Tropical Animal Health and Production (2020) 52:2529–2539
https://doi.org/10.1007/s11250-020-02284-9

REGULAR ARTICLES

Semen production and semen quality of indigenous buffalo breeds under hot semiarid climatic conditions in India

Kaustubh Bhave[1] · Thilak Pon Jawahar Koilpillai[1] · Venkataraman Ragothaman[1] · Shivaji Sontakke[2] · Gautami Joshi[2] · Vincent Ducrocq[3]

Received: 30 December 2019 / Accepted: 13 May 2020 / Published online: 22 May 2020

Abstract

Semen data comprising of 97,023 ejaculates of 197 bulls from 6 buffalo breeds were analyzed. The traits considered were as follows: ejaculate volume, sperm concentration, mass activity, initial and post-thaw motility, total sperm, and total motile sperm before and after thawing as well as a composite trait equal to the theoretical number of doses which can be produced from each given ejaculate. The objective was to measure the semen production potential of indigenous buffalo bulls and identify factors affecting these traits. A linear mixed model was used, including a random bull effect along with other fixed factors: the order of the ejaculate on a particular day, the interval between collections, the time of collection, the breed, the age at collection, the semen collector, and the year and month of collection. The study showed breed wise variation for all traits. The first ejaculate of a bull on a particular day was superior to the second for nearly all the traits. Longer collection intervals are better than shorter intervals for all the parameters, although short collection interval of 2 to 4 days produced higher yield in terms of total semen doses without hampering semen quality. The study also showed a slight decrease of semen quality with time of collection within a day. The Murrah breed showed comparatively consistent performance during their whole life compared with the other breeds. Repeatability estimates for semen traits were found to be low (0.09 for mass activity) to relatively large (> 0.4 for volume and concentration). A negative correlation was found between bull effects for semen volume and concentration while a high positive correlation was found between mass activity, initial motility, and post-thaw motility. Results of the study will help in suggesting suitable management and breeding plans for semen production traits.

Keywords Buffalo · Semen production · Mixed linear model · Repeatability

Introduction

Milk production in India has increased steadily in recent years with almost half (49.2%) of total milk yield contributed by buffaloes (Department of animal husbandry and dairying, Government of India 2019). The breeding policy of Government of India made a huge impact on the total milk production of the country: in particular, it involved the upgrading of the "non-descript" buffaloes (i.e., crosses of undetermined breed(s) origin) using semen from purebred buffalo bulls of well-defined breeds. The breeding policy relies on the use of Artificial Insemination (AI) technology.

Semen production traits are economically important in conjunction with female reproductive traits such as age at first calving and conception rate. The objective of the AI industry is to maximize the output of good quality semen from bulls with better milk production potential. This is assessed by focussing on semen volume, sperm concentration, and motility and functional sustainability of the semen against cryopreservation. Selection of bulls exclusively on milk production related traits may not be effective if semen traits as selection criteria are not accounted for: elite bulls with poor semen production are not desirable. Furthermore, knowing the variations of semen traits due to different environmental and management characteristics is necessary to ensure sufficient semen

✉ Kaustubh Bhave
kaustubhbhave89@gmail.com

[1] Department of Animal Genetics and Breeding, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai 600007, India

[2] BAIF, Development Research Foundation, Dr. Manibhai Desai Nagar Warje, Pune 411058, India

[3] Université Paris-Saclay, INRAE, AgroParisTech, GABI, 78350 Jouy-en-Josas, France

production in the bull studs and to study within and across breed genetic variability. Also, the information on genetic parameters, i.e., transmissible bull characteristics corrected for environmental effects, can help in exploring the possibility of including semen evaluation traits in breeding programs for bulls, but this objective will not be addressed in this paper.

Studies addressing semen production of buffaloes exist (Singh et al. 2013; Bhakat et al. 2015; Ramajayan 2016) but are far less frequent than for cattle. Most of these focused on seasonal and age variation of different semen parameters among Murrah buffalo bulls. There are many other management practices such as frequency of collection and length of interval between collections and effect of semen collector and handler. which could also contribute to variation in semen production. The present study analyzes and compares semen production of different indigenous breeds of buffalo bull semen collected under semiarid conditions since 2010 at the Central Research Station of the BAIF Development Research Foundation (BAIF Development Research Foundation 2019), a large NGO located close to Uruli Kanchan near Pune, India. BAIF provides door-to-door cattle and buffalo AI service to about 5 million families of small farmers in over 100,000 villages in 13 states.

Materials and methods

Data

Semen quantity and quality data spanning over 9 years (from August, 2010 to December, 2018) maintained at BAIF's frozen semen station, Uruli Kanchan, Pune, India, were utilized for the study. Data included 97,023 ejaculates from 197 bulls of 6 water buffalo (*Bubalus bubalis*) breeds (Banni, Bhadawari, Jaffarabadi, Murrah, Pandharpuri, and Surti). Information on breed characteristics is available on the national portal of the NBAGR website (National Bureau of Animal Genetic Resources 2019). During the period study, the bulls collected were of variable ages, i.e., some bulls were culled and replaced by younger ones, according to semen demand.

Farm location and climate

The BAIF frozen semen station is located in Maharashtra state, India, in the outskirts of Pune city (18.5° N latitude 73.8° E) and at an altitude of 559 m above sea level. The region experiences three different seasons, viz. summer (March to May), monsoon (June to October), and winter (November to February). In summer, the maximum temperature reaches 42 °C while in winter, the minimum temperature goes to as low as 6 °C. The region receives an annual rainfall of 722 mm on average, mostly during the months of June to September. The average monthly rainfall ranges from 0.5 (January) to 187.2 (July) mm. The average relative humidity varies from 36% (in April) to a maximum of 82% (in August).

