Original Research

Relationship of Age, Body Condition Score and Rump Fat Thickness with Semen Quality in Murrah Buffalo Breeding Bulls

Satendra Kumar Yadav[1], P. Singh[2], M. Bhakat[3]*, T. K. Mohanty[4], A. Kumar[5], A. Singh[6], S. Mondal[7], V. K. Upadhyay[8] and S. Tomar[9]

[1&8]Department of LPM, Post Graduate Institute of Veterinary Education and Research, Jaipur-302031, INDIA
[2]LPM Section, ICAR-National Dairy Research Institute, Karnal-132001, Haryana, INDIA
[3]ABRC, ICAR-National Dairy Research Institute, Karnal-132001, Haryana, INDIA
[4]ABRC, ICAR-National Dairy Research Institute, Karnal-132001, Haryana, INDIA
[5]Department of VGO, GADVASU, Ludhiana-141004, Punjab, INDIA
[6&7]LPM Section, ICAR-National Dairy Research Institute, Karnal-132001, Haryana, INDIA
[9]Veterinary Officer, Frozen Semen Station, Yamunanagar, Haryana, INDIA

***Corresponding author:** bhakat.mukesh@gmail.com

Rec. Date:	Jan 16, 2018 13:55
Accept Date:	Mar 16, 2018 17:48
DOI	10.5455/ijlr.20180116015543

Abstract

The present study was undertaken to understand the role of age, body condition score and rump fat thickness on quality semen production of Murrah bulls. One hundred thirty Murrah buffalo breeding bulls which were in regular semen collection were selected from five different semen stations of Haryana and Punjab. Age of bull was recorded by calculating the days between date of birth to day of experiment. Body condition score was recorded by visual observation and palpation of seven skeletal check points on 1-5 scale. Rump fat thickness was measured in 38 bulls by ultrasonography. Semen quality attributes viz. ejaculate volume (EV, ml), mass motility (MM, 0-5), sperm concentration (SPC, million/ml), sperm motility (SM, %), sperm viability (%) and sperm abnormality (SA, %) of each ejaculate were recorded. The bulls were divided into three groups on the basis of Age; and two on the basis of Body Condition Score (BCS) and Rump Fat Thickness (RFT). Results revealed that EV, MM ($P<0.01$) and SM ($P< 0.05$) increased up to 88 months of age after that there was no significant changes were observed. No significant changes were also observed in semen quality parameters between the two BCS groups. Wherever, between the RFT groups, bulls with thinner RFT produced ejaculates with lower ($P<0.05$) percent sperm abnormalities as compared to bulls with thicker RFT. It can be concluded that the Murrah buffalo bulls up to 7 years of age and thinner rump fat thickness produced better quality semen, therefore these parameters can also be an important criterion while selection of buffalo breeding bulls for quality semen production.

Key words: Age, Body Condition Score, Rump Fat Thickness, Murrah, Semen Quality

How to cite: Yadav, K. S., Singh, P., Bhakat, M., Mohanty, K. T., Kumar, A.,Singh, A., Mondal, S., Upadhyay, V. K. & Tomar, S. (2018). Relationship of Age, Body Condition Score and Rump Fat Thickness with Semen Quality in Murrah Buffalo Breeding Bulls. International Journal of Livestock Research, 8(8), 110-120. doi: 10.5455/ijlr.20180116015543

