

## EFFECT OF FEED ENERGY LEVELS ON SEMEN QUALITY AND FREEZABILITY OF YOUNG MURRAH BUFFALO BULLS

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### ABSTRACT

Effect of feed energy levels on semen quality and freezability of Murrah buffalo young bulls was investigated. Eighteen bulls (age ~24 mo and bwt ~428 kg) were randomly allotted to three groups of six in each. Group 1 was kept as control and bulls in this group were fed as per NRC (2001) recommendations, while feed of Group 2 and Group 3 bulls was same as in control and additional 10% and 20% higher energy was provided through molasses to bulls of Group 2 and Group 3, respectively. Monthly feed intake of every bull was recorded. Semen was collected using AV technique at weekly interval; reaction time of bulls was recorded at collection. Ejaculate volume (ml), mass motility (0 to 5 scale), individual sperm motility (%), sperm concentration (m/ml); percent viability, acrosome integrity and morphological abnormality in fresh semen and post thaw sperm motility, viability and acrosome integrity in frozen semen were evaluated with microscopic methods. Results showed that the DM intake was higher ( $P<0.01$ ) in Group 3 than Group 2 and control. Reaction time (second) was the lowest ( $P<0.01$ ) in Group 2 followed by Group 1 and Group 3. Sperm concentration (m/ml) and mass motility was found to be higher ( $P<0.05$ ) in Group 2 than Group 3 and

control. Similar trend ( $P<0.05$ ) of percent sperm motility, viability and acrosome integrity among the groups was observed. Whereas, significantly ( $P<0.05$ ) higher percent sperm abnormality was obtained in Group 3 followed by Group 1 and Group 2. Post-thaw percent sperm motility, viability and acrosome integrity were significantly higher ( $P<0.05$ ) in Group 2 than control and Group 3. From this study it can be concluded that 10% higher energy provided to the bulls in Group 2 than the control improved quality significantly of fresh and frozen semen in comparison to the control and other treatment group.

**Keywords:** buffaloes, *Bubalus bubalis*, cryopreservation, feed energy, Murrah buffalo bulls, semen quality, reaction time

### INTRODUCTION

Buffalo is main dairy animal in India. For enhancing its productivity further, superior quality male germplasm of high genetic potential can play a significant role. However, this is a matter of great concern the availability of sufficient quality buffalo male germplasm to achieve the desired progress. Artificial insemination is an important

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tool for the improvement of milk productivity in buffalo by the multiplication of animal with high genetic potential (Baruselli and Carvalho, 2005). In recent decade, in view of high demand of buffalo germplasm in the country, emphasis for conservation and multiplication of superior Murrah buffalo germplasm has increased (Singh, 2009). In general, buffalo bulls start semen production at about 24.9 to 34.0 months of age (Ahmad *et al.*, 1984; Sethi, 1999). However, poor libido and reduced semen quality are major causes of poor reproductive performance of young bulls and availability of buffalo germplasm. Therefore, to obtain quality frozen semen of young bulls is desirable to increase AI coverage and early sire evaluation. Energy is the major dietary element which is responsible for the utilization of other nutrients and in turn it enhances the growth and productivity of animal (Hosseini *et al.*, 2008). High level of dietary energy intake influenced reproductive performance of young bulls through a direct effect on the age at puberty and growth of scrotal circumference (Pruitt and Corah, 1985). Bull's diet deficient in protein and energy generally result in extreme weight loss which may adversely affect the libido and semen quality (Mecham *et al.*, 1963). Feeding of high energy in diet to young bulls increased body growth and testicular development (Barth *et al.*, 2008). The quality semen produced by individual bulls is the product of the bull inherent genetic makeup, less any detrimental influence induced by the environment in which the bull was reared and maintained (Coulter, 1994). Therefore, the optimum energy level for growth and sexual maturity of young buffalo male needs to be precisely understood, so that male attains puberty in time and starts production of quality semen at an early age.