Collection of semen

All the bulls were washed and cleaned in early morning before semen collection. Handling and semen collection of a particular bull were performed by a same person responsible for a same group of bulls. Dummy bulls were used for sexual stimulation, and every bull was usually allowed 2 to 3 false mounts before actual semen collection. The time between false mounts varied among the bulls. One or two semen ejaculate was collected using individual artificial vagina according to a standard procedure. After collecting semen, the semen tube was kept in a water bath for 37 °C after recording the ejaculate volume. The sperm concentration was estimated by digital photometer (IMV technologies). Mass activity (motility score) was measured as a score from 0 to 5 on the basis of swirling pattern of undiluted semen, while the initial motility (percent progressive motile sperm) was assessed subjectively using diluted semen. The ejaculates which did not fulfill minimum criteria set by standards were removed from production, although the corresponding data was kept in the analysis. After initial assessment, 0.25 ml semen doses were prepared with 20×10^6 sperms per dose (i.e., with the aim to reach approximately 10 million motile sperms after thawing per dose), sealed, and printed using IS4 instrument of IMV technologies (IMV Technologies, L'Aigle, France). Semen straws were cooled to 4 °C for 3 h and step by step frozen down to − 140 °C for 7 to 8 min in a programmable freezer (IMV Technologies) followed by submersion and storage in liquid nitrogen at − 196 °C. Post-thaw motility was then carried out after 24 h (after freezing) using 2–3 straws. Standardized initial and post-thaw motility assessments were carried out by different technicians. The assessment of mass activity, initial, and post-thaw motility was carried out using phase contrast microscope (Nikon ECLIPSE E400, Tokyo, Japan).

Traits studied and influencing factors

The semen traits studied were the following: ejaculate volume (ml), sperm concentration (10^9/ml), mass activity (defined as the motility score measured on a scale of 0 to 5 with the undiluted semen after collection), initial, and post-thaw motility (% of motile sperms after dilution and before/ after thawing). Other composite traits included total sperms (10^9/ejaculate, as the product of volume and sperm concentration), total motile sperm (10^9/ejaculate, as the product of proportion of initial motility and total sperm per ejaculate), and total motile sperms per ejaculate after thawing (10^9/ejaculate, as the product of proportion of post-thaw motility and total sperms per ejaculate). An extra combined variable was also defined, as in Humblot et al. (1993): the theoretical number of semen doses produced (hereafter called TNSDP) equal to the number of motile sperm cells (NMS = volume × concentration × post-thaw motility, divided by the objective of this NMS in each semen dose (10 million in our case)). TNSDP summarizes the main characteristics of each ejaculate into a meaningful number reflecting its output in terms of semen doses produced.

Whatever the variable considered, values over and below the raw mean of the trait ± 4 standard deviations were considered outliers and discarded. Young bulls' data during their testing period was not included in the study. As the first collection of every bull from the data did not have any interval value, it was removed from the analysis. The various factors influencing semen characteristics which were considered in the study were as follows: the order of ejaculates on a same day (first and second), the interval between two consecutive days of collection (1 to 3 days; 4 to 6 days; 7 and above), year (2010 to 2018), month (January to December), collection time (before 6 am, then on an hourly basis up to 10 am and after 10 am), age at collection in months (classified per year from 36 to 180 months, 12 year classes), and semen collector. Months were also grouped to form three seasons, viz. winter (January, February, November, and December), summer (March to May), and monsoon (June to October). Season and age in months as a covariate were used in a preliminary analysis.

Statistical analysis

As the data consisted of many repeated measurements for each bull, a linear mixed repeatability model was used for the analysis of the various traits: a random bull effect was included to take care of the between bulls' variability. This effect was supposed to be constant over time for a given bull. Various combinations of the factors described above were tested in order to find a proper model. All models included an age effect and a breed effect. The age effect was included either as a class variable or as a covariate through a quadratic or cubic polynomial. Seasonal effects were analyzed either as season or month effects within each year. Two-way interactions between breed and age at collection and between semen collector and year of collection were also considered. In total, 9 models combining these different options were analyzed using the WOMBAT software (Meyer 2007) to get solutions for fixed and random effects. BIC (Bayesian Information Criterion) and MSE (mean square error) values for model comparison and significance of fixed effects were obtained using the "lmerTest" package of the R software (R Core Team 2019).

Model (1) showed the lowest mean square error against other models for all traits. BIC results for the 9 models were slightly less consistent between traits, but differences between best models were not large and for the sake of simplicity, model (1) was selected as the model of choice for all traits. It can be written as:

$$Y_{\mathrm{ijklmnpqr}} = E_{\mathrm{i}} + I_{\mathrm{j}} + T_{\mathrm{k}} + (B \times A)_{\mathrm{lm}} + (C \times Y)_{\mathrm{pq}} + (M \times Y)_{\mathrm{np}} + U_{\mathrm{r}} + e_{\mathrm{ijklmnpqr}} \quad (1)$$

where

$Y_{\mathrm{ijklmnpqrs}}$	semen production trait
E_{i}	fixed effect of the i^{th} order of the ejaculate on a same day ($i = 1, 2$)
I_{j}	fixed effect of the j^{th} collection interval ($j = 1$ to 3)
T_{k}	fixed effect of the k^{th} collection time ($k = 1$ to 6)
$(B \times A)_{\mathrm{lm}}$	fixed effect of the lm$^{\mathrm{th}}$ breed × age class interaction ($l = 1$ to 6, $m = 1$ to 12)
$(C \times Y)_{\mathrm{pq}}$	fixed effect of the pq$^{\mathrm{th}}$ semen collector × year of collection interaction ($p = 1$ to 12, $q = 1$ to 9)
$(M \times Y)_{\mathrm{nq}}$	fixed effect of the np$^{\mathrm{th}}$ month × year of collection interaction ($n = 1$ to 12)
U_{r}	random effect of the r^{th} bull ($r = 1$ to 197), supposed to follow a normal distribution with mean 0 et and variance σ^2_{b}
$e_{\mathrm{ijklmnpqrs}}$	random residual associated with each observation, supposed to follow a normal distribution with mean 0 and variance σ^2_{e}

Results and discussion

Table 1 displays for each breed the number of bulls evaluated and the phenotypic mean and standard deviation of their semen production and semen quality parameters.

