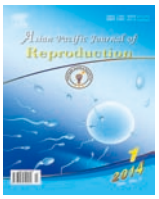

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# Effects of pedigree and exotic genetic inheritance on semen production traits of dairy bulls

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ABSTRACT

**Objective:** To study the effects of different levels of exotic inheritance on ejaculate quality in bulls and its passage through different generations. **Methods:** Data on semen production traits and ejaculate quality were obtained for 38 crossbred bulls and grandsire-sire-progeny relationship in relation to semen quality was studied. The bulls were classified into three groups based on the level of exotic inheritance viz. F1, 50.0%-62.5% exotic germplasm and >75% exotic germplasm. **Results:** Results of the present study indicated that about 40% of the ejaculates obtained from the crossbred bulls were rejected from further processing due to poor ejaculate quality. The F1 bulls produced significantly higher proportions (57.00±10.00) of poor quality ejaculates compared to the interse mated bulls. The age at first semen collection in crossbred bulls ranged from 567 to 1 010 days with an average of 738.89±18.18 days while the mean age at first semen freezing was 865.72±34.60 days. **Conclusions:** It may be inferred that the "acceptable quality semen producing ability" decreased from grandsire through sire to male progeny and among the increasing exotic genetic levels of CB cattle, F1 bulls produced significantly higher "low grade ejaculates" that were unfit for cryopreservation.

## 1. Introduction

Artificial insemination of indigenous cattle with semen from high-producing exotic bulls has been in practice in several developing countries including India. Although this practice has resulted in improved milk productivity, it has been reported that the crossbred offspring are more susceptible to reproductive problems [1]. Available reports suggest that that more than 50% crossbred young bulls inducted for semen collection programme had problems with semen quality, libido and cryotolerance of spermatozoa [2, 3]. The freezability and fertility of spermatozoa in crossbred bulls is lower than purebred or indigenous bulls. There is consensus among researchers that quite a number of differences exist in semen production not only between crossbred bulls and other bulls but also among the crossbred bulls [4, 5]. In crossbred bulls, at least 50%-55% of the ejaculates were unsuitable for freezing due to poor initial semen quality [6, 7], however the reason(s) for

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production of such low quality semen, even during the best breeding season, has not been identified yet [8]. Several attempts are being made to identify the etiology of poor semen production in crossbred bulls at genetic, andrological, seminological and possible environmental levels but with varied success [5].

The impaired semen production capacity has been shown to be the major reason for rejection of CB bulls in several semen stations [8]. Among the different genetic levels, it was reported that higher percentage (68.50%) of interse mated crossbred bulls produced poor quality semen compared to the bulls of HF X lower crosses origin [9]. These observations indicate that the level of exotic inheritance could influence the quality of ejaculates produced by a crossbred bull. Further it is not clearly known if these characters are passed from one generation bull to the other. It is in this backdrop, the present investigation was carried out on Karan Fries (Holstein Friesian X Tharparkar) crossbred bulls to (i) study the semen production attributes among bulls belonging to three subsequent generations and (ii) to investigate the effect of different levels of exotic inheritance on the quality of ejaculates.

## 2. Materials and methods

The present study was carried out at Artificial Breeding Research Centre of the Institute. The study place is located at an altitude of 250 meters above the mean sea level on 29.43°N latitude and 72.2°E longitude, in the bed of Indo-Gangetic alluvial plain. A subtropical climate prevails in the area.

### 2.1. Experimental bulls and their management

A total number of 38 crossbred (Holstein Friesian X Tharparkar) were utilized for the study. The bulls were kept in individual pens under loose housing system on concrete floor with adequate ventilation. The bulls were fed concentrate ration with 21 percent crude protein and 70 percent TDN at the rate of 2.5 kg per bull per day. Seasonal green fodders such as maize, cowpea, berseem, jowar etc., depending on their availability, along with mixture of maize and oat silage was fed *ad lib* to the animals. The bulls had free access to clean drinking water throughout the day. Vaccination, de-worming, regular check-up for communicable diseases and other herd-health programmes were followed as per the farm schedule, to protect the animals from diseases and to produce quality semen. The bulls were exercised once a week, the day prior to semen collection in the rotatory exerciser so as to maintain the sexual behaviour of bulls and ensure quality semen production.

### 2.2. Data on semen production and ejaculate characteristics

The data spread over a period of 30 years (1980-2010) related to semen production traits of all the 38 bulls were collected from records. The parameters included were the total number of ejaculates per bull, total number of ejaculates frozen, ejaculate rejection rate (ERR), good ejaculates (mass activity $\geq 3$), poor ejaculates (mass activity $\leq 2$), total volume of semen produced, semen production period (SPP), age at first semen collection (AFSC) and age at first semen freezing (AFSF).