## MATERIALS AND METHODS

Eighteen Murrah buffalo bulls (age ~24 mo and bwt ~428 kg) selected for the study and kept at Artificial Breeding Research Centre of National Dairy Research Institute, Karnal. They were randomly allotted to three groups of six bulls in each based on their body weight and age. Group 1 was kept as control and fed as per NRC (2001) recommendations while in Group 2 and Group 3 were fed same as in control plus 10 and 20% higher energy supplied through molasses. The ration containing concentrate mixture, green fodder (Berseem and Oats) and dry roughage (wheat straw) was supplied (DM basis) throughout the trial of five months. Dry matter intake was calculated at monthly interval by weighing offered and residual quantity of concentrate mixture, green fodder and roughages on two consecutive days and then DM intake was determined for whole month. Blood samples were collected by puncturing of jugular vein in vacutainer tube with heparin (20 IU/ml blood) of all the bulls at monthly interval for estimation of testosterone. The plasma was separated within one hour after sampling following centrifugation (3000 rpm for 20 minutes) at 4°C and stored at -20°C till the estimation of testosterone. Testosterone concentration was estimated with the Bovine Testosterone ELISA kit. For semen collection, bulls were thoroughly washed, cleaned, and dried before making semen collection in early morning. Semen was collected at weekly interval (single ejaculate) by using bovine Danish model artificial vagina (IMV model-005417) (41 to 42°C) over a dummy bull. Reaction time in seconds was recorded with the help of stop watch at the time of semen collection as described by Anzar *et al.* (1993) for buffalo bulls. Immediately after collection each ejaculate was placed in a water

bath (30°C) and standard laboratory tests for semen evaluation were performed. Quality of fresh semen ejaculates was assessed for ejaculate volume (ml), sperm concentration (m/ml) (Haemocytometer, Improved Neubauer's method), mass motility (0 to 5 scale), and individual motility (%) by using phase contrast microscope (Nikon Eclipse E600, Tokyo, Japan) with a heating stage. Live and dead sperm counts in semen sample were assessed by Eosin-Nigrosine modified method. Assessment of intact acrosome proportion of sperm in semen sample was performed by staining as described by Hancock (1952). Semen was processed for cryopreservation as per the standard protocol followed for buffalo semen processing and cryopreservation at the laboratory of Artificial Breeding Research Centre. Assessment of post thaw sperm motility was done by putting aliquot of 10 µl of thawed semen on a cleaned warmed slide and then covered it with a warmed cover slip. Sperm motility then was assessed under 200x magnification of a phase contrast microscope on a heating stage (37°C). Post-thaw sperm viability of every semen sample was determined using Eosin-Nigrosin stain and acrosome integrity of post-thaw spermatozoa was determined by Giemsa staining technique procedure as explained above under initial evaluation of semen.

### Statistical analysis

The data obtained during this study was analyzed using ANOVA as described by Snedecor and Cochran (1967) and Tukey's Studentized Range (HSD) between feed groups and compared means using DMRT using SAS (9.3) package at computer centre the institute.

## RESULTS AND DISCUSSION

### Effect of feed energy levels on dry matter intake (dmi)

The mean DM intake (kg/100 kg bwt/day) was 2.16±0.02, 2.23±0.02, 2.29±0.20 in Group 1, Group 2 and Group 3, respectively, it was significantly ( $P<0.01$ ) different among the groups (Table 1). Higher DM was found to be in higher feed energy groups (Group 3) this might be due to increased palatability of the feed with higher energy levels (Puri *et al.*, 2004; Tovar-Luna *et al.*, 2011). The lower DMI was obtained in Group 1 among the groups and it might be explained in the light of the fact that diet low in energy level reduced DMI because of their slow clearance from the rumen and passage through the digestive tract (Allen, 1997). These results are also in agreement with those obtained by Girdher *et al.* (2008) in Frieswal and Swanepoel *et al.* (2008) in Bonsmara and Patil (2013) in Murrah buffalo bull calves they found that the dry matter intake was significantly increased by increasing energy level in the ration. In the present study also the higher DM intake was recorded which could be again due to the increased palatability of the ration through the supplementation of molasses in the diets of the bulls of higher energy groups.

### Effect of feed energy levels on testosterone profile

Testosterone concentration (ng/ml) was 1.29±0.32, 1.46±0.28 and 1.81±0.34 in Group 1, Group 2 and Group 3, respectively (Table 1). It showed significant ( $P<0.05$ ) difference among the groups and higher value was obtained in Group 3 (1.81±0.34) followed by Group 2 (1.46±0.28) and Group 1 (1.29±0.32). High nutrition has effects

on hypothalamus-pituitary-testis axis through the influence on GnRH and LH pulse generator and direct effect on leydig cells in turn it increases IGF-1 production which then increases proliferation and differentiation of leydig cells then testosterone production increases (Cailleau *et al.*, 1990). Additional nutrients intake also alters leydig cells function directly to increase serum testosterone production (Nolan *et al.*, 1990). In the present study also significantly higher concentration of testosterone was found in the higher feed energy group (20% HE) than other two groups. The above results are in accordance with the findings (Brito *et al.*, 2007a, c; Selvaraju *et al.*, 2012; Shahzad *et al.*, 2013); they found that bulls fed on high energy diets had higher level testosterone.