The highest number of bulls (154) in the dataset was for the Murrah breed, while Banni, Pandharpuri, and Surti breeds were represented by 3 to 7 bulls only, reflecting a much lower demand. In the following analyses, these three breeds were considered together as "other breeds." Raw means of the 6 breeds for volume ejaculate ranged between 4.09 (Banni) and 5.10 ml (Jaffarabadi). These means are in the same range as those reported by Javed et al. (2000), for the Nili Ravi breed and El-wishky (1978) for Iraqi buffalo bulls. Ramajayan (2016) reported much lower means (2.56 ml) for Murrah buffalo bulls, but this lower volume could be due to the hot and humid weather in Tamil Nadu state of India.

Sperm concentration was higher in the Murrah, Banni, and Surti breeds. Similar results of concentration were reported by Ramajayan (2016) in Murrah buffaloes.

Mass activity showed limited variation across breeds with a mean close to 2.5. Many studies in Murrah bulls (Singh et al. 2013; Kumar and Krupakaran 2014; Bhakat et al. 2015; Ramajayan 2016) and Surti buffalo bulls (Bhosrekar et al.

Table 1 Number of observations, mean, and standard deviation of semen production traits, per breed

Breed	No. of bulls	No. of observations before and after thawing	Ejaculate volume	Sperm concentration (10^9/ml)	Total sperms (10^9/ejaculate)	Mass activity (0–5 score)	Initial motility (%)	Total motile sperms (10^9/ejaculate)	Post-thaw motility (%)	Total motile sperms (10^9/ejaculate) after thawing	Theoretical number of semen doses
Banni	4	3643–3148	4.09 (1.59)	1.31 (0.48)	5.31 (2.93)	2.52 (0.84)	74.14 (11.31)	3.95 2.40)	48.47 (3.06)	3.08 (1.44)	308.6 (2.58)
Bhadawari	15	7758–6130	4.11 (1.57)	1.16 (0.48)	4.75 (3.09)	2.43 (0.91)	71.57 (12.89)	3.40 (2.77)	47.80 (3.42)	2.76 (1.36)	276.6 (1.74)
Jaffarabadi	14	7929–6919	5.10 (1.80)	1.18 (0.46)	6.00 (3.03)	2.53 (0.87)	74.70 (11.10)	4.56 (2.72)	48.37 (3.43)	3.50 (1.67)	352.0 (2.01)
Murrah	154	72,457–61,000	4.48 (1.87)	1.31 (0.53)	5.67 (2.93)	2.55 (0.89)	74.05 (11.38)	4.22 (2.36)	48.45 (7.16)	3.32 (1.64)	332.2 (0.65)
Pandharpuri	3	933–809	4.79 (1.80)	0.93 (0.36)	4.56 (2.68)	2.42 (0.98)	73.22 (10.49)	3.37 (2.19)	48.93 (2.73)	2.78(1.48)	278.9(5.23)
Surti	7	2659–2633	4.68 (1.73)	1.27 (0.50)	5.85 (2.83)	2.55 (0.97)	73.21 (12.45)	4.29 (2.31)	48.34 (2.89)	3.42 (1.63)	336.5 (3.44)

Figures in parenthesis show standard deviation

1992) reported similar results. Buffalo bulls usually show lower motility when semen is undiluted.

Average initial motility ranged between 71 and 75% among the breeds, similar to that reported by Misra et al. (1994) and Rao et al. (1996) in Murrah, although some studies reported slightly higher motility (above 78%) in Murrah and Surti buffaloes (Sahu and Pandit 1997; Dhami and Kodagali 1988; Singh et al. 2013). Average post-thaw motility did not vary among the breeds with values close to 48%. Similar mean values were reported by Dhami and Kodagali (1988). Other studies (Ravimurgan 2001; Tiwari et al. 2011) reported slightly higher post-thaw motility means (50.5% and 53.7%) in Murrah buffalo bulls. The motilities are measures with subjective evaluations which strongly depend on the evaluator, so slight variations among different studies are expected.

An average total number of sperms of 4.56 to 6 billion per ejaculate is in close accordance with the report of Dhami and Kodagali (1988) in Surti buffalo bulls. This total number of sperms tends to increase with the volume of the ejaculate as was observed in the present study. Ramajayan (2016) reported much lower total sperms per ejaculate (3.12 billion per ml) in Murrah, which reflected a lower average ejaculate volume in their study.

The order of the ejaculate (first or second on a same day of collection) significantly affected ($P < 0.01$) semen production traits in buffalo bulls (Table 2). Except for post-thaw motility, all semen production traits showed lower solutions for the second ejaculate. The observed differences between the two ejaculates on the other composite traits are essentially the consequences of a lower volume and a lower concentration for the second ejaculate. The overall effect is large, with a decrease in semen doses produced of 133.4 between the first and the second ejaculate collected. These findings are in agreement with studies conducted on Murrah buffalo bulls in Tamil Nadu state of India (Ramajayan 2016).

The higher values for the first ejaculate are well documented in cattle for sperm concentration, ejaculate volume, total sperm per ejaculate, mass activity, and initial motility (Everett et al. 1978; Everett and Bean 1982; Taylor et al. 1985; Fuerst-Waltl et al. 2006; Bhakat et al. 2011; Murphy et al. 2018). The basic reason for collection of two ejaculates on a same day is to increase the number of semen doses produced without affecting sperm quality. The very similar post-thaw motilities of the two ejaculates in the present study suggest that sperm integrity remains satisfactory with short collection intervals on a same day.