### 2.3. Grandsire-sire-progeny relationship

Out of the 38 bulls utilized in the study, 24 bulls were still under semen collection during the period of experiment. From the record, the sire ($n$=10) and grandsire ($n$=4) of all the 24 bulls were traced. All the above mentioned parameters (in previous section) were collected from the existing records and the relationship among the grandsire, sire and progeny was studied.

### 2.4. Effect of different levels of exotic inheritance on semen production

The bulls were classified into three groups based on the level of exotic inheritance; Group I with 50% exotic germplasm (F1), Group II with >75% (≥3/4) exotic germplasm and Group III with 50-62.5% (inter-se) exotic germplasm and the semen production details were compared among the bulls belonging to different groups.

### 2.5. Statistical analysis

Descriptive statistics were calculated for age at first semen collection (AFSC), age at first semen freezing (AFSF), total semen production period (SPP), number of ejaculates collected per month, total volume of ejaculate per month and ejaculate rejection rate (ERR), with all means are represented in mean±SEM format. Comparison between 50%, >75% and 50-62.5% exotic germplasm for above parameter was done using one-way ANOVA. Similarly comparison between grand sire, sire and progeny was also done using one-way ANOVA. For One-way ANOVA means were separated on the basis of the actual mean and pair wise multiple comparisons was performed using Tukey test as post hoc test. When the $P$ value is $< 0.05$, means were considered to be significantly differing. All the analyses were performed using SigmaPlot 11® software (Systat software Inc., USA).

## 3. Results

### 3.1. Semen production details and Ejaculate Rejection Rate (ERR)

The details of age at first semen collection (AFSC), age at first semen freezing (AFSF), total semen production period (SPP), number of ejaculates collected/month and ejaculate rejection rate (ERR) in crossbred (CB) bulls are given in Table 1. The AFSC in CB bulls ranged from 567 to 1010 days with an average of 738.89±18.18 days while the mean AFSF was 865.72±34.60. The average number of ejaculates/bull/month ranged from 1.51 to 8.01 numbers. About 40% of the ejaculates obtained from the CB bulls were straight away rejected from further processing due to poor ejaculate quality.

**Table 1**
Semen production details and ejaculate rejection Rate (ERR) in CB bulls ($n$=38).

| Parameters | Values |
|---|---|
| AFSC (days) | 738.89±18.18 |
| AFSF (days) | 865.72±34.60 |
| SPP (days) | 838.64±55.99 |
| Number of ejaculates/month | 5.05±0.21 |
| Total volume of ejaculate/month (mL) | 23.54±1.29 |
| ERR (%)* | 39.00±3.00 |

AFSC-Age at first semen collection, AFSF-Age at first cemen freezing, SPP – Semen production period and ERR-Ejaculate rejection rate, *Rejected based on initial semen qualities (mass activity <3; individual sperm motility <60%).

### 3.2. Effect of genetic levels of CB bulls on semen production traits and ERR

The influence of level of exotic germplasm on AFSC, AFSF, SPP and average number of ejaculates in CB bulls was studied. Although not enough to be statistically significant, it was observed that the AFSC was lower in the bulls which had 50% exotic germplasm (F1) compared to those bulls who had >75% (≥3/4) and 50.0%-62.5% (interse) exotic germplasm. There was no significant difference between F1, ≥3/4 and interse bulls in terms of AFSF (Table 2). The time interval between AFSC and AFSF was higher in F1 bulls compared to the others; being around 218 days in the F1 bulls compared to 140 days in ≥3/4 bulls and 166 days in interse mated bulls. The ERR was significantly higher in F1 bulls (57.00±10.00) compared to the interse mated bulls (Figure 1).

### 3.3. Grandsire-sire-bulls relationship in terms of semen production traits and semen quality

The mean AFSC was 718.14±13.18 days in grand sires (GS), which increased to 783.86±25.46 days in sires that in turn decreased to 716.08±40.03 days (Table 3). But the AFSF showed different trend. There was a linear increase in AFSF from GS to bulls. In the GS the AFSF was 767.14±25.82 days, which increased to 831.43±31.17 days in sires. The AFSF further increased in bulls (871.25±61.82 days), which was higher compared to both GS and sires. On contrary to the AFSF that increased from GS to bulls, the SPP showed a significantly ($P$<0.05) decrease. The SPP was significantly ($P$<0.05) higher in GS (1058.71±129.72 days) than bulls but there was no significant difference between sire and bulls in terms of SPP (Figure 1). The ERR was significantly ($P$<0.01) higher in bulls (47.76±3.49) compared to GS but there was no significant difference between sire and bulls (Figure 2).

