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Semen quality and production potential of Indian buffalo breeds

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Bull with high breeding value for the milk producing ability would be useless if the bull could not produce superior quality semen. The optimum semen production and quality traits are crucial for assessing the bull's fertility. The interest of the AI industries is to produce more semen doses with sufficiently good quality from their high demand bulls. However semen traits are often influenced by environmental effects than genetic effects. There are very limited studies available regarding semen traits on different buffalo breeds (Bhakat et al. 2015, Ramajayan 2016, Pathak et al. 2018). Knowing the across breed variations of semen traits due to different environmental effects, it is also necessary to study within and across breed genetic variability. The present study was designed to study the effect of different breeds, season and age at collection on semen traits in different Indian breeds of buffalo.

Semen quantity and quality data spanning over 7 years [January, 2012 to December, 2018] maintained at BAIF's frozen semen station, Uruli Kanchan, Pune, India, was utilised for the study. Data included 85,068 ejaculates of semen production and 3,230 records of semen quality from 185 and 203 bulls, respectively of 5 water buffalo (*Bubalus bubalis*) breeds (Banni, Bhadawari, Jaffarabadi, Murrah, and Surti). The semen traits studied were the following: ejaculate volume, sperm concentration, mass activity, initial, and post-thaw motility, total sperms per ejaculate, hypo osmotic swelling test (HOST), acrosome integrity test fresh and frozen semen. The factors studied were: breed of buffalo, season of collection (winter, summer, and monsoon) and the age at collection in months (classified as: 36 to 156 months, 10 classes). The semen processing

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and quality control test were carried out as per the guidelines of GoI (2012). Per cent data was transformed using the arcsine transformation. The statistical analysis was carried out using least square analysis by "*lm*" function and "emmeans" package in R statistical software (R Core Team, 2019). The model for the analysis is given below:

$$Y_{ijkl} = \mu + B_i + S_j + A_k + e_{ijkl}$$

where, Y_{ijkl} , Semen production trait; μ , Overall mean; B_i , Effect of i^{th} breed (i=1 to 5); S_j , Effect of j^{th} season of collection (j=1 to 3); A_k , Effect of k^{th} age at collection of a bull (k=1 to 10); e_{ijkl} , Random error associated with Y_{ijkl} which is assumed to be normally and independently distributed with mean zero and constant variance.

The overall and least square means were presented in Table 1. There was a significant breed effect found for all the semen parameters except acrosome integrity of frozen semen. All the breeds differed with each other in ejaculate volume where Jaffarabadi bulls produced highest volume followed by Surti and Murrah, while lowest was found in Banni. In contrast to volume, Banni produced higher concentration while Jaffarabadi produced comparatively lower. The results of concentration and volume were due to negative relationship of two traits. Jaffarabadi and Surti showed higher means for total sperms as compared to other breeds. Despite of having high statistical significance, three motility patterns showed marginal differences among the breeds. Study carried out on Murrah by Bhakat et al. 2015 for volume, concentration, and mass activity were in close accordance with present study. Pathak et al. (2018) reported lower concentration in Surti and Murrah while Kumar and Sharma (2018) reported lower volume in Bhadawari than the present study. Bhakat et al. (2015) reported lower total sperm and initial motility in Murrah than the present study. The differences were due to different management practices carried out at different locations of study. Bhakat et al. (2015) reported slightly lower means for HOST in Murrah, while Ramajayan (2016) reported slightly higher means for HOST than the present study. Lower acrosome integrity of frozen semen was reported by Bhakat et al. (2015), while Ramajayan (2016) found higher means. All the studies were carried out on Murrah buffalo breeds. No literature was