Interval between two collection days had a significant effect ($P < 0.01$) on all semen production traits in buffalo bulls. Out of the three classes defined, the first one (interval of 1 to 3 days) was considered the reference because it was the most frequent one. Among the traits considered, semen concentration, volume, total sperms per ejaculate, total mo-

Table 2 Solutions of the fixed effects influencing semen characteristics

Levels	No. of observations (n)	Ejaculate volume	Sperm concentration (10^9 / ml)	Total sperms (10^9/ejaculate)	Mass activity (on scale of 0 to 5)	Initial motility (%)	Total motile sperms(10^9/ ejaculate)	Post-thaw motility (%)	Total motile sperms (10^9/ ejaculate) after thawing	Theoretical number of semen doses
Order of ejaculate**										
1	61,544–70,357	0*	0*	0*	0*	0*	0*	0*	0*	0*
2	20,775–25,022	− 0.29 ± 0.03	− 1.76 ± 0.29	− 3.17 ± 0.37	− 0.61 ± 0.09	− 0.16 ± 1.49	− 2.00 ± 0.30	0.96 ± 0.55	− 1.24 ± 0.22	− 133.4 ± 21.0
Collection interval**										
1 to 3 days	44,474–50,833	0*	0*	0*	0*	0*	0*	0*	0*	0*
4 to 6 days	32,769–37,782	0.06 ± 0.01	0.20 ± 0.01	0.56 ± 0.01	0.026 ± 0.01	0.10 ± 0.07	0.42 ± 0.01	−0.096 ± 0.03	0.30 ± 0.01	28.7 ± 0.9
≥ 7 days	5076–6764	0.20 ± 0.01	0.50 ± 0.02	1.43 ± 0.03	−0.070 ± 0.01	−0.88 ± 0.17	0.86 ± 0.03	−0.76 ± 0.07	0.70 ± 0.02	66.0 ± 2.3
Time of collection**										
≤ 6 am	2479–2655	0*	0*	0*	0*	0*	0*	0*	0*	0*
6 to ≤ 7	35,573–40,071	− 0.013 ± 0.01	0.03 ± 0.02	− 0.02 ± 0.04	− 0.04 ± 0.01	− 0.30 ± 0.22	− 0.072 ± 0.03	− 0.23 ± 0.09	− 0.01 ± 0.02	− 1.5 ± 2.7
7 to ≤ 8	21,244–24,488	− 0.020 ± 0.01	0.19 ± 0.02	0.15 ± 0.05	− 0.09 ± 0.01	− 0.42 ± 0.23	0.041 ± 0.04	− 0.50 ± 0.09	0.09 ± 0.03	8.3 ± 2.9
8 to ≤ 9	15,151–18,267	− 0.046 ± 0.01	0.19 ± 0.03	0.03 ± 0.05	− 0.16 ± 0.01	− 1.12 ± 0.25	− 0.071 ± 0.04	− 0.54 ± 0.10	0.03 ± 0.03	2.6 ± 3.1
9 to ≤ 10	6016–7505	− 0.064 ± 0.01	0.11 ± 0.03	− 0.10 ± 0.06	− 0.29 ± 0.02	− 1.6 ± 0.27	− 0.19 ± 0.04	− 0.63 ± 0.11	−0.02 ± 0.03	− 2.7 ± 3.5
> 10 am	1856–2393	− 0.075 ± 0.01	0.09 ± 0.04	− 0.13 ± 0.07	− 0.45 ± 0.02	− 1.62 ± 0.33	− 0.24 ± 0.05	− 0.82 ± 0.14	−0.014 ± 0.04	− 1.8 ± 4.3

0*These solutions are considered reference values, **indicates $P < 0.01$ significance level tile sperms per ejaculate, and total motile sperms after thawing showed higher solutions for collections with an interval of 7 days or more, while lower values were found in shorter intervals. In the case of mass activity and motility traits, intervals of more than 7 days led to a slight decline, but overall, the number of doses produced increase with the interval between collection (+ 28.7 and + 66.0 doses for the second and third intervals considered).

To increase semen production at the semen station, collection is carried out at least twice a week from every bull. It may go up to thrice a week for selected bulls having higher demand in the field. Our results are consistent with the findings of Everett et al. (1978), Mathevon et al. (1998a, b), Fuerst-Waltl et al. (2006), Murphy et al. (2018). Even with smaller collection intervals, post-thaw motility was marginally better than longer intervals. In commercial AI industry, having intervals of more than 7 days is impractical: to cope with a high demand of semen doses from adult bulls, a longer interval between collections and a lower number of doses produced are not economically viable. To illustrate this, a theoretical comparison was carried out: based on our results, an average bull with collection intervals of either 7, 4, or 2 days produces a total of 470, 976, and 1350 doses a week. Therefore, it can be concluded that with intervals between 2 to 4 days, it is possible to produce a substantially larger number of doses without hampering semen quality.

Time of collection had a highly significant effect ($P < 0.01$) on all semen production traits. The "before 6 a.m." time class was used as the reference. Sperm concentration, mass activity, and motility traits decreased with time, while volume and total sperm increased up to 9 am and then decreased gradually. Usually, testicular temperature is lower than body temperature, which is required for production of fertile spermatozoa. The increase of ambient temperature causes a rise in the testicular temperature subsequently decreasing semen quality. Our results illustrate the change in semen parameters as time passed. However, the overall impact on number of semen doses produced is small, with a variation of TNSDP of 11 doses between extreme categories. Fuerst-Waltl et al. (2006) also reported that mass activity and initial motility in cattle were affected by an increase in ambient temperature. Taylor et al. (1985) found that semen volume and total sperms were affected by higher and lower classes of ambient temperature in cattle, through a non-linear relationship, i.e., with larger decreases for more extreme temperatures.

The 3 to 4-year of age class of Murrah bulls was considered the reference for the breed × age class interaction solutions for all semen production traits. Age at semen collection × breed interaction was found to have a significant effect ($P < 0.01$) for all semen production parameters. The average ejaculate volume of the Jaffarabadi and Bhadawari breeds showed a quadratic pattern with an increasing trend until 8 to 9 years of age and then a slow decline (Fig. 1). Similar trends were found in other traits of Jaffarabadi and Bhadawari breeds. In contrast, the Murrah bull performance was quite constant throughout the production period except after the age of 8 to 9 years from where it showed slight decline up to 10 years and then rose up to remain constant. In general, all the breeds showed a higher ejaculate volume at 8 to 9 years. Total sperms per ejaculate, total motile sperm per ejaculate, and total motile sperms after thawing followed similar trends. A slight opposite trend was observed in case of the sperm concentration of Murrah after the age of 8 to 9 years (Fig. 2) with a slight positive increasing trend. In the case of mass activity and the other motility traits, all breeds showed more or less a stable pattern over the production period. Overall, the TNSDP reflects the combination of these factors (Fig. 3): the Murrah and Jaffarabadi breeds produce more doses per ejaculate during their whole productive life (+2 0 to + 60 doses during most of it) compared with the Bhadawari breed, the other breeds being even worse.