#### **Effect of feed energy levels on reaction time**

The reaction time (second) was  $80.13 \pm 13.26$ ,  $62.02 \pm 9.96$  and  $118.90 \pm 21.06$  in Group 1, Group 2 and Group 3, respectively (Table 1). It showed a significant ( $P < 0.01$ ) difference among the groups. Group 2 bulls showed the lowest reaction time ( $62.02 \pm 9.96$ ) where as Group 1 ( $80.13 \pm 13.26$ ) and Group 3 ( $118.90 \pm 21.06$ ) showed the higher reaction time. High feed energy diet in adult bulls tends to increase fat deposition which makes bull lethargic and may reduce sex drive (Girdher *et al.*, 2008). The above results are also in accordance with the findings of Morrow *et al.* (1981) and Pruitt *et al.* (1986), where higher dietary energy was found to reduce the reaction time. However, Chase *et al.* (1993) and Mandal *et al.* (2008) found that there was no effect of feed energy on reaction time or sexual behaviour in cross bred bulls. In the present findings, though no set trend of reaction time with dietary energy levels was obtained, however, shorter reaction time was recorded in the medium energy group (10% HE)

than the higher (20% HE) and control. Therefore, it could be due to species differences as libido is mainly regulated by the genetics. As this trait has high heritability ( $h = 0.6$ ), hence there is little scope of effect of total environmental factors in regulation of reaction time, including nutrition.

#### **Effect of feed energy levels on fresh semen quality:**

##### **Ejaculate volume**

The ejaculate volume (ml) was  $2.02 \pm 0.20$ ,  $2.25 \pm 0.20$  and  $2.57 \pm 0.22$  in Group 1, Group 2 and Group 3, respectively. Ejaculate volume showed no significant difference among the groups, however, higher volume was obtained in Group 3 as compared to other groups (Table 2). High energy diet might have increased the scrotal size, number of secretary tissue and testosterone production which then might have enhanced the ejaculate volume. Similar findings were also reported by Mandal *et al.* (2008); Girdhar *et al.* (2008); Azizunnesa-Zohara *et al.* (2013) and Shahzad *et al.* (2013) where increased ejaculate volume was recorded. However there are contrary reports to the present findings, Lunstra and Coulter (1997) and Swanepoel *et al.* (2008) have found that feeding of high energy diet reduced the ejaculate volume.

##### **Mass motility**

Mean value of mass motility was  $2.13 \pm 0.20$ ,  $3.01 \pm 0.06$  and  $2.22 \pm 0.13$  in Group 1, Group 2 and Group 3, respectively (Table 2). It showed a significant ( $P < 0.05$ ) difference among the groups and higher value was found to be in Group 2 as compared to other groups. The above results are in accordance with the findings by Fourie *et al.* (2004) in ram; Swanepoel *et al.* (2008) in beef bulls. In contrary to these results, findings of

Mandal *et al.* (2008) and Azizunnesa *et al.* (2013) have reported increased in the mass motility. The mass motility, in the present findings, was found to be significantly higher in the 10% HE group than the 20% HE and control. This indicates that slightly higher feed energy than the control (NRC, 2001) has worked better and enhanced the semen quality in terms of mass motility in buffalo bulls. It could be because of the combined effect of lower scrotal size, higher IGF-1 and testosterone profiles in bulls of Group 2. Small size scrotum might have had better thermoregulation, and higher IGF and testosterone concentrations might have provided conducive milieu to sperms in the testis and hence Group 2 bulls gave significantly higher mass motility than other two groups.

### Sperm concentration

The sperm concentration (m/ml) (Mean $\pm$ SE) was 895.61 $\pm$ 73.0, 1165.27 $\pm$ 46.70 and 935.18 $\pm$ 51.20 in Group 1, Group 2 and Group 3, respectively (Table 2). This showed significant ( $P<0.05$ ) difference among the groups and higher value was obtained in Group 2 as compared to other groups. Sperm concentration (m/ml) obtained in this study was in the range as reported by different workers in Murrah buffalo bulls (Shukla and Mishra, 2005). The bulls of Group 2, medium energy (10%), produced higher sperm concentration than the higher energy (20%) and the control. From this, it appeared that feeding of higher energy did not give any benefit in terms of sperm concentration; however, the high energy group (Group 2) could produce higher sperms per ejaculate. Effect of feeding of high energy diets to increase the concentration of spermatozoa have been reported in beef bulls (Rekwot *et al.*, 1997), dairy bulls (Mandal *et al.*, 2008) and buffalo bulls (Shahzad *et al.*, 2013). However, Seidel *et al.*

(1980) and Bester *et al.* (2004) have not found any significant effect of change in feed energy levels on sperm concentration in bulls. It has been found that feeding of high energy diet increases the deposition of fat in the neck of scrotum which impairs the thermoregulation and increased the scrotal temperature then reduced the sperm concentration (Lunstra and Coulter, 1997).