Table 1. Least square Mean \pm SE of semen traits of buffalo bulls

Levels of factors No. of records	No. of records					Mean ± SE				
		Volume (ml)	Concentration (109/ml)	Total sperm (10%) Ejaculate)	Mass Activity (0 to 5)	Initial Motility (%)	Post Thaw Motility (%)	HOS Test (%)	Acrosome Integrity of fresh semen (%)	Acrosome Integrity of frozen semen (%)
Overall mean Breed	* *	4.39±0.01 **	1.29±0.001 **	5.48±0.01 **	2.55±0.003 **	73.41±0.0004 **	55.9±0.0002 **	59.01±0.001 NS	84.98±0.002 **	71.13±0.002
Banni	139–3167	3.84 ± 0.032^{e}	1.317 ± 0.009^{a}	$5.01\pm0.051^{\circ}$	2.51 ± 0.016^{a}	73.95 ± 0.0005^{a}	56.14 ± 0.0008^{a}	58.68±0.005ab	85.12 ± 0.003^{a}	$70.3\pm0.004^{\rm bc}$
Bhadawari	236–7472	4.18 ± 0.021^{d}	1.172 ± 0.006^{b}	4.87 ± 0.034^{d}	2.46 ± 0.010^{b}	72.27 ± 0.0002^{c}	54.96±0.0003°	60.89 ± 0.003^{a}	86.17 ± 0.002^{a}	74.48 ± 0.003^{a}
Jaffarabadi	387-6756	4.90 ± 0.022^{a}	$1.187\pm0.006^{\text{b}}$	5.74 ± 0.035^{a}	2.52 ± 0.011^{a}	74.19 ± 0.0002^{a}	55.82±0.0003 ^b	58.2±0.001 ^b	85.44 ± 0.001^{a}	$70.22\pm0.004^{\circ}$
Murran Surti	2351-65004 117-2480	$4.42\pm0.00\%$ 4.669 ± 0.035^{b}	$1.316\pm0.002^{\circ}$ 1.307 ± 0.010^{a}	$5.63\pm0.012^{\circ}$ $5.96\pm0.056^{\circ}$	$2.5/\pm0.003^{\circ}$ $2.58\pm0.018^{\circ}$	73.80±0.0006 ^b	$56.01\pm0.0004^{\circ}$ $55.72\pm0.0001^{\circ}$	59.38±0.006ab	85.09±0.002° 85.09±0.003°	$70.92\pm0.001^{\circ}$
Season of collection	ion	* *	* *	* *	NS	* *	* *	* *	* *	NS
Winter	1103-28026	4.31 ± 0.014^{b}	1.25 ± 0.004^{b}	$5.30\pm0.023^{\circ}$	2.53 ± 0.007^{a}	$73.27\pm0.0009^{\circ}$	55.36 ± 0.0001^{b}	59.88 ± 0.001^{a}	85.75 ± 0.001^{a}	71.76 ± 0.002^{a}
(November to February)										
Summer (March to May)	891–20619	4.46 ± 0.015^{a}	1.30 ± 0.004^{a}	5.67 ± 0.025^{a}	2.54 ± 0.007^{a}	74.06±0.0001 ^a	55.97±0.0002ª	58.33 ± 0.001^{b}	85.03±0.001 ^b	71.29 ± 0.001^{a}
Monsoon (June to October)	1236–36234	4.44 ± 0.013^{a}	$1.24\pm0.004^{\circ}$	5.36±0.022 ^b	2.53 ± 0.006^{a}	73.66±0.0008 ^b	55.86 ± 0.0001^{a}	59.68 ± 0.001^{a}	85.66 ± 0.001^{a}	71.72 ± 0.001^{a}
Age at collection (months)	(months)	* *	* *	* *	* *	* *	*	* *	* *	*
>36£48	341–6818	3.83±0.024 ^h	1.31 ± 0.007^{a}	5.03 ± 0.039^{e}	2.37±0.012 ^g	72.69 ± 0.0002^{f}	55.97 ± 0.0004^{a}	57.3±0.002c	$84.6\pm0.001^{\text{def}}$	69.54±0.002e
>48£60	554-12517	4.10 ± 0.019 g	1.27±0.005 ^b	5.21 ± 0.031^{d}	2.54 ± 0.010^{cd}	73.38 ± 0.0001^{d}	55.95 ± 0.0003^{a}	57.99±0.002bc	84.09±0.001ef	70.66±0.001 ^{cde}
> 60£72	514–9626	4.23 ± 0.021^{1}	1.28±0.006°	5.28±0.034 ^d	2.59±0.010 ^p	73.41±0.0002 ^{de}	55.60±0.0003 ^d	58.85±0.001 ^{bc}	84.35±0.001 ^r	70.18±0.002cde
>72£84	431–9699	$4.25\pm0.020^{\text{r}}$	1.24±0.006 ^a	5.16 ± 0.033^{e}	$2.45\pm0.010^{\text{r}}$	73.07±0.0002 ¹	55.23±0.0003 ^d	59.03±0.002°	85.72±0.001 ^{bc}	70.02±0.002 ^{de}
>84£96 >96£108	359–11311 298–9071	4.4 ± 0.0193^{e} 4.52 ± 0.020^{d}	$1.22\pm0.005^{\rm e}$ 1 29 $\pm0.006^{\rm b}$	5.26 ± 0.031^{d} 5.63 ± 0.033^{c}	2.50 ± 0.009^{e}	73.85 ± 0.0001^{c} 73.13 ± 0.0007^{ef}	55.73±0.0003cd 55.90±0.0003abc	59.19 ± 0.002^{6} 58.33 ± 0.003^{6}	85.96±0.001 ^{pc} 85.24±0.002 ^{cde}	70.65±0.002cae 71.29±0.002cd
>108£120	232–7570	4.50±0.022 ^d	1.27±0.006 ^b	5.59±0.036°	2.52±0.011 ^{de}	73.58±0.0002 ^{cd}	55.80±0.0004bc	59.02±0.004 ^b	86.21±0.002 ^b	71.28±0.003 ^{cd}
>120£132	165-6429	4.62 ± 0.024^{c}	1.27 ± 0.007^{b}	5.74±0.039 ^b	2.57 ± 0.012^{bc}	74.40±0.0002 ^b	55.79 ± 0.0004^{abc}	61.41 ± 0.005^{a}	85.35±0.003bcd	71.76 ± 0.004^{c}
>132£144	132–4655	4.8 ± 0.027^{b}	$1.26\pm0.008^{\circ}$	5.96 ± 0.044^{a}	2.64 ± 0.013^{a}	74.76±0.0003 ^a	55.47±0.0006°	62.43 ± 0.006^{a}	85.72 ± 0.003^{bc}	74.69±0.005 ^b
>144£156	204–7183	4.79 ± 0.022^{a}	1.18 ± 0.006^{f}	$5.56\pm0.037^{\circ}$	2.63 ± 0.011^{a}	74.32 ± 0.0002^{b}	55.83 ± 0.0004^{ab}	59.71 ± 0.004^{b}	87.5 ± 0.002^{a}	76.05 ± 0.003^{a}