Introduction

Buffalo is one of the main dairy animals of our country as it contributes maximum milk in the country's milk pool. Buffalo population is increasing day by day and it contributes about 54.4% of total world buffalo population (FAO, 2015). Out of total buffalo population 43.37% are non-descript in the country (DADF, 2015). Therefore, huge scope is there to upgrade the nondescript buffaloes using superior germplasm to improve the productivity of these types of animals to fulfill the growing demand of milk. The superior quality of high genetic potential male germplasm can play a significant role. A technique artificial insemination has played an important role for improvement in productivity through use of cryopreserved semen of superior bulls. Importance of artificial insemination in buffaloes increasing at faster rate, as a result demand of buffalo bull semen is also increasing. It is essential that maximum number of females be served with the quality frozen semen of superior genetic constitution (Singh and Pant, 2000). Therefore, AI with quality frozen semen from genetically superior bulls is the only way through which a sizable population can be covered and genetically improved. Adoption of artificial insemination in buffaloes has increased in last two decades due to significant remarkable progress in cryopreservation of buffalo bull semen. But not enough work has been carried out on breeding bull management practices and also standardization of bull selection criteria. Therefore, there is need to standardize the management and selection criteria for augmenting the reproductive efficiency of buffalo breeding bulls. Rump fat thickness and body condition scoring are the emerging techniques which determine the deposition of fat in the body of bull that reflects the semen production performance and their quality. However, scanty information is available regarding relationship of body condition scoring (BCS) and rump fat thickness with semen production performance of Murrah buffalo bulls. Therefore, the present study was undertaken to determine the relationship of age, body condition score and rump fat thickness with semen quality in Murrah buffalo breeding bulls.

Materials and Methods

The present study was conducted on 130 Murrah buffalo breeding bulls in winter season (November-February), maintained at different institutes of Haryana and Punjab *viz*. Artificial Breeding Research Centre (ABRC), ICAR-National Dairy Research Institute (NDRI), Karnal, Haryana; ICAR-Central Institute for Research on Buffalo (CIRB), Hisar, Haryana; Frozen Semen Stations of Haryana Livestock Development Board (HLDB) situated at Jagadhri and Hisar of Haryana and Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India. The bulls were kept in individual bull pens. The pens were

separated by cemented wall to prevent direct contact of the bull from adjacent pens. One third of the area of pen was covered with corrugated asbestos sheets and two third was open. The bulls had free access to fresh drinking water throughout the day with continuous supply of *ad lib* drinking water. All the Bulls were fed according to standard feeding schedule along with *ad lib* seasonally available green fodder. The shed was cleaned once daily in the morning. The bulls were made to exercise, the day prior to semen collection in the bull exerciser. Vaccination, deworming and other herd-health programme were followed as per the standard schedule. The age of bulls was determined by calculating the number of months on the day of experiment from the date of birth.

Body Condition Scoring

To assess the nutritional status or body condition of bull, a score chart was establish by visual observation and palpation of seven skeletal check points. These seven check points were identified which includes spinous processes of lumbar vertebrae, transverse processes of lumbar vertebrae, space between 12^{th} and 13^{th} ribs, sacral crest, shape between sacral crest and hooks, hooks and pins and tail head region; and observed the prominences, sharpness, flesh covering in between 12^{th} and 13^{th} ribs and prominence of depression between backbone and pins of tail head region by palpation and visual observation (Fig. 3 and 4). Based on the amount of fat reserves the bulls were scored in a 1 to 5 scale. A score of 1 indicates emaciated, 2 indicates thin, 3 indicates average, 4 indicates fat and 5 indicates obese condition. Considering the above skeletal check points, Anitha *et al*. (2011) formulated a score chart for she buffaloes, which was adopted with some modifications in the present study. The modifications are-

1. Showed 7-skeletal check points, in place of 8-skeletal check points.
2. There was not considered 0.5, 1.5, 2.5, 3.5 and 4.5 body score in the score chart during scoring.

During experiments the body condition of bull was scored two times at one week interval on each five bull stations and then the average value were taken for the study.

Rump Fat Thickness

The diameter of rump fat thickness was measured by ultrasonography (KAIXIN KX 2600, Xuzhou Kaixin Electronic Instrument Co. Ltd. China) of 38 bulls at ABRC, ICAR-NDRI, Karnal; ICAR-CIRB, Hisar and GADVASU, Ludhiana. The ultrasonographic probe of 6.5 MHz was used, placed longitudinally and slightly dorsal to the centre point between hook and pin bone and recorded the thickness of fat on rump in mm, as reported by Singh *et al*. (2015) (Fig. 1 and 2).