### Sperm motility

Percent sperm motility in fresh semen was 46.36 $\pm$ 4.66, 66.84 $\pm$ 1.52 and 47.86 $\pm$ 3.09 in Group 1, Group 2 and Group 3, respectively (Table 2). Sperm motility showed significant ( $P<0.05$ ) difference among the groups and it was higher (66.84 $\pm$ 1.52) in Group 2 followed by Group 3 (47.86 $\pm$ 3.09) and Group 1 (46.36 $\pm$ 4.66). The percent sperm motility obtained across the groups in the present study was similar to that reported by several workers (Mandal, 1998; Pandey, 2001; Shivahare, 2013) in Murrah buffalo bulls. Bulls in Group 2 showed significantly higher sperm motility as compared to the other two groups; the positive effect of higher energy in this group reflected in terms of higher mass motility. The possible reason for that has been explained under mass motility that it could be because of the combined effect of lower scrotal size and higher testosterone profiles in bulls of Group 2. In beef bulls increased dietary energy shown to enhance scrotal fat accumulation in scrotum neck and skin which affects the scrotal thermoregulation that increases the scrotal temperature and decreases the sperm motility (Coulter *et al.*, 1997). Similar finding were also obtained in the previous studies in beef bulls (Swaneopoel *et al.*, 2008), ram (Fourie *et al.*, 2004) and bulls (Girdheret *et al.*, 2008) who have found that feeding of high energy diet reduced the sperm motility. In contrary to these findings, Mandal *et al.* (2008) have obtained highersperm

motility by feeding high energy diet to the bulls.

### **Viability**

The percent value of sperm viability was  $73.97 \pm 2.7$ ,  $81.59 \pm 0.75$  and  $74.92 \pm 1.59$  in Group 1, Group 2 and Group 3, respectively (Table 2). Sperm viability showed significant ( $P < 0.05$ ) difference among the groups and higher value was obtained in Group 2 as compared to other feed groups. Values of sperm viability obtained in the present study in different groups were in accordance with the values reported by earlier researchers in Murrah buffalo bulls (Mandal, 1998; Pandey, 2001). The benefit of higher (10%) feed energy level, explained above, provided to the bulls of Group 2 has not only reflected in higher value of sperm concentration, mass motility and sperm motility in this group but in higher sperm viability also. The present results are also in agreement with the findings of Coulter (1997) and Swaneopel *et al.* (2008) where they have observed higher sperm viability in the bulls fed with medium energy diets.

### **Acrosome integrity**

The percent value of acrosome integrity was  $69.91 \pm 2.92$ ,  $81.26 \pm 0.91$  and  $74.26 \pm 1.77$  in Group 1, Group 2 and Group 3, respectively (Table 2). Acrosome integrity showed significant ( $P < 0.05$ ) difference among the groups and higher value was obtained in Group 2 ( $81.26 \pm 0.91$ ) followed by Group 3 ( $74.26 \pm 1.77$ ) and Group 1 ( $69.91 \pm 2.92$ ). The values of acrosome integrity observed in this study in buffalo sperm are accordance with reported by earlier workers in Murrah buffalo bulls (Pant *et al.*, 2002; Shivahre, 2013). Like other sperm quality parameters (percent sperm motility and viability) sperm acrosome integrity of bulls in the Group 2 found to be significantly higher than other two groups.

### **Morphological sperm abnormalities**

The percent head sperm abnormalities were ( $6.84 \pm 0.48$ ,  $5.25 \pm 0.36$  and  $7.18 \pm 0.50$ ), mid-piece ( $3.78 \pm 0.36$ ,  $3.55 \pm 0.37$  and  $5.03 \pm 0.42$ ) and tail ( $4.73 \pm 0.58$ ,  $3.96 \pm 0.38$  and  $5.27 \pm 0.76$ ) in Group 1, Group 2 and Group 3, respectively. The percent total sperm abnormality was  $15.36 \pm 1.16$ ,  $12.78 \pm 0.83$  and  $17.48 \pm 1.33$  in Group 1, Group 2 and Group 3, respectively. The total morphological sperm abnormalities showed significant ( $P < 0.05$ ) difference among the groups and higher value was obtained in Group 3 ( $17.48 \pm 1.33$ ) followed by Group 1 ( $15.36 \pm 1.16$ ) and Group 2 ( $12.78 \pm 0.83$ ) show in (Table 2). The values of percent sperm abnormality obtained in the this study in different groups is in accordance with the values reported by various researchers (Singh, 1987; Shukla *et al.*, 2005), whereas lower values have reported in Murrah buffalo bulls (Shivahre, 2013). In the present study also the percent value of all types of sperms abnormalities and total abnormality were lesser than the acceptable range in the bulls of Group 2, whereas, in other groups these were slightly higher but they were also with the acceptable range. The higher values of total abnormalities in Group 3 and control could be due the fact that bulls of these groups had higher scrotal size which might have affected thermoregulation of the scrotum required for normal sperm production. Deposition of highly insulative lipids within the scrotal tissue due to high energy diets and might have reduced the radiation of heat from the scrotal surface, impairs the thermoregulation of the testis there by increasing testicular temperature (Coulter and Kozub, 1989). Fat deposits may decrease the capacity for counter current heat exchange within the testicular vascular cone (Cook *et al.*, 1994), limit scrotal thermoregulation and causes substantially more sperm abnormalities (Swaneopel *et al.*,