These results could be useful in formulating tailored culling policies for different breeds of bulls. There is a breed variation in the way semen production performances of bulls vary with age, so it could be wise to fix a culling age specific to each breed. However, in addition to the breed differences, preference for breeds based on semen demand is likely to lead to an extent of their use beyond an objective culling age which may affect their performance, especially after the age of 9–10 years.

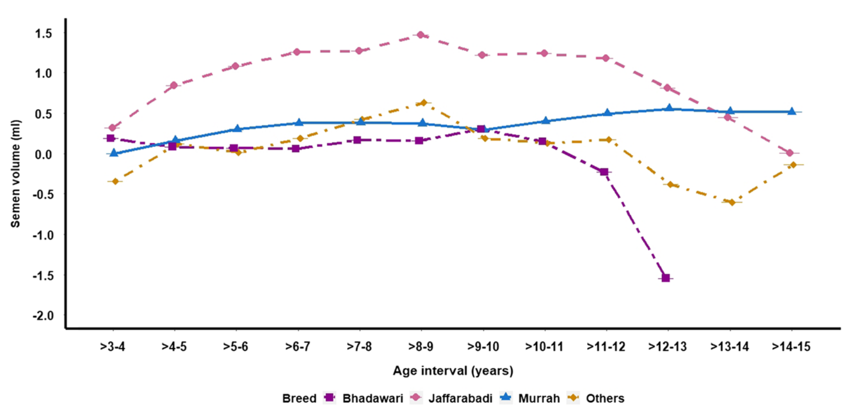

Fig. 1 Effect of age at semen collection on ejaculate volume (subtitle: 0 = reference solution as Murrah × age (> 3 to 4 years))

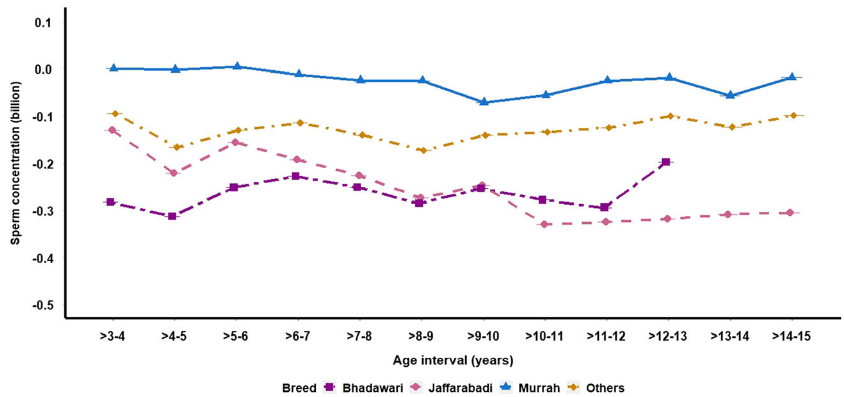

Fig. 2 Effect of age at semen collection on sperm concentration (subtitle: 0 = reference solution as Murrah × age (> 3 to 4 years))

This is evident from the trend for Bhadawari. Compared with other breeds studied, Bhadawari bulls were poor performers after 10 years of age, when there was a marked decline in all the semen traits. However, breeds other than Murrah were represented by a small set of bulls. Hence, it is advised to treat the results with caution.

Almquist (1978) indicated that the increased semen output due to a rise in the activity of the hypothalamic pituitary testicular axis, and the simultaneous development of the testis and their accessory glands with sexual maturity. This leads to an increase in volume of semen as the bull gets older and consequently, to a greater semen output. This increase was observed until the age of 9–10 years after which most of the traits started to decline. Nevertheless, Murrah bulls showed a steady performance, even up to 15 years of age. However, the number of Murrah bulls after 10 years of age was very low indicating that a majority of them were culled or removed from the bull stud: after 10 years of age, only 27 out of 154 Murrah bulls were maintained in the bull stud for semen production program. This shows a high culling rate (83%) of old Murrah bulls. This could be attributed to a decrease in performance of most bulls by the age of 10 years and selection of high demand selected bulls with better semen production. Furthermore, it seems wise to avoid the use of older bulls as this may lead to possible inbreeding in progeny unless the animals' pedigree is well traced. Moreover, if bulls are coming from an efficient breeding scheme, the higher genetic level of younger bulls is a strong initiative to stop collection of older bulls.

In the present study, both the handling and semen collection were carried out by a same person. Semen collector 1 with 2010 year subclass was considered the reference for other solutions to be compared with. The collector × year of collection interaction was found to have a significant effect ($P < 0.01$) for all semen production parameters. It was observed that some groups of traits showed particular patterns over the studied period. For volume, concentration, total sperms, total motile sperms, and total motile sperms after thawing, the collector × year effects showed increasing solutions until 2014 where they reached the highest values and then showed sharp decline up to 2017. The collector × year effects for volume and semen concentration may be relative large on a same year (e.g., with differences between collectors of 0.5 ml for volume and 0.15 10^9/ml for concentration) but are negatively correlated, leading to changes in TNSDP much less variable within year than between years (Fig. 4). After 2014, this effect showed a declining trend for all traits which could be due to an increase in number of young bulls collected during this period, potentially resulting in less time for each collection.