 DOI 10.5455/ijlr.20180116015543

Fig.1

Fig. 2

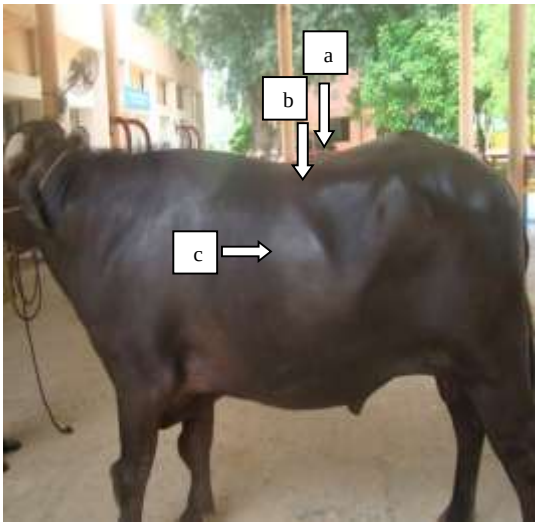

Fig.3

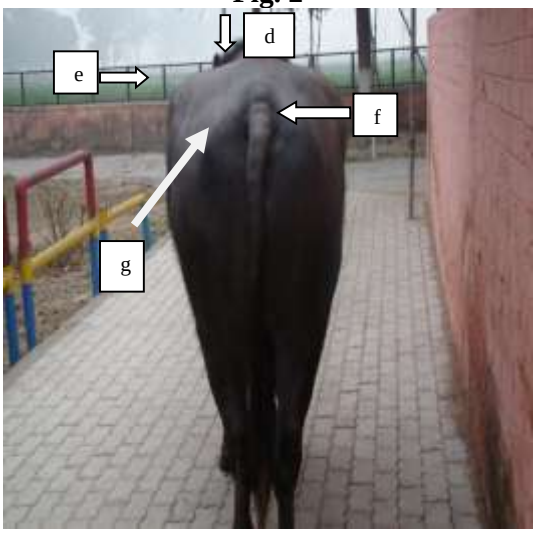

Fig.4

Fig. 1: Point on rump region at which ultrasonographic linear probe placed to record thickness of rump fat, **Fig. 2:** Ultrasonographic image showing thickness of rump fat, **Fig. 3 and 4:** Showing seven skeletal check points for scoring body condition of bull, a. Spinous processes of lumbar vertebrae b. Transverse processes of lumbar vertebrae c. Space between 12^{th} and 13^{th} ribs d. Sacral crest e. Hook bone f. Pin bone g. Tail head region.

Semen Collection and Their Quality Evaluation

Semen was collected twice a week in artificial vagina with smooth neoprene liner (IMV-005331) and artificial vagina was kept in incubator at 45-48°C till use. At least 30 minutes before semen collection the bulls were thoroughly washed, cleaned and dried in early morning. The temperature of AV was maintained at 42°C during semen collection with sufficient pressure and proper lubrication with sterilized KY jelly (Johnson & Johnson) for proper thrust and ejaculatory reflex of bull. Immediately after collection, each

DOI 10.5455/ijlr.20180116015543

ejaculates was placed in a water bath at 32°C and various standard laboratory tests of semen were performed. The quality of fresh semen was assessed in terms of ejaculate volume (ml), mass motility (0-5 scale) and sperm motility (%) (Kiani *et al*., 2014); sperm concentration (million/ml) (Improved neubauers chamber and IMV photometer); sperm viability (%) and sperm abnormality (%) (eosin-nigrosine staining) by using phase contrast microscope equipped with a heating stage (37°C) as per standard procedure. The different other seminal attributes *viz*. sperm concentration per ejaculates (SPCE), total motile sperms per ejaculates (TMSE) and total live sperms per ejaculates (TLSE) were also calculated for each bull by using following formula-

$$SPCE = Ejaculate\ volume(ml) \times Sperm\ concentration(\frac{million}{ml})$$

$$TMSE = \frac{\%sperm\ motility\ x\ ejaculate\ volume(ml) x\ sperm\ concentration(\frac{million}{ml})}{100}$$

$$TLSE = \frac{\%sperm\ viability\ x\ ejaculate\ volume\ (ml) x\ sperm\ concentration\ (\frac{million}{ml})}{100}$$

Statistical Analysis

The data with respect to age, BCS, RFT and semen attributes was analyzed by one way ANOVA using SPSS version 20 (statistical analysis software) and the means were compared by Duncan multiple range test. The significance level was set at 95%. The relationship among these parameters was also calculated by Pearson's correlation coefficient (r) using above statistical software.