Table 1. Effect of feed energy levels on dry matter intake, testosterone and reaction time in Murrah buffalo bulls.

<b>Parameters</b>	<b>Group 1 (Control)</b>	<b>Group 2 (10% HE)</b>	<b>Group 3 (20%HE)</b>
DMI <sup>**</sup> (kg/day)	2.16±0.02	2.23±0.02	2.29±0.20
Testosterone* (ng/ml)	1.29±0.32	1.46±0.28	1.81±0.34
Reaction time <sup>**</sup> (sec)	80.13±13.26	62.02±9.96	118.9±21.06

Values with different superscripts in a row differ significantly (\*P<0.05, \*\*P<0.01).

Table 2. Effect of feed energy levelson fresh semen quality of Murrah buffalo bulls.

<b>Parameters</b>	<b>Group 1 (Control)</b>	<b>Group 2 (10% HE)</b>	<b>Group 3 (20%HE)</b>
Ejaculate volume (ml)	2.02±0.2	2.25±0.2	2.57±0.22
Mass motility* (0-5)	2.13±0.23	3.01±0.06	2.23±0.13
Spermconcentration* (million/ml)	895.61±73.0	1165.27±46.70	935.18±51.20
Sperm motility *(%)	46.36±4.66	66.84±1.52	47.86±3.09
Viability* (%)	73.97±2.7	81.59±0.75	74.92±1.59
Acrosomal integrity* (%)	69.91±2.92	81.26±0.91	74.26±1.77
Morphological abnormality* (%)	15.36±1.16	12.78±0.83	17.48±1.33

Values with different superscripts in a row differ significantly (\*P<0.05).

Table 3. Effect of feed energy levelson frozen semen quality of Murrah buffalo bulls.

<b>Parameters</b>	<b>Group 1 (Control)</b>	<b>Group 2 (10% HE)</b>	<b>Group 3 (20%HE)</b>
Post-thaw motility* (%)	46.44±1.74	53.37±1.13	44.55±1.06
Viability* (%)	70.87±1.5	71.55±0.66	67.69±1.3
Acrosome integrity* (%)	70.32±1.6	73.83±0.82	69.90±1.24

Values with different superscripts in a row differ significantly (\*P<0.05).

2008). Similar finding with significantly higher sperm abnormalities on high energy diets in buffalo bulls (Bhosrekaret *et al.*, 1988), in beef bulls (Kedbe *et al.*, 2007; Swanepoel *et al.*, 2008). However, non-significantly higher sperm abnormalities were found on high energy diets fed to the bulls (Barth *et al.*, 2008; Azizunnesa *et al.*, 2013; Shahzad *et al.*, 2013).

### **Effect of feed energy levels on post-thaw sperm motility, viability and acrosome integrity**

The percent post-thaw sperm motility was  $46.44 \pm 1.74$ ,  $53.37 \pm 1.13$  and  $44.55 \pm 1.06$  in Group 1, Group 2 and Group 3, respectively (Table 3). Post-thaw motility showed significant ( $P < 0.05$ ) difference among the groups and higher value was found in Group 2 ( $53.37 \pm 1.13$ ) followed by Group 1 ( $46.44 \pm 1.74$ ) and Group 3 ( $44.55 \pm 1.06$ ). Values of post-thaw sperm motility obtained in the present study in different groups were in accordance with the values reported in Murrah buffalo bulls (Singh *et al.*, 2013; Kumar *et al.*, 2014).

