International Journal of Livestock Research eISSN : 2277-1964 NAAS Score -5.36 Vol 9 (11) Nov '19

Original Research

Analysis of Sperm Morphometric Traits and Estimation of Genetic Distance in Cattle

Sandeep Kumar Barik, Gangadhar Nayak and Chinmoy Mishra*

Department of Animal Breeding and Genetics, College of Veterinary Science and Animal
Husbandry, Orissa University of Agriculture and Technology, Bhubaneswar, Odisha, INDIA

***Corresponding author:** drchinmoymishra@gmail.com

Rec. Date:	Aug 03, 2019 06:02
Accept Date:	Sep 15, 2019 06:24
DOI	10.5455/ijlr.20190803060237

Abstract

A total of 180 semen samples from 6 different breeds i.e. Holstein Friesian (HF), Crossbred Jersey (CBJ), Jersey, Gir, Sahiwal and Red Sindhi were examined for head length, head width, head base, middle piece, tail length, acrosome cap length and acrosome cap width. The highest value for total length in Sahiwal (71.61±0.49 µm), head length in Red Sindhi (11.00±0.08 µm), head width in Sahiwal (6.20±0.08 µm), head base in Jersey (3.15±0.07 µm), middle piece in Red Sindhi (15.33±0.14 µm) and tail length in Jersey (46.18±0.35 µm) was observed. The fertility of the spermatozoa was found to be significantly negatively correlated with acrosome cap length (P<0.05) and acrosome width (P<0.01). Crossbred Jersey, Holstein Friesian, Sahiwal, Jersey and Red Sindhi were found in one cluster and Gir was in a separate cluster. A crossbred male of Gir and either of other breed viz. Holstein Friesian, Sahiwal, Jersey and Red Sindhi may produce heterosis.

Key words: Acrosome, Breed, Cattle, Crossbred, Semen, Sperm

How to cite: Barik, S., Nayak, G., & Mishra, C. (2019). Analysis of Sperm Morphometric Traits and Estimation of Genetic Distance in Cattle. International Journal of Livestock Research, 9(11), 153-161. doi: 10.5455/ijlr.20190803060237

Introduction

Selection of bull is the prime factor in any breeding program as it determines the performance potential of the future progeny. Progeny testing program for bull evaluation is an essential part of state breeding policy. Sperm morphology, motility, volume and concentration are considered to be the three most important parameters for assessing the semen quality (Yathish *et al*., 2018). Morphological features of sperm serve as a reliable indicator in predicting the fertilizing capacity of sperm and also reflect certain disorders of spermatogenesis (Padrik and Jaakma, 2002 and Esteso *et al*., 2006). Fertilization efficacy also depends on the structures of middle piece, tail length and its progressive movement. An intact plasma membrane is a requisite for the normal sperm functions. For conventional morphology evaluation, sperm are fixed and classified by observers as either normal or abnormal (Barth and Oko, 1989). The variations in sperm morphometric traits and their association with the fertility were studied extensively. The report of reduced reproductive efficiency due to various morphological abnormalities of the spermatozoa in bulls is available (Walters *et al*., 2005). Morphometric analysis of sperm heads has been shown to be an indicator *in vitro* fertility (Thompson *et al*., 1994). Various reports on classical sperm morphometry of different cattle breeds are also available (Kant, 1991; Kumar *et al*., 1997; Beletti *et al*., 2005).

Sperm abnormalities have traditionally been classified by location of the defect (head, mid piece, tail), or its site of origin (primary: testis; secondary: epididymis; tertiary: accessory glands/post-ejaculation). The sperm abnormalities were also classified according to their effect on fertility (Blom, 1950), major defects include abnormalities of the head and mid piece, proximal cytoplasmic droplets and single abnormalities present in a high percentage, whereas minor defects include looped tails, detached sperm heads, and distal cytoplasmic droplets. The present research work thus has been designed with the objectives to analyze the sperm morphometry of Holstein Friesian, Crossbred Jersey X Holstein Friesian, Jersey, Gir, Sahiwal and Red Sindhi along with the association between sperm morphometric traits and fertility.