Results and Discussion

The results of relationship of age, body condition score and rump fat thickness with semen quality of Murrah buffalo breeding bulls were presented in Table 1, 2 & 3 respectively.

Age and Semen Quality

The results revealed that EV, MM ($p<0.01$) and SM ($p<0.05$) improved significantly with increase in age up to 88 months, and there after the parameters did not improved significantly with age (Table 1). The results are in agreement with finding observed by Kiani *et al*. (2014) in Kundhi buffaloes. Similar results were also observed by Javed *et al*. (2000), and Nordin *et al*. (1990) in which the ejaculate volume in adult was found to be higher than young and older bulls. Contrary to our finding, the ejaculate volume did not show any increase with the age in Murrah buffalo bulls reported by various authors (Kanchan and Matharoo, 2015; Kanchan and Singh, 2005). The result showed that ejaculate volume in Murrah buffalo bulls increases

DOI 10.5455/ijlr.20180116015543

up to 7 years of age and after that it does not increase significantly. Similar trend of increase in MM was found in Kundhi buffalo bulls by Kaini *et al*. (2014).

Table 1: Relationship of age and semen quality of Murrah breeding bulls

Parameter	Group 1 (≤60 mo) (47.27±1.10 mo) (N=46)	Group 2 (61-88 mo) (75.49±1.19 mo) (N=51)	Group 3(>89 mo) (108.15±2.67 mo) (N=33)
EV** (ml)	3.20^{A}±0.13	3.73^{B}±0.14	3.80^{B}±0.12
MM**	3.30^{A}±0.09	3.59^{B}±0.05	3.62^{B}±0.06
SPC (m/ml)	1130.76±44.79	1163.35±27.60	1136.05±48.59
SM*(%)	74.69^{a}±1.60	78.31^{b}±0.61	77.97^{b}±0.86
Viability (%)	82.95±1.07	84.76±0.61	84.34±0.74
SA (%)	9.21±0.67	8.34±0.46	8.91±0.79
SPCE*(million)	3609.34^{a}±198.12	4257.85^{b}±154.44	4280.73^{b}±209.00
TMSE**(million)	2767.71^{A}±172.91	3349.69^{B}±129.16	3364.17^{B}±181.00
TLSE*(million)	3034.77^{a}±178.81	3620.35^{b}±137.47	3628.22^{b}±189.24

*Mean values bearing different superscripts A, B, C (P<0.01) or a, b, c (P<0.05) in row differ significantly; EV= Ejaculate volume, MM= Mass motility, SPC= Sperm concentration, SM= Sperm motility, SA= Sperm abnormality, SPCE= Sperm concentration per ejaculate, TMSE= Total motile sperms per ejaculate, TLSE= Total live sperms per ejaculate, **Indicates level of significant at the 0.01 (2-tailed) and * indicates level of significant at 0.05 (2-tailed)*

In buffalo bulls, Saeed (1988) and Younis (1996) were also observed similar to present study that higher (P<0.05) mass activity in adult bulls than young and old bulls. Contrary to the present finding, MM did not show any increase with the increase in age from < 24 mo to > 33 mo in Murrah buffalo bulls (Kanchan and Matharoo, 2015), it may be due to the bulls were of younger age than the bulls of present study. The higher mass activity in adult bulls could be probably due to the presence of higher sperm concentration and lower abnormalities in their ejaculates (Dhami and Kodagali, 1988), because mass motility primarily depends on sperm concentration and their motility.