Significance level, **; P<0.01; NS, Not Significant.

available on other buffalo breeds. In the present study, Banni, Murrah, and Surti breed did not differ from each other while, Jaffarabadi and Bhadawari differed from each other for HOST. In case of acrosome integrity of frozen semen, Banni and Surti did not differ from each other, while Murrah, Bhadawari, and Jaffarabadi differed from each other.

Season of collection had significant effect (P<0.01) on all semen production and quality traits except mass activity and acrosome integrity of frozen semen which showed no significant variation. For all semen production traits, summer had highest means compared to other seasons. However in case of volume and post thaw motility, summer, and monsoon season did not differ significantly, while in remaining production traits, all three seasons differed significantly from each other. However the difference among seasons for motility traits were very meagre due to subjective assessment of the traits. The higher volume in summer were reported in Surti and Murrah (Ramjayan, 2016 and Pathak et al. 2018). Higher means during the summer season were due to effect of climatic condition during the early days of spermatogenesis and epididymal maturation process which takes 65 days to produce a sperm. Winter and monsoon season showed higher means followed by summer season in all three quality traits, although differences within a season were very low. Bhakat et al. (2015) reported similar results in Murrah for HOST and acrosome integrity of frozen semen. On contrary, Ramajayan (2016) reported higher values in summer.

Age at the semen collection showed significant effect on all semen production and quality traits. As the age progressed, the quadratic pattern of growth was observed in the volume and total sperm up to 96 months, while all three semen quality traits showed gradual increase in means. On contrary, sperm concentration fell from early age and kept declining. The quadratic pattern were also reported by Fuerst-Waltl et al. (2006), and Murphy et al. (2018) in cattle. Post-thaw motility showed irregular pattern over the age with marginal changes. Although semen production traits except concentration and post-thaw motility, again improved after 96 months just to fall during age of 144 to 156 month. It was observed that only high demand bulls were kept for longer duration, while 84% of bulls were culled after age of 108 to 120 months. The least square means were low during the younger age than the older. Similar results for HOST and acrosome integrity were observed in study conducted by Ramajayan (2016). The present study showed that keeping the bull for a longer period did not hamper their semen quality traits. It can be concluded that breed wise differences for semen traits exist among the five buffalo breeds which would help to understand the genetic variability.

SUMMARY

The objective of the study was to evaluate non-genetic factors on semen traits. The overall means for the semen quality traits: ejaculate volume, sperm concentration, total sperm, mass activity, initial and post thaw motility, hypoosmotic swelling test, acrosome integrity of fresh and frozen test were 4.39±0.01 ml, 1.29±0.001 billion, 5.48±0.01 billion, 2.55±0.003, 73.41±0.0004%, 55.9±0.0002%, 59.01±0.001%, 84.98±0.002% and 71.13±0.002%, respectively. Breeds were found to differ in all the semen production and quality traits studied except acrosome integrity of fresh semen. Summer for semen production, and winter and monsoon for semen quality traits were comparatively better season. The quality traits improved with age and declined slightly in the later life.

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