Materials and Methods

Sample Collection and Semen Evaluation

A total of 180 semen samples, 3 semen samples each from 10 different animals from 6 different breeds Holstein Friesian, Crossbred Jersey, Jersey, Gir, Sahiwal and Red Sindhi bull were collected from Frozen Semen Bank, Cuttack, Odisha. The semen sample was stained with Rose Bengal stain for visualisation of head length, head base, head width, middle piece, tail length and total length. For measurement of acrosome cap length and acrosome cap width, Giemsa stain was used. The spermatozoa were visualized using trinocular microscope with fluorescent and dark field camera attachment at 100x oil immersion lens.

Estimation of Fertility of Bulls

All the data related to fertility was collected from OLRDS, Odisha (Table 2). Total AI was recorded as 1156859. Fertility was calculated using the following formula -

$$\text{Fertility} = \text{No. of Calf Born/Total No. of AI} \times 100$$

Statistical Analysis

Statistical analysis of the data obtained during the present course of investigation was carried out as per the methods laid down by Becker (1975). The least square means and standard error for each trait was calculated through software package SPSS 22.0 (SPSS Inc, Chicago, IL, USA). Duncan's multiple range test was conducted using software SAS 9.13 for each character. Pearson's correlation coefficient (r) was

DOI 10.5455/ijlr.20190803060237

obtained between seminal characters through the software SPSS 22.0 (SPSS Inc, Chicago, IL, USA). Multiple regressions were done to examine how multiple independent variables are related to a dependent variable.

$$Y = a + b1X1+b2X2+b3X3+b4X4+b5X5$$

Where,
Y=Total length
a = is the "Y Intercept"
b1, b2, b3 are called as regression coefficient
X1= head area, X2 = head base area, X3= acrosome area, X4 = tail length, X5= middle piece

The principal component analysis was done for the important uncorrelated principal component through MATLAB software (MAT lab version12.0 inc USA). D^2 statistics for group distance based on principal component was carried out (Singh and Choudhary, 1985). It measured the genetic distance among groups or varieties based on multiple characters. The purpose was to identify genotypes which can be grouped together as one genetic group. If there are p characters measured on each individual and D^2 are difference between means of two groups, then D^2 statistics can be defined as follows-

$$PD^2=b1d1+b2d2+\ldots\ldots\ldots\ldots\ldots\ldots\ldots +bpdp$$

Where, the bi values are to be estimated such that the F ratio of variance between groups and within groups is maximized.

The computation was very much simplified when the characters under study were independent (Singh and Chaudhary, 1985). It was therefore preferred first to transform the correlated variables to uncorrelated ones to work out the D^2 values. In such a case D^2 values were reduced to simple summation of difference in mean values of various characters of the two populations. The populations were arranged in increasing order according to D^2 values for cluster formation.

Results and Discussion

Variation in Sperm Morphological Parameters

The highest head length was observed in Red Sindhi (11.00±0.08 µm) followed by Gir and Jersey (Table 1). The lowest value of head length was observed in Jersey (10.53±0.11 µm) followed by Holstein Friesian (HF) and Sahiwal. Statistically significant difference ($P<0.05$) of head length was identified between Red Sindhi as compared to other studied breeds (Table 1). The head width was found to be the highest in Sahiwal (6.20±0.08 µm) followed by Red Sindhi and Jersey. The lowest head width was observed in Crossbred Jersey (CBJ) ((5.76±0.11 µm) followed by Gir and HF (Table 1). Statistically significant difference ($P<0.05$) of head width was observed in CBJ, Gir, HF with Sahiwal (Table 1). Any significant difference

DOI 10.5455/ijlr.20190803060237

of head width could not be observed between other breeds. The head base of sperm was found to be the highest in Jersey (3.15±0.07 µm) followed by Sahiwal and Red Sindhi (RS) (Table 1). The lowest value of head base was observed in Gir (2.90±0.08 µm) followed by CBJ and HF. Statistically significant difference ($P<0.05$) of head base was observed between Gir and other breeds like Sahiwal and Jersey (Table 1). The length of the middle piece was found to be the highest in Red Sindhi (15.33±0.14 µm) followed by Sahiwal and Jersey (Table 1). However, the lowest value for the length of the middle piece was recorded in Gir (12.91±0.31 µm) followed by CBJ and HF (Table 1). Significant difference ($P<0.05$) of length of middle piece was observed in Gir and other bulls like CBJ, HF, Sahiwal, Jersey and Red Sindhi (RS). Similarly, significant difference between the length of middle piece was observed in RS and other bulls like CBJ and HF. The tail length was found to be the highest in Jersey (46.18±0.35 µm) followed by Sahiwal and RS (Table 1). Similarly, tail length was found to be the lowest in CBJ (43.85±0.61 µm) followed by HF and Gir. Statistically significant difference ($P<0.05$) of tail length was observed in CBJ and other bulls like Sahiwal and Jersey (Table 1). Any significant difference of tail length could not be observed in other breeds. Total length of sperm was found to be the highest in Sahiwal (71.61±0.49 µm), followed by Red Sindhi and Jersey. The total length of sperm was found to be the lowest in Gir (68.46±0.62 µm) Jersey and HF (Table 1). Statistical significant difference between total length of sperm was observed between Gir and CBJ and other bulls like RS, Jersey, Sahiwal and HF. Any significant difference ($P<0.05$) between total length could not be observed in other breeds.