**Table 2**
Effect of genetic level of CB bulls on semen production and ERR.

| Bulls ($n$=38) | AFSC (Days) | AFSF (Days) | SPP (Days) | Number of ejaculates/month | Total volume/month (mL) |
|---|---|---|---|---|---|
| F1 ($n$=6) | 663.83±130.98 | 881.50±55.12 | 860.40±179.55 | 5.17±0.76 | 24.08±4.19 |
| ≥3/4 ($n$=7) | 729.43±41.64 | 869.43±53.84 | 641.14±87.03 | 5.01±0.30 | 22.86±2.84 |
| Interse ($n$=25) | 730.00±105.13 | 896.80±33.84 | 889.60±67.99 | 5.03±0.25 | 23.63±1.50 |

AFSC-Age at first semen collection, AFSF-Age at first semen freezing, SPP-Semen production period and ERR- Ejaculate rejection rate.

**Table 3**
Semen production characteristics and ERR in CB bulls belonging to three generations.

| Generations | AFC (Days) | AFF (Days) | SPP (Days) | Total volume of ejaculate/month (mL) | Total ejaculates/month (no) |
|---|---|---|---|---|---|
| Bulls | 716.08±40.03 | 871.25±61.82 | 671.08±68.61$^{ac}$ | 21.49±1.65$^{ac}$ | 4.48±0.30$^{ac}$ |
| Sires | 783.86±25.46 | 831.43±31.17 | 891.50±59.93$^{bc}$ | 30.48±2.48$^{b}$ | 6.27±0.34$^{b}$ |
| GS | 718.14±13.18 | 767.14±25.82 | 1058.71±129.72$^{b}$ | 27.91±2.34$^{bc}$ | 5.60±0.10$^{bc}$ |

Value containing different superscript within a column vary significantly ($P$<0.01), AFC-Age at first collection, AFF-Age at first freezing, SPP-Semen production period, GS-Grand sires of bulls and ERR-Ejaculate rejection rate.

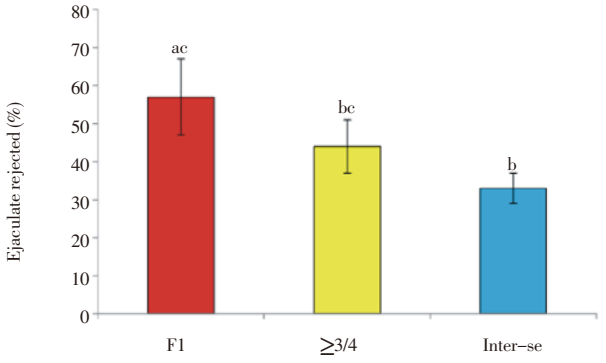


**Figure 1.** Ejaculate rejection rate in crossbred bulls with different levels of exotic inheritance (Columns containing different superscript vary significantly at $P<0.05$).

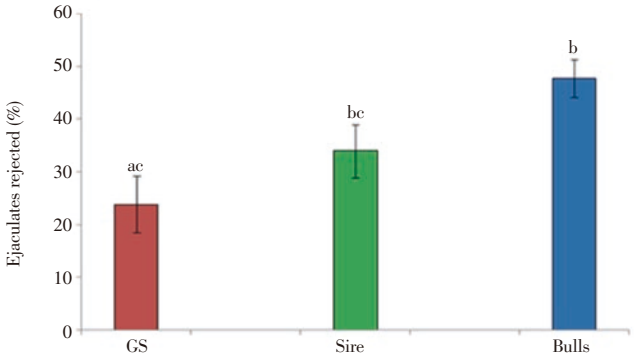


**Figure 2.** Ejaculate rejection rate in crossbred bulls of three subsequent generations (Columns containing different superscript vary significantly at $P<0.05$).

## 4. Discussion

Sub-fertility/infertility is a major problem in crossbred bulls. Due to poor quality semen, large numbers of crossbred bulls are rejected from semen collection program in semen stations. Since rejection rate in breeding bulls has been reported to have some concerns with the genetic makeup of their sire [9], the present study investigated the effects of generations and exotic inheritance on semen production attributes in crossbred bulls.

The AFSC in CB bulls ranged from 567 to 1 010 days with an average of (738.89±18.18) days, which is lower than those reported earlier [3], where it was observed that the AFSC was (872.06±19.12) days in CB bulls. However lower values have also been reported by several researchers [10-12] in CB bulls. The time interval between AFSC and AFSF was higher in F1 bulls compared to the others, indicating that the semen produced during initial days in F1 bulls were of poor quality. The AFSC indirectly indicates the sexual maturity of the male; however, it is influenced by several factors including the experience of the training personnel, genetic makeup of the bull and other managemental practices. The values observed of the present study for the CB bulls were lower than those reported on Bos indicus but higher than those reported on Bos taurus cattle. In Sahiwal cattle, it has been reported that the AFSC was 973.04±24.65 days [3] which was higher than the values observed in the present study in case of CB bulls.