In accordance to the present findings, Kaini *et al*. (2014) reported that sperm motility increased up to 8 years with the increase in age and thereafter it decreased in Kundhi buffalo bulls, and Kanchan and Matharoo (2015); Sekharam and Rao (1986) reported in Murrah bulls. In similar line Younis *et al*. (1998) reported that young and adult buffalo bulls produced semen with higher sperm motility than the older. Whereas, in contrary to our findings Javed *et al*. (2000) reported that the sperm motility did not show any significant change with the age in Nili Ravi bulls. In present study the SPCE, TMSE and TLSE improved significantly with age up to 88 months, and there after no significant change was observed. Whereas, SPC, viability and SA did not vary significantly with age among the groups but similar increasing trend of SPC and viability; and decreasing trend of SA was observed up to 88 months of age (Table 1). Similar to the present study, trend of SPC with age was obtained in buffalo bulls in India (Kanchan and Matharoo, 2015; Sekharam and Rao, 1986) and in Pakistan (Younis *et al*., 1998). It shows that SPC does not change significantly with the change in the age of bulls. Whereas, in the present study when the SPCE was

 DOI 10.5455/ijlr.20180116015543

calculated, it showed significant variation among the groups. The significant increase in SPCE with age among the groups was due to the increase in EV. The similar trend in increment was also obtained in case of total live sperm and total motile sperm per ejaculate because it depends on ejaculate volume that varies significantly with age of bulls (Table 1). The age of bull is important because a physiological change that occurs with age as bulls grow to sexual maturity which affects the quality semen production. The quality of semen deteriorates after certain age may be due to onset of senility.

Body Condition Score (BCS) and Semen Quality

All the semen quality attributes *viz*. EV, MM, SPC, SM, viability, SA, SPCE, TMSE and TLSE did not show any significant ($p<0.05$) difference between the groups along with BCS (Table 2).

Table 2: Relationship of body condition score and semen quality of Murrah breeding bulls

Parameter	Group 1 (≤3.5) (3.32±0.02) (N=63)	Group 2 (>3.5) (3.81±0.02) (N=67)
EV (ml)	3.52±0.12	3.60±0.11
MM	3.50±0.05	3.49±0.07
SPC (m/ml)	1150.09±32.41	1139.99±31.94
SM (%)	77.21±0.61	76.69±1.16
Viability (%)	83.76±0.56	84.25±0.79
SA (%)	8.30±0.54	9.25±0.48
SPCE (million)	3981.71±147.20	4083.53±162.03
TMSE (million)	3093.65±123.20	3198.00±142.39
TLSE (million)	3349.26±130.26	3477.09±147.11

EV= Ejaculate volume, MM= Mass motility, SPC= Sperm concentration, SM= Sperm motility, SA= Sperm abnormality, SPCE= Sperm concentration per ejaculate, TMSE= Total motile sperms per ejaculate, TLSE= Total live sperms per ejaculate.

In the present study the BCS of bulls in both the groups fallen with the normal range (between 3 - 4), therefore, no significant variation in any of the semen parameters between the groups was observed as it was evident from the previous studies (Hossain *et al*., 1990; Sarder, 2008) that non-significant association between body condition (average, good, very good and excellent) of breeding bulls and sperm abnormalities was obtained. It was also observed that abnormalities of the spermatozoa could occur due to disorder of the semniferous tubules or germinal epithelium (Hossain *et al*., 1990). Body condition had significant ($P < 0.05-0.01$) effect on qualitative and quantitative traits of bull's sperm (Beran *et al*., 2011). In previous study on rams Maurya *et al*. (2010) observed that reproductive efficiency was better of moderate BCS rams (3.0 and 3.5) than lower (2.5) and higher BCS (4.0). They also observed that semen volume, mass motility and progressive sperm motility were significantly ($p<0.05$) higher in moderate group and lowest in lower group of BCS. However, the sperm concentration did not differ significantly between the groups. The variation in the results may be due to breed variation.

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Rump Fat Thickness (RFT) and Semen Quality

The results showed that SA decreased significantly ($p<0.05$) with decrease in RFT. SPC showed decreasing trend with increase in RFT. However, other seminal attributes *viz.* EV, MM, SM, sperm viability, SPCE, TMSE and TLSE did not show any significant variation between groups along with RFT (Table 3).