The acrosome cap length was found to be the highest in Sahiwal (5.68±0.12 µm) followed by Gir and Red Sindhi (Table 1). The acrosome cap length was found to be the lowest in CBJ (4.86±0.09 µm) followed by Jersey and HF. Statistically significant difference ($P<0.05$) of acrosome cap length was observed between CBJ and breeds like HF, Sahiwal, Gir and Red Sindhi, Jersey and breeds like HF, Sahiwal, Gir and Red Sindhi (Table 1). Similarly, significant difference ($P<0.05$) between acrosome cap length was observed in HF and other breeds like Sahiwal, Gir, CBJ and Jersey. Any significant difference of acrosome cap length could not be observed in HF and Red Sindhi, Sahiwal and Gir, Sahiwal and Red Sindhi (Table 1). Acrosome cap width was found to be the highest in Gir (5.25±0.09 µm) followed by Sahiwal and HF (Table 1). The lowest value of acrosome cap width was recorded in CBJ (4.66±0.09 µm) followed by Jersey and Red Sindhi. Statistically significant difference ($P<0.05$) of acrosome cap width was observed between CBJ and HF, CBJ and Sahiwal, CBJ and Gir, HF and Gir (Table 1).

Earlier study showed that the length of sperm head of bull was 8.85 µm and width of sperm head of bull was 4.40 µm (Gravance *et al*., 1996). The dimension of spermatozoa was found to be independent of biological and environmental sources of variation but dependent upon genetic variation (Beatty, 1971; Maroto-Morales, 2016). The present experiment was also in agreement with these earlier studies. All the breeds except Gir had high values of head length, head width, middle piece, tail length and total length. The

DOI 10.5455/ijlr.20190803060237

larger values of sperm morphometric traits were associated with sub-fertility (Sunderaraman and Kalatharan, 2007), so Gir may be the better breed in terms of fertility. The total tail length of 65 µm was considered sufficient (Bahr and Zeitler, 1964). The length of tail varied from 45 to 65 µm which was in close agreement with the present study for bulls of all breeds.

The sperms of *Bos taurus* bulls were greater in length as compared to *Bos indicus* breeds (Bellati *et al*., 2005). However, the climatic factors could account for greater morphometrical variations in *Bos taurus* without impacting bull fertility in Brazil. The tropical climate of Brazil is similar to India and hence result is having greater significance to Indian conditions. The mean head length, head base, tail length, head width, middle piece length in earlier study were very much near to the calculated values of the present study (Saini, 2015). The acrosome cap length and cap width were significantly different with respect to breeds. This was in close agreement with that of earlier study (Beatty, 1971).

Table 1: Variation in sperm morphometric parameters expressed as Mean ± SE (µm)