The percent viability was  $70.87 \pm 1.50$ ,  $71.55 \pm 0.66$  and  $67.69 \pm 1.30$  in Group 1, Group 2 and Group 3, respectively (Table 3). Sperm viability showed significant ( $P < 0.05$ ) difference among the groups and higher value was obtained ( $71.55 \pm 0.66$ ) Group 2 followed by Group 1 ( $70.87 \pm 1.50$ ) and in Group 3 ( $67.69 \pm 1.30$ ). Value of sperm viability before freezing was higher so it could maintain it after freezing also. Values of viability obtained in the present study in different groups were in accordance with the values reported in Murrah buffalo bulls (Singh *et al.*, 2013; Kumar *et al.*, 2014).

The percent acrosome integrity was  $70.32 \pm 1.60$ ,  $73.83 \pm 0.82$  and  $69.90 \pm 1.24$  in Group 1, Group 2 and Group 3, respectively (Table 3). Acrosome integrity showed significant ( $P < 0.05$ )

difference among the groups and higher value was obtained ( $73.83 \pm 0.82$ ) in Group 2 followed by ( $70.32 \pm 1.60$ ) Group 1 and in Group 3 ( $69.90 \pm 1.24$ ). Similar finding was also obtained in a study in Murrah buffalo bulls (Nitharwal, 2013). The benefit of higher (10%) feed energy level, in bulls of Group 2, has not only reflected in higher sperm motility, viability and acrosome integrity in fresh semen but also in post-thaw frozen semen. As in general, there is a positive relationship between the qualities of fresh semen sample with the post-thaw quality.

### **CONCLUSION**

From the findings of the present study, it can be concluded that the buffalo bulls provided with higher energy (10%) than the recommended level in their diet, not only produced superior quality fresh semen but also maintained it even after cryopreservation as compared to other groups, thereby suggesting the scope of its incorporation in management of high genetic merit bulls for quality germplasm production.

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### **REFERENCES**

Ahmad, M.L.M., M.Q. Ahmad, N. Sahir and M. Arsian. 1984. Age related changes in body

- weight, scrotal size and plasma testosterone levels in buffalo bulls (*Bubalus bubalis*). *Theriogenology*, **22**(6): 651-656.
- Allen, M.S. 1997. Relation between fermentation acid production and gonadotropins concentrations in the ram. *Anim. Prod.*, **34**: 265-277.
- Anzar, M., M. Ahmad, M. Nazir, N. Ahmad and I.H. Shah. 1993. Selection of buffalo bulls: Sexual behaviour and its relationship to semen production and fertility. *Theriogenology*, **40**: 1187-1198.
- Azizunesa-Zohara, F.B., F.Y. Bari and S.G. Alam. 2013. Effects of concentrate supplementation on reproductive performances and semen quality of indigenous rams in Bangladesh. *J. Emb. Trans.*, **28**(4): 325-335.
- Barth, A.D., L.F.C. Brito and J.P. Kastelic. 2008. The effects of nutrition on sexual development of bulls. *Theriogenology*, **70**: 485-494.
- Baruselli, P.S. and N.A.T. Carvalho. 2005. Biotechnology of reproduction in buffaloes (*Bubalus bubalis*). *Brazilian J. Anim. Breed.*, **29**(2): 4-17.
- Bester, N., L.M.J. Schwallbach, H.J.V. Merwe, J.P.C. Greyling and M.D. Fair. 2004. The influence of dietary concentrations on scrotal, testicular and semen characteristics of young Dorper rams. *S. African J. Anim. Sci.*, **34**(2): 53-55.
- Bhosrekar, M.R., J.R. Purohit, A.B. Pande and B.R. Mangurkar. 1988. Effect of different seasons on frozen semen production in Murrah buffalo bulls. *Indian J. Anim. Sci.*, **58**: 1073-1074.
- Brito, L.F.C., A.D. Barth, N.C. Rawlings, R.E. Wilde, D.H.Jr. Crews, P.S. Mir and J.P. Kastelic. 2007a. Effect of improved nutrition during calfhoo and peri-pubertal period on serum metabolic hormones, gonadotropins, and testosterone concentrations and on sexual development in bulls. *Domest. Anim. Endocrin.*, **33**(1): 1-18.
- Brito, L.F.C., A.D. Barth, N.C. Rawlings, R.E. Wilde, D.H.Jr. Crews, P.S. Mir and J.P. Kastelic. 2007c. Effect of improved nutrition during calfhoo on serum metabolic hormones, gonadotropins and testosterone concentrations and on testicular development in bulls. *Domest. Anim. Endocrin.*, **33**(4): 460-469.
- Cailleau, J., S. Vermeire and G. Verhoeven. 1990. Independent control of the production of insulin-like growth factor-1 and its binding protein by cultured testicular cells. *Mol. Cell Endocrinology*, **69**: 79-89.
- Chase, C.C., R.E. Larsen, A.C. Hammond and R.D. Randel. 1993. Effect of dietary energy on growth and reproductive characteristics of Angus and Senepol bulls during summer in Florida. *Theriogenology*, **40**: 43-63.
- Cook, R.B., G.H. Coulter and J.P. Kastelic. 1994. The testicular vascular cone, scrotal thermoregulation and their relationship to sperm production and seminal quality in beef bulls. *Theriogenology*, **41**: 653-671.
- Coulter, G.H. 1994. Beef bull fertility: Factors affecting seminal quality, p. 307-315. In Michael, J.F. and S.S. Robert (eds.) *Factors Affecting Calf Crop*. CRC Press. Boca Raton London, New York, Washington, D.C., Florida, USA.
- Coulter, G.H. and G.C. Kozub. 1989. Efficacy of methods used to test fertility of beef bulls used for multiple-sire breeding under range conditions. *J. Anim. Sci.*, **67**: 1757-1766.
- Coulter, G.H., R.B. Cook and J.P. Kastelic. 1997.