Unlike AFSC, the AFSF observed in the study (865.72±34.60 days) was lower compared to those reported earlier (1044.86± 20.26 days [3]) in CB bulls. This difference might be attributed to the number of bulls used and the period of the study. The period between the AFSC and AFSF indicates the time taken to obtain an ejaculate that meets the quality standards for cryopreservation. In our study, the period between AFSC and AFSF was around 127 days which is again lower than those reported by earlier worker [3], who observed 172 days between AFSC and AFSF. The total SPP (838.64±35.99 days) observed in the present study is also comparable with reports of previous reports [3, 10], who observed 884.24±52.37 days and 27.6±5.3 months, respectively but the SPP was lower compared to earlier works [11, 13], who earlier reported (33.0±3.2) and 52.7 months, respectively.

The average number of ejaculates/bull/month ranged from 1.51 to 8.01 numbers; in the experimental station where the work was conducted the routine procedure was to collect two ejaculates from a bull on a single day/week with 15-20 minutes gap between ejaculates. If all the bulls gave two ejaculates on all collection days then the mean number of ejaculates/bull/month would be around eight, but the mean number of ejaculates/bull/month observed in the study was 5.05±0.21 only. This indicates that some of the bulls either did not give any ejaculate or gave only one ejaculate at a particular day of collection which indirectly mean that the libido of CB bulls is comparable less than the exotic bulls in which the number of ejaculates/bull/month average around 8 [14]. Reduced libido has already been reported in CB bulls compared to exotic or zebu bulls [3].

An interesting observation of the present study is that about 40% of the ejaculates are straight away rejected for use in AI either as liquid semen or after cryopreservation owing to poor semen quality. The semen quality of CB bulls have been reported to be poor than the indigenous breeds or exotic cattle [7]. They reported that about 50% of the CB bulls produce semen, which did not meet the quality standards for cryopreservation. Low-grade ejaculates (unfit to be processed for cryopreservation) were reported to be higher in CB bulls [2-4, 7, 15]. In a study conducted on Frieswal CB bulls, it has been reported that 55% of the ejaculates produced by the CB bulls were unsuitable for cryopreservation [6]. The findings of the present study are in agreement with these earlier reports. Among the three levels of exotic inheritance compared in the present study, in the F1 bulls, it was observed that 57.00±10.00 percent of ejaculates were rejected based on the poor quality. The results of the present study clearly indicates that the problem of "poor

ejaculate quality" or "Low-grade ejaculates" is higher in F1 bulls compared to ≥3/4 or inter-se mated bulls, the ERR observed in the latter bulls was 44.00±7.00 and 33.00±4.00, respectively. The ERR was significantly higher ($P$<0.05) in F1 than inter-se mated bulls. It is of high importance in the line that not only the period between AFSC and AFSF was high among the F1 bulls, but the ERR was also high in these bulls during the SPP. Taken together the above results indicate that the F1 bulls during their productive life produce high number of ejaculates that are unfit for freezing compared to other bulls (≥3/4 or interse).

Studying the semen quality details over generations would give an idea about how semen quality is being altered through generations. It was observed that the AFSC was lower in grand sires, which increased in sires that in turn decreased in bulls while the AFSF increased in a linear fashion from grand sires to bulls. These results indicate that although the crossbred bulls (3rd generation) produced semen at an early age (comparable with that of grand sires), the quality of semen was not good at that moment and they required some more days to produce ejaculates that meet the quality standards of cryopreservation. On contrary to the AFSF that increased from GS to bulls, the SPP showed a significantly ($P$<0.05) decrease from GS to bulls. The SPP observed in the study is comparable with those reported earlier [3, 10, 13]. Differences in AFSC, AFSF and SPP between CB bulls grown during different periods have been reported [3]. The differences in AFSC, AFSF and SPP among GS, sire and bulls might be due to the different managemental procedures followed during different periods.

From the findings of the study it may be inferred that the ERR is very high in CB bulls and the "acceptable quality semen producing ability" decreased from grandsire through sire to male progeny. Among the different exotic genetic levels (F1, ≥3/4, Interse) of CB cattle, F1 bulls produced significantly higher "low grade ejaculates" that were unfit for cryopreservation.

## Conflict of interest statement

We declare that we have no conflict of interest.

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