Breed	Head Length	Head Width	Head Base	Middle Piece	Tail Length	Total Length	Acrosome cap length	Acrosome cap width
Crossbred Jersey	10.56^{a}±0.11	5.76^{a} ±0.11^{a}	2.96^{ab} ±0.07	14.38^{b} ±0.23	43.85^{a} ±0.61	68.80^{ab} ±0.70	4.86^{a} ±0.09	4.66^{a} ±0.09
Holstein Friesian	10.58^{a} ±0.11	5.90^{ab} ±0.01	3.03^{ab} ±0.07	14.53^{b} ±0.24	45.13^{ab} ±0.37	70.25^{bc} ±0.47	5.26^{b} ±0.09	4.98^{bc} ±0.06
Sahiwal	10.66^{a} ±0.09	6.20^{c} ±0.08	3.13^{b} ±0.07	14.91^{bc}±0.23	46.03^{b} ±0.40	71.61^{c} ±0.49	5.68^{c} ±0.12	5.08^{cd} ±0.07
Jersey	10.5^{a} ±0.11	6.10^{bc} ±0.09	3.15^{b} ±0.07	14.73^{bc}±0.23	46.18^{b} ±0.35	71.45^{c} ±0.40	4.95^{a} ±0.09	4.80^{ab} ±0.08
Gir	10.80^{ab} ±0.11	5.76^{a} ±0.09	2.90^{a} ±0.08^{a}	12.91^{a} ±0.31	44.75^{ab} ±0.56	68.46^{a} ±0.62	5.65^{c} ±0.14	5.25^{d} ±0.09
Red Sindhi	11.00^{b} ±0.08	6.15^{bc}±0.08	3.10^{ab}±0.07	15.33^{c} ±0.14	45.15^{ab}±0.54	71.48^{c}±0.54	5.45^{bc} ±0.09	4.8^{ab} ±0.08

The values with different superscripts differ significantly.

Fertility

The fertility for all the breeds was calculated (Table 2). The Gir cattle had the highest fertility (0.75) and HF had the lowest fertility (0.25).

Table 2: Fertility % in different breeds

Breed	AI	Calf-Born	Y=Fertility %	X=Sin^{-}√Y (Angular Transformed Value)
Holstein Friesian	7436	1872	25.1748	0.525615
Jersey	415908	182661	43.9186	0.724433
Crossbred Jersey	420732	185087	43.9917	0.725169
Red Sindhi	282803	107794	38.1163	0.665413
Sahiwal	21247	11959	56.2847	0.533556
Gir	8733	6587	75.4356	0.931342

The angular transformed values will be used for calculation of correlation.

DOI 10.5455/ijlr.20190803060237

Correlation between Sperm Morphometric Traits

The correlation value between different sperm morphometric parameters were calculated using these angular transformed data (Table 3).

Table 3: Correlation values between different sperm morphometric parameters

	Fertility	**Head length**	**Head width**	**Head base**	**Middle piece**	**Tail length**	**Total length**	**Acrosome cap length**	**Acrosome width**
Fertility	1	-0.03	0.11	0.16	0.06	-0.06	-0.03	-0.59*	-0.90**
Head length	-0.03	1	0.58*	0.24	0.91	-0.01	0.45**	0.81*	0.02
Head width	0.11	0.58*	1	0.93**	0.86**	0.79*	0.98**	0.53	0.23
Head base	0.16	0.24	0.93*	1	0.62**	0.94**	0.96**	0.27	0.25
Middle piece	0.06	0.91**	0.86**	0.62*	1	0.39	0.78**	0.75**	0.1
Tail length	-0.06	-0.01	0.79**	0.94	0.39	1	0.88**	0.2	0.47
Total length	-0.03	0.47	0.98**	0.96**	0.78**	0.88**	1	0.54*	0.4
Acrosome cap length	-0.60**	0.81**	0.53*	0.27	0.75**	0.2	0.54*	1	0.60*
Acrosome width	-0.91**	0.02	0.23	0.25	0.1	0.47	0.4	0.60*	1

**P < 0.05; **P <0.01*

Fertility of the spermatozoa were found to be negatively significantly correlated with acrosome cap length (P<0.05) and acrosome width (P<0.01). Total length of the spermatozoa was found to be positively significantly correlated with head length (P<0.01), head width, head base, middle piece, tail length as well as with acrosome cap length (P<0.05). A statistically significant positive correlation was found between head length with head width, acrosome cap length (P<0.05) and also with middle piece, acrosome cap length (P<0.01). Head width was positively significantly correlated with head base, middle piece (P<0.01) and tail length, acrosome cap length (P<0.05). Middle piece was also having significant positive correlation with acrosome cap length (P<0.01) as well as with middle piece and total length. Any statistically significant difference correlation value couldn't be identified among other traits as mention above.