Table 3: Relationship of Rump fat thickness and semen quality of Murrah breeding bulls

Parameters	Group 1 (4.5-7.8 mm) (6.13±0.21 mm) (N=18)	Group 2 (7.9-12.6 mm) (9.39±0.27 mm) (N=20)
EV (ml)	2.88±0.27	3.32±0.21
MM	2.97±0.12	3.18±0.12
SPC (m/ml)	1160.50±48.82	983.91±90.83
SM (%)	71.23±2.86	74.09±2.37
Viability (%)	79.26±1.92	83.96±1.68
SA*(%)	7.73^{a}±1.00	11.05^{b}±1.09
SPCE (million)	3279.89±288.78	3203.78±321.59
TMSE (million)	2360.43±224.12	2446.06±270.02
TLSE (million)	2607.59±236.10	2727.72±284.97

*Mean values bearing different superscripts a, b ($P<0.05$), in a row differ significantly; EV= Ejaculate volume, MM= Mass motility, SPC= Sperm concentration, SM= Sperm motility, SA= Sperm abnormality, SPCE= Sperm concentration per ejaculate, TMSE= Total motile sperms per ejaculate, TLSE= Total live sperms per ejaculate, * indicates level of significant at 0.05 (2-tailed)*

Average rump fat thickness was significantly ($P<0.05$) lower in high libido bulls as compared to low libido Murrah buffalo bulls (Singh *et al*., 2015). Increased rump fat thickness in poor libido bulls lead to increase in conversion of testosterone to estrogen (Ellem and Risbridger, 2010); testosterone and estrogen are negatively correlated (Javed *et al*., 2000).Testosterone is responsible for supporting spermatogenesis and essential for male fertility and the maintenance of spermatogenesis. Males become infertile in absence of testosterone because spermatogenesis does not proceed beyond the meiosis stage (Walker, 2011). This is further proved that high libido bulls produced more ejaculates, had lesser reaction time and refractory period as compared to low libido bulls (Bury *et al*., 2011) and better fertility (Coulter and Kozub, 1989). In the present study also group of bulls with lower RFT produced lesser percentage of abnormal sperm than the group of bulls with higher RFT. Generally fatty tissues are deposited in all parts of body. Along with increase in fat thickness of rump region, thickness of scrotum was also increased due to deposition of fatty tissues. It was evident that Rump fat thickness was positively correlated with testicular covering thickness (Yadav *et al*., 2017). Increase fat deposition in scrotum acts as insulator of testicular heat exchange. Due to improper testicular heat exchange normal thermoregulatory process of testis was disturbed and the quality of semen was deteriorated.

DOI 10.5455/ijlr.20180116015543

Body Condition Score and Rump Fat Thickness

The results revealed that a significant ($p<0.01$) positive correlation ($r=0.604$) between BCS and RFT was obtained in present study. Similar to this study, Schroder and Staufenbiel (2006) also observed that the relationship between back fat thickness (BFT) and total body fat (TBF) content was highly significant and it showed that a change in BFT of 1 mm equates to approximately 5 kg of TBF content. Since, scanty/no information was available on these parameters in breeding bulls in general and buffalo in particular, hence the values obtained in the present study could not be compared. However, it was a novel approach to correlate the BCS and RFT in buffalo bulls.

Conclusion

It can be concluded that buffalo breeding bulls having less fat deposition on rump region and up to seven years of age produced better quality semen. Body condition score within normal range is an indicator of health status of the animal for quality semen production. Therefore, RFT along with age can be used as important criterion while selecting breeding bulls for quality semen production.

Acknowledgements

The authors are thankful to Head LPM Section and In-charge Artificial Breeding Research Centre of NDRI, Karnal for providing necessary information and facilities. The authors are also thankful to the In-charge officers of different frozen semen stations *viz.* ICAR-CIRB, GADVASU and HLDB Hisar and Yamunanagar for allowing us to utilize the data of their breeding bulls. We are highly grateful to Director ICAR-NDRI, Karnal, Haryana, (India) for providing necessary facilities and financial support for carrying out this present work.

Conflict of Interest

The authors declare that they don't have any conflict of interest.

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