- Effects of dietary energy on scrotal surface temperature, seminal quality and sperm production in young beef bulls. *J. Anim. Sci.*, **75**: 1048-1052.
- Fourie, P.J., L.M. Schwalbach, E.W.C. Naser and C.V. Westhuizen. 2004. Scrotal, testicular and semen characteristics of young dorper rams managed under intensive and extensive conditions. *Small Rumin. Res.*, **54**: 53-59.
- Girdher, N., R. Prasad, M. Kumar and K. Singh. 2008. Effect of different planes of nutrition and mineral supplementation on nutrient utilization and semen quality of Frieswal bulls. *Indian J. Anim. Sci.*, **78**(7): 764-768.
- Hancock, J.L. 1952. The morphology of bull spermatozoa. *J. Exp. Biology*, **29**: 445-453.
- Hosseini, S.M., S.M. Akbary, N. Maherisis and A.M. Aghsaghali. 2008. Effect of different energy levels of diet on feed efficiency, growth rate and carcass characteristics of fattening lambs. *Iranian J. Anim. Vet. Adv.*, **7**: 1551-1554.
- Kebede, M., J.P.C. Greyling and L.M.J. Schwalbach. 2007. Effect of season and supplementation on percent live sperm and sperm abnormalities in Horro (Zebu) bulls in sub-humid environment in Ethiopia. *Tropical Animal Health and Production*, **39**(2): 149-154.
- Kumar, P., D. Kumar, P. Singh, P.S. Yadav and S.K. Sarkar. 2014. Characteristics of frozen thawed semen in predicting the fertility of buffalo bulls. *Indian J. Anim. Sci.*, **84**: 389-392.
- Lunstra, D.D. and G.H. Coulter. 1997. Relationship between infrared scrotal temperature pattern and natural mating fertility in beef bulls. *J. Anim. Sci.*, **75**: 767-774.
- Mandal, D.K., K. Singh and S. Tyagi. 2008. Effect of plane of nutrition on body growth, testicular characteristics and sexual behaviour of young Frieswal bulls. *J. Remount Vet. Corps.*, **47**: 227-242.
- Mandal, D.K. 1998. *Effect of surface cooling on reproductive performance of Murrah buffalo bulls*. Ph.D. Thesis, National Dairy Research Institute, Deemed University, Karnal, India.
- Meacham, T.N., T.J. Cunha, A.C. Warnick, J.F. Hentges and D.D. Hargrove. 1963. Influence of low protein rations on growth and semen characteristics of young beef bull. *J. Anim. Sci.*, **22**: 115-120.
- Morrow, R.E., R.G. Elmore and A.L. Brooks. 1981. Growth and reproductive development of beef bulls tested on two levels of energy. *J. Anim. Sci.*, **53**: 188-204.
- Nitharwal, K. 2013. *Post thaw sperm functions of Murrah buffalo bull semen frozen during various seasons*. M.V.Sc. Thesis, National Dairy Research Institute, Deemed University, Karnal, Haryana, India.
- Nolan, C.J., D.A. Necendorf and R.W. Godfrey. 1990. Influence of dietary energy intake on prepubertal development of Brahman bulls. *J. Anim. Sci.*, **68**: 1087-1096.
- NRC. 2001. *Nutrient Requirements of Dairy Cattle*, 7<sup>th</sup> ed. National Dairy Research Institute.
- Pandey, A.K. 2001. *Effect of blood serum and caffeine on cryopreservation of buffalo spermatozoa*. M.V.Sc. Thesis, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, India.
- Pant, H.C., L.R. Barot, R. Kabiraj, A.K. Mishra and J.H. Prabhakar. 2002. Acrosomal integrity of buffalo spermatozoa and its relationship to fertility. *Indian J. Anim. Reprod.*, **23**(2): 110-112.