Acrosome measurement were significantly but negatively correlated (P<0.01) with fertility. The penetration of sperm into ovum is accomplished by acrosomal enzyme acrosin and hyaluronidase. So optimum acrosome cap length and width would result in higher penetration and fertility. The correlation between head, mid piece and tail length was not significantly correlated with fertility. The normal sperm morphology may be an indicator of the fertility potential of a male (Saini, 2015). Sperm head was associated with fertility. However, the acrosome was significantly correlated with fertility in the present study. Acrosome is a part of sperm head; however, sperm head length was strongly correlated (P<0.01) with head width, middle piece and acrosome cap. Similarly, head length was also correlated significantly (P<0.01) with middle piece, tail length and total length. This was in close agreement with the earlier study. The total length was also highly correlated (P<0.01) with head measurements and middle piece along with tail

DOI 10.5455/ijlr.20190803060237

measurements. So, sperm morphometry in combination with other objective traits can be useful for developing a fertility index (Roy, 2014). The association of increased morphological abnormality of spermatozoa with reduced reproductive efficiency had been reported (Saini, 2015). Morphometric analysis of sperm head had been also shown to be an indicator of *In vitro* fertility.

Regression Analysis of Sperm Morphometric Traits

The regression analysis for prediction of total length of sperm was done for each breed. The regression coefficient for head area (X_1), head base (X_2), acrosome area (X_3), tail length (X_4), middle piece length (X_5) and corresponding X-intercept value for each breed (CBJS, Jersey, Gir, Sahiwal, Red Sindhi, Holstein Friesian) were estimated (Table 4). The regression equations for each breed are given below-

For Crossbred Jersey, $Y_{CBJS} = 8.07 + 0.06(X_1) + 0.01(X_2) - 0.04(X_3) + 1.01(X_4) + 0.87(X_5)$

For Sahiwal, $Y_{SAHIWAL} = 4.86 + 0.08(X1) - 0.50(X2) + 0.005(X3) + 1.03(X4) + 1.03(X5)$

For Jersey, $Y_{JERSEY} = 8.65 + 0.08(X_1) - 0.58(X_2) + 0.0003(X_3) + 0.97(X_4) + 0.98(X_5)$

For Gir, $Y_{GIR} = 8.20 + 0.06(X_1) - 0.27(X_2) - 0.02(X_3) + 0.10(X_4) + 1.01(X_5)$

For Red Sindhi, $Y_{RED\ SINDHI} = 4.67 + 0.08(X_1) - 0.70(X_2) - 0.02(X_3) + 1.03(X_4) + 1.15(X_5)$

For Holstein Friesian, $Y_{HF} = 6.72 + 0.05(X_1) + 0.08(X_2) + 0.003(X_3) + 0.99(X_4) + 1.03(X_5)$

Table 4: Regression values of bovine sperm morphometric parameters

Breed	Intercept = a	Head Area (X1)	Head Base (X2)	Acrosome Area (X3)	Tail Length (X4)	Middle Piece (X_5)	R^2 value
Crossbred Jersey	8.07±1.16	0.06±0.01	0.01±0.02	-0.04±0.23	1.01±0.02	0.87±0.06	0.99±0.35
Sahiwal	4.86±2.08	0.08±0.01	-0.50±0.24	0.005±0.01	1.03±0.03	1.03±0.06	0.98±0.38
Jersey	8.65±2.50	0.08±0.01	-0.58±0.26	0.0003±0.02	0.97±0.04	0.98±0.07	0.97±0.45
Gir	8.20±1.02	0.067±0.07	-0.27±0.14	-0.02±0.01	0.10±0.02	1.01±0.03	0.10±0.25
Red Sindhi	4.67±2.07	0.08±0.02	-0.70±0.24	-0.02±0.01	1.03±0.02	1.15±0.10	0.99±0.35
Holstein Friesian	6.72±2.02	0.05±0.01	0.08±0.25	0.003±0.03	0.99±0.04	1.03±0.06	0.98±0.41

Principal Components Analysis of Sperm Morphometric Traits

Out of nine semen morphometric characters only three (piece length, tail length and total length of sperm) characters were found as principal components. Based on eigen factors and eigen values, the other morphometric characters were correlated to the above said three characters.

Diversity Analysis using D^2 Statistics

D^2 values were obtained (Table 5) using the three principal characters of each breed through Torchers method of grouping. It was found that HF, Jersey, CBJ, Sahiwal and Red Sindhi were in one cluster and Gir was under separate cluster.