Bull handlers and semen collectors have a large impact on the semen production of bulls (Fuerst-Waltl et al. 2006). How

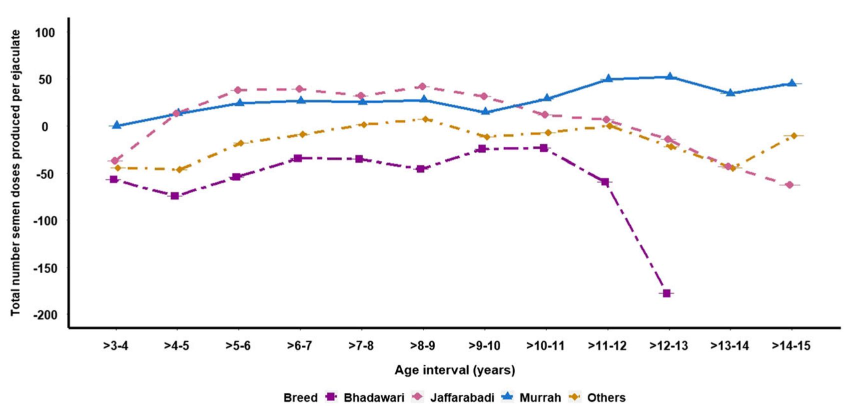

Fig. 3 Effect of age at semen collection on theoretical number semen doses per ejaculate (subtitle: 0 = reference solution as Murrah × age (> 3 to 4 years))

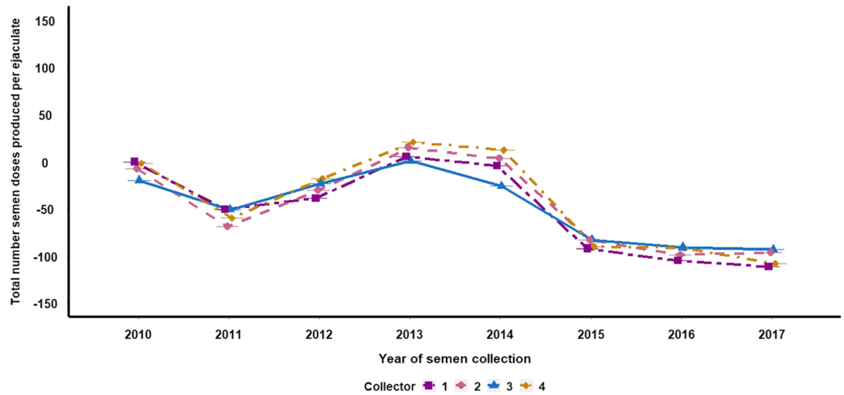

Fig. 4 Effect of the collector on theoretical number semen doses over the years (subtitle: 0 = reference solution as collector 1 × 2010 year; for clarity, only 4 major collectors are reported)

bulls are treated, the method of restraining, and their sexual preparation are strongly dependent on the collector and handler. The effect of the semen collector was also reported by Dominguez et al. (1994) for the ejaculate volume, by Komisrud and Berg (1996) for the volume and post-thaw motility by Mathevon et al. (1998a) for concentration, volume and total sperms per ejaculate, and by Fuerst-Waltl et al. (2006) for concentration, volume, total sperms per ejaculate and initial motility.

The monthly variations of the traits studied did not follow a similar trend over the years. There was a large variation ($P < 0.01$) of the month effect observed among the traits during the course of 9 years. This effect for traits like volume, concentration, total sperms, and total motile sperm after thawing had higher values during the years 2011 and 2015, although the differences among month effects over the years appeared not too large. However, their combined influence led to differences in theoretical number of semen doses produced per ejaculate up to 150 doses (Fig. 5). These traits showed higher solutions in summer months (April to May). Such results were in agreement with the reports of Stalhammar et al. (1989), Snoj et al. (2013), and Murphy et al. (2018) who found higher values in summer months. The increasing number of the theoretical number of doses produced can be explained on the basis of the spermatogenesis process which takes 65 days on average. This means that a more favorable climatic conditions during the winter and their subsequent effect on the sensitive stage of spermatogenesis resulted in better sperm concentration, ultimately resulting in higher numbers of semen doses. On the contrary, the hot climate in summer resulted in lower semen output during the monsoon season and led to a lower production of semen doses. On the other hand, the month effects for mass activity and motility traits were consistently showing better values in year 2013 than in any other years. It was also observed that there were large differences in mass activity and motility traits over years. The differences could be attributed to variation in the production targets set by the frozen semen station and the semen demands over the years. For example, BAIF frozen semen lab achieved its highest number of doses produced in 2015.

The bull effect reflects the similarity of the semen characteristics it produces throughout its productive lifespan. This similarity is the sum of its own genotype (genetic component) and specific non-genetic characteristics (e.g., its early growth) which influence its semen production (permanent environment component). A thorough search of literature revealed very few (Taraphder et al. 2001; Ramajayan 2016) studies with estimates of repeatability of bull effects for buffaloes, under the assumption that the bull effect remains constant over its whole productive life. Table 3 displays the different variance components for each trait. The repeatability estimates computed analyzing each trait separately ranged from 0.09

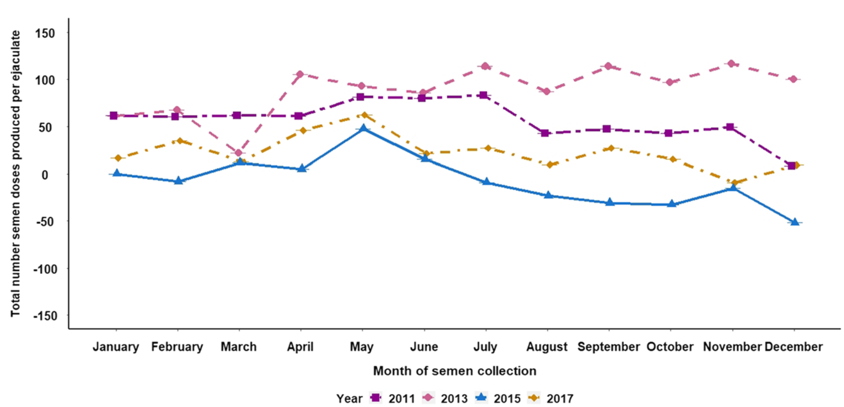

Fig. 5 Effect of the month of semen collection on theoretical number semen doses over the year (subtitle: 0 = reference solution as 2015 year × January month; for clarity, only 4 alternate years are reported)