- Patil, L.N. 2013. *Effect of feed energy level on nutrient utilisation and growth in buffalo male calves*. M.V.Sc. Thesis, National Dairy Research Institute, Deemed University, Karnal, Haryana, India.
- Pruitt, R.J. and L.R. Corah. 1985. Effect of energy intake after weaning on the sexual development of beef bulls. Semen characteristics and serving capacity. *J. Anim. Sci.*, **61**: 1186-1193.
- Pruitt, R.J., L.R. Corah and J.S. Stevenson. 1986. Effect of energy intake after weaning on the sexual development of beef bulls. II Age of first mating, age at puberty, testosterone and scrotal circumference. *J. Anim. Sci.*, **63**: 579-585.
- Puri, J.P., S.S. Grewal, S. Sindhu and M.K. Rose. 2004. Effect of feeding of protected protein supplemented with urea treated straw on performance of buffalo calves. *Indian J. Anim. Sci.*, **74**: 319-320.
- Rekwot, P.I., E.O. Oyedipe, P.M. Dawuda and V.O. Sekoni. 1997. Age and hourly related changes of serum testosterone and spermogram of prepubertal bulls fed two levels of nutrition. *Vet. J.*, **153**: 341-347.
- Seidel, G.E., B.W. Jr. Pickett, C.O. Wilsey and S.M. Seidel. 1980. Effect of high level of nutrition on reproductive characteristics of Angus bulls. In *Proceeding of the 9<sup>th</sup> International Congress on Animal Reproduction and Artificial Insemination*, Madrid, Spain.
- Selvaraju, S., T. Sivasubramani, B.S. Raghavendra, P. Raju, S.B.N. Rao, D. Kumar and J.P. Ravindra. 2012. Effect of dietary energy on seminal plasma insulin-like growth factor-1 (IGF-1), serum IGF-1 and testosterone levels, semen quality and fertility in adult rams. *Theriogenology*, **78**(3): 646-655.
- Sethi, R.K. 1999. Breeding for higher genetic improvement. *National Symposium, Animal Production in Next Millennium*, CIFMA, Bangalore, India.
- Shahzad, A., M. Sajjad, N. Ullah, A. Shamim, I. Khan and M. Khan. 2013. Serum testosterone level, scrotal circumference and semen characteristics in Nili-Ravi buffalo bulls fed two levels of feed. *International Livestock Nutrition Conference*, Pakistan.
- Shivahre, P.R. 2013. *Disposal pattern analysis and evaluation of Murrah buffalo males on the basis of conventional semen evaluation and in vitro fertility tests*. M.V.Sc. Thesis, National Dairy Research Institute, Karnal, Haryana, India.
- Shukla, M.S. and A.K. Mishra. 2005. Correlation between seminal characteristics in Murrah bulls. *Indian J. Anim. Sci.*, **75**(3): 263-266.
- Shukla, M.K., A.K. Mishra and H.P. Gupta. 2005. Studies on the biochemical constituents of Murrah buffalo seminal plasma, p. 23. *Souvenir 21<sup>st</sup> Annual Convention of ISSAR and National Symposium*.
- Singh, I. 2009. Buffalo reproduction an Indian perspective. *Rev. Bras. Repro. Anim. Sup. Belo Horizonte*, **6**: 91-97.
- Singh, N.P. 1987. *Studies on cryopreservation of buffalo semen in relation to various extenders and initial freezing temperature*. M.Sc. Thesis, National Dairy Research Institute, Karnal, Haryana, India.
- Singh, P., D. Kumar, P. Kumar, I. Singh and P.S. Yadav. 2013. Cryopreservation and quality assessment of buffalo bull semen collected from farmer's doorstep. *Agric. Res.*, **2**(2): 148-152.
- Snedecor, G.W. and Cochran, W.G. 1967. *Statistical Methods*. Iowa State University

Press, Ames, USA.

Swanepoel, F.J.C., G.J. Taylor, E.C. Webb and A. Stroebel. 2008. Effect of nutrition on testicular traits of tropically adapted yearling beef bulls. *Aust. J. Exp. Agr.*, **48**: 950-953.

Tovar-Luna, I., R. Puchala, T. Sahlu, H.C. Freetly and A.L. Goetsch. 2011. Effect of level of feeding on energy utilisation by Angora goat. *J. Anim. Sci.*, **89**(1): 142-149.