Table 3 Univariate estimates of variance and repeatability estimates

Trait	Phenotypic variance	Bull variance	Residual variance	Repeatability estimates
Ejaculate volume (ml)	2.94	1.20	1.75	0.41 ± 0.029
Concentration (10^9/ml)	0.26	0.104	0.15	0.40 ± 0.029
Total sperm (10^9/ejaculate)	6.80	1.55	5.25	0.23 ± 0.021
Mass activity (scale of 0 to 5)	0.73	0.065	0.66	0.09 ± 0.010
Initial motility (%)	127.2	20.46	106.7	0.16 ± 0.016
Total motile sperms (10^9/ejaculate)	4.51	0.99	3.51	0.22 ± 0.020
Post-thaw motility (%)	19.86	2.33	17.52	0.12 ± 0.013
Total sperms after thawing (10^9/ejaculate)	2.21	0.52	1.68	0.24 ± 0.021
Theoretical number of semen doses produced	22,052.7	5239.8	16,812.9	0.24 ± 0.021

for mass activity to 0.41 for semen concentration, with a value of 0.21 for the combined measure TNSDP. Ramajayan (2016) also found low to moderate estimates (0.22 to 0.34) for semen production characters of Murrah buffalo while Taraphder et al. (2001) found lower estimates for volume in Murrah buffaloes. Estimates for mass activity and motility traits were found lower than those reported by Ramajayan (2016) while estimates of sperm concentration and ejaculate volume were found higher. The estimates reported in the present study show that the future performance of a bull can be explained with some accuracy for sperm concentration and ejaculate volume, and with moderate accuracy for TNSDP. The bull effects (genetic + permanent environmental correlation) analyzing three major traits (volume, concentration, and post-thaw motility) together with the combined TNSDP are presented in Table 4. Here, only the first ejaculate was included in the analysis for a given day, to avoid the specification of a particular residual correlation structure between the first and the second ejaculate of a same animal on a given day. The estimate of the bull effect correlations between TNSDP and either concentration or ejaculate volume is moderate of the same order of magnitude (respectively, 0.379 and 0.459) while its correlation with post-thaw motility is small (0.166).

In this study, it was assumed that the bull effect remained the same during the whole productive life of an animal. To test this rather strong hypothesis, a random regression model describing the bull effect as a continuous function of age (for example with Legendre polynomials) could be used to account for the fact that bulls may become better or worse as they are aging.

The repeatability and bull effect correlations estimates can be regarded as a higher bound of the heritability of the traits and of the genetic correlation between these traits, which could not be estimated here because of limited availability of pedigree relationships, even in Murrah, the largest breed.

In conclusion, our study showed that despite the high differences between solutions of the ejaculate numbers, collection of a second ejaculate is an important practice to increase daily semen output. Similarly, collection at intervals of 2 to 4 days can result in a 2- to 3-fold increment of semen output per week. The way each semen collector deals with sexual preparation and handling of a bull had a significant impact on all traits. A regular training of the least talented collectors and performance incentives may encourage them to improve their performance. The repeatability estimates might be helpful to explain the future performances of the bulls with some accuracy. Knowledge of the different factors can help an AI stud to achieve a high semen production of good quality with

Table 4 Multivariate bull parameters of four major traits considering only the first ejaculate of each bull on a given day

Traits	Concentration (10^9/ml)	Ejaculate volume (ml)	Post-thaw motility (%)	Theoretical number of semen doses per ejaculate
Concentration (10^9/ml)	*0.463 ± 0.029*	−0.523 ± 0.061	0.1183 ± 0.082	0.379 ± 0.072
Ejaculate volume (ml)	−0.267 ± 0.032	*0.401 ± 0.029*	0.026 ± 0.086	0.459 ± 0.067
Post-thaw motility (%)	0. 046 ± 0.020	0.003 ± 0.019	*0.121 ± 0.013*	0.166 ± 0.085
Theoretical number of semen doses per ejaculate	0.139 ± 0.024	0.161 ± 0.022	0.127 ± 0.013	*0.180 ± 0.018*

The italicized values in the table are repeatability estimates ± standard errors and placed in the diagonal cells of the table; correlations between bull (genetic + permanent environmental) own effects ± standard error on the upper triangle; phenotypic correlations ± standard error on the lower triangle desired profit. A measure such that the theoretical number of semen doses produced per ejaculate (TNSDP) can help to summarize the overall impact of the different semen traits on overall semen output and to objectively compare bulls' performance.

Acknowledgments The authors thank the President, Senior Vice president, and colleagues of frozen semen station of BAIF Development Research Foundation for permission to utilize their data and the constant support during the study. The authors acknowledge the Department of Animal Genetics and Breeding, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai for the facilities provided.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Almquist, J.O., 1978. Bull semen collection procedures to maximize output of sperm. Proc. 7th Tech. Conference. Artificial Insemination Reproduction of the National Association of Animal Breeders, Columbia. 33–36.

BAIF Development Research Foundation. 2019. http://www.baif.org.in/. Accessed 1 December, 2019.

Bhakat, M., Mohanty, T.K., Raina, V.S., Gupta, A.K. Khan, H.M., Mahapatra, R.K. and Sarkar, M., 2011. Effect of age and season on semen quality parameters in Sahiwal bulls. Tropical Animal Health and Production, 43(6), 1161–1168.

Bhakat, M., Mohanty, T.K., Gupta, A.K., Prasad, S., Chakravarty, A.K. and Khan, H.M., 2015. Effect of season on semen quality parameters in Murrah buffalo bulls. Buffalo Bulletin, 34(1), 100- 112.

Bhosrekar, M.R., Purohit, J.R., Gokhale, S.B. and Mangurkar B.R., 1992. Effect of seasons on production performance of Surti buffalo bulls. Indian Journal of Animal Sciences, 62(5), 443-447.

Department of animal husbandry and dairying, Government of India, 2019. Animal Husbandry statistic Division. Basic animal husbandry and fisheries statistics, 2017. Ministry Of Fisheries, Animal Husbandry & Dairying. http://www.dahd.nic.in/Division/statistics/animal-husbandry-statistics-division/. Accessed 1 December, 2019.

Dhami, A.J. and Kodagali, S.B., 1988. Seminal characteristics and their interrelationships in Surti buffalo. Indian Veterinary Journal, 65(1), 61-64.

Dominguez, J.C., Alegre, B., Molero, R., Carbajo, M., Anel, L. and Boixo, J.C., 1994. Effect of management on semen quality of Brown Swiss AI bulls. Veterinary Medicine, 11, 332–340.

El-Wishky, A.B., 1978. Reproduction performance of Iraqi buffaloes. Seasonal variation in sexual desire and semen characteristics. Reproduction in Domestic Animals. 13(1), 28-32.

Everett, R.W. and Bean, B., 1982. Environmental influences on semen output. Journal of Dairy Science, 65(7), 1303–1310.

Everett, R.W., Bean, B. and Foote, R.H., 1978. Sources of variation of semen output. Journal of Dairy Science, 61 (1), 90–95.

Fuerst-Waltl, B., Schwarzenbacher, H., Perner, C. and Sölkner, J., 2006. Effects of age and environmental factors on semen production and semen quality of Austrian Simmental bulls. Animal Reproduction Science, 95, 27–37.

Humblot, P., Ducrocq, V., Nemeth, C. (1993) Relationship between semen characteristics of young and adult bulls in the Normande breed. Livestock Production Science, 35, 265-281.

Javed M.T., Khan, A. and Kausar, R., 2000. Effect of age and season on some semen parameters of Nili-Ravi buffalo (Bubalus bubalis) bulls. Veterinarski Arhiv, 70 (2), 83-94.

Komisrud, E. and Berg, A.K., 1996. The influence of duration of sexual preparation on bovine semen characteristics and fertility rates. Reproduction in Domestic Animals, 31(2), 369–371.

Kumar, A.S. and Krupakaran, R.P., 2014. Comparative study on physio-morphological characteristics of semen from Murrah buffaloes and Jersey crossbred cattle. CIBTech Journal of Bio Protocols. 3(3), 12-14.

Mathevon, M., Buhr, M.M. and Dekkers, J.C., 1998a. Environmental, management, and genetic factors affecting semen production in Holstein bulls. Journal of Dairy Science, 81(12), 3321–3330.

Mathevon, M., Dekkers, J.C.M. and Buhr, M.M., 1998b. Environmental, management and genetic factors affecting semen production in French Montbeliard bulls. Livestock Production Science, 55(1), 65–77.

Meyer, K., 2007. WOMBAT- A tool for mixed model analyses in quantitative genetics by restricted maximum likelihood (REML). Journal of Zhejiang University-Science B. 8(11), 815–821.

Misra, A.K., Patel, S.H., Joshi, B.V., Jaiswal, R.S. and Trivedi, K.B., 1994. Buffalo semen characters and its freezability under Indian conditions. In proceedings, 4th world buffalo congress, Sao Paulo Brazil, 27-30.

Murphy, E.M., Kelly, A.K., O'Meara, C., Eivers, B., Lonergan, P. and Fair, S., 2018. Influence of bull age, ejaculate number, and season of collection on semen production and sperm motility parameters in Holstein Friesian bulls in a commercial artificial insemination centre. Journal of Animal Science, 96(6), 2408-2418.

National Bureau of Animal Genetic Resources, 2019. Animal genetic resources of India (AGRIS-IS). http://www.nbagr.res.in/, Accessed 1 December, 2019.

R Core Team, 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Version: 3.6.1.). https://www.R-project.org/. Accessed 15 July, 2019.

Ramajayan, P., 2016. Genetic studies on semen production in Murrah buffalo bulls reared in Tamil Nadu. M.V.Sc dissertation. Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India.

Rao, A.V.N., Sreemannarayana, O. and Rao, C. 1996. Studies on sex behaviours and seminal traits in Ongole, Jersey, Jersey × Ongole and Murrah bulls. Indian Veterinary Journal, 73(3), 284-287.

Ravimurgan, T., 2001. Semen production capacity of Murrah buffalo bulls in Tamil Nadu. M.V.Sc dissertation. Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India.

Sahu, S.B. and Pandit, R.K., 1997. Grading of Murrah buffalo bulls on sexual behaviour and seminal characteristics including cry preservability. Indian Veterinary Journal, 74(7), 577-579.

Singh, A.P., Singh, R., Singh, A.K., Gupta A.K. and Raina, V.S., 2013. Influence of microclimate modification on sexual behaviour and semen characteristics of Murrah buffalo bull during the hot humid period in India. Indian Journal of Animal Sciences, 83(4), 431-434.

Snoj, T., Kobal, S. and Majdic, G., 2013. Effects of season, age, and breed on semen characteristics in different *Bos taurus* breeds in 31 years of retrospective study. Theriogenology, 79, 847–852.

Stalhammar, E.M., Janson, L. and Philipsson, J., 1989. Genetic studies on fertility in A.I. bulls. I. Age, season and genetic effects on semen characteristics in young bulls. Animal Reproduction Science, 19, 1–17.

Taraphder, S., Gupta A.K. and Tomar S.S., 2001. Repeatability estimates of seminal characteristics of Murrah buffalo bulls. Indian Journal Animal Research, 35(1), 71-72.

Taylor, J.F., Bean, B., Marshall, C.E. and Sullivan, J.J., 1985. Genetic and environmental components of semen production traits of artificial insemination Holstein bulls. Journal of Dairy Science, 68, 2703–2722.

Tiwari, R., Mishra, G.K., Shukla, M.K., Singh, R.B., Saxena, S.K. and Sidiqui, M.U., 2011. Seasonal variation in semen production of Murrah buffalo bulls. Indian Journal of Animal Reproduction, 32(2), 5-7.