DOI 10.5455/ijlr.20190803060237

Table 5: D^2 Value arranged in ascending order for each breed

Crossbred Jersey	Holstein Friesian	Sahiwal	Jersey	Gir	Red sindhi
0.2599 (Holstein Friesian)	0.2156 (Jersey)	0.0684 (Jersey)	0.0684 (Sahiwal)	1.5764 (Crossbred Jersey)	0.5209 (Sahiwal)
0.9031 (Sahiwal)	0.2218 (Sahiwal)	0.2218 (Holstein Friesian)	0.2156 (Holstein Friesian)	1.899 (Holstein Friesian)	0.7723 (Holstein Friesian)
0.931 (Jersey)	0.2591 (Crossbred Jersey)	0.5209 (Red Sindhi)	0.931 (Crossbred Jersey)	2.8703 (Jersey)	0.953 (Jersey)
1.1659 (Red Sindhi)	0.7723 (Red Sindhi)	0.9031 (Crossbred Jersey)	0.953 (Red Sindhi)	2.959 (Sahiwal)	1.1659 (Crossbred Jersey)
1.5764 (Gir)	1.899 (Gir)	2.959 (Gir)	2.8703 (Gir)	3.5108 (Red Sindhi)	3.5108 (Gir)

So, there is a possibility of finding heterosis when two different homogenous clusters are crossed (Singh and Choudhary, 1985). The heterosis with respect to fertility exists (Beatty, 1971). So, probably a crossbred male out of Gir and either of other breed *viz*. HF, Sahiwal, Jersey and Red Sindhi may produce highest heterosis.

Conclusion

From the above study it was concluded that in terms of fertility Gir may be the best breed. A crossbred male out of Gir and either of other breed *viz.* HF, Sahiwal, Jersey and Red Sindhi may produce highest heterosis. Gir is separate from other breed in all morphometric characters. It was also found that sperm length and fertility are inversely related which can be incorporated in breed characterization.

Acknowledgements

The authors are thankful to Frozen Semen Bank, Cuttack for their help and support for this study.

References

1. Bahr, G.F., Zeitler, E. (1964). Study of bull spermatozoa. *The Journal of Cell Biology*. 21(2), 175-189.
2. Barth, A.D., Oko, R.J. (1989). Defects of the Sperm Head in Abnormal Morphology of Bovine Spermatozoa. Iowa State University Press, Ames, 15, 130-192.
3. Beatty, R.A. (1971). The genetics of size and shape of spermatozoan organelles. In Edinburg symposium on genetics of spermatozoa ed. Beatty RA. Gluecksohn Waelsch S:97-191.
4. Becker, G.S. (1975). Human capital and the personal distribution of income: An analytical approach. In Human capital: a theoretical and empirical analysis, with special reference to education (2 ed.). *New York: National Bureau of Economic Research.*
5. Beletti, M.E., Costa, L.F., Guardieiro, M.F. (2005). Morhometric features and chromatin condensation abnormalities evaluated by toluidine blue staining in bull spermatozoa. *Brazilian Journal of Morphological Sciences*, 22(2), 85-90.
6. Blom, E.A. (1950). One-minute live-dead sperm stain by means of eosin-nigrosin. *Journal of Fertility Sterility*, 1,176-177.
7. Esteso, M.C., Soler, A.J., Fernandez, M.R., Quintero-Moreno, A.A., Garde, J.J. (2006). Functional significance of the sperm headmorphometric size and shape for determining freezability in Iberian red deer (*Cervuselaphushis panicus*) epididymal sperm samples. *Journal of Andrology*, 27(5), 662-670.

DOI 10.5455/ijlr.20190803060237

8. Gravance, C.G., Vishwanath, R., Pitt, C., Casey, P.J. (1996). Computer automated morphometric analysis of bull sperm heads. *Elsevier Science Inc*, 46, 1205-1215.
9. Kant, R. (1991). Genetic and Phenotypic Studies on Biometry of Spermatozoa of Karan Swiss Bulls. M.Sc. Thesis, NDRI, Karnal, Haryana.
10. Kumar, P., Kumar, S., Ahlawat, S.P.S., Joshi, B.K., Raina, V.S. (1997). Biometrics of spermatozoa of Sahiwal bulls. *Indian Journal of Animal Production*, 29(1), 32-34.
11. Maroto-morales, A. (2016). Current status and potential of morphometric sperm analysis. *Asian Journal of Andrology*, 18, 863-870.
12. Padrik, P., Jaakma, U. (2002). Sperm morphology in Estonian Holstein dairy bulls and factors affecting it and relation to fertility. *Agraateadus. Journal*. 13, 243-256.
13. Roy, B. (2014). A Comparative study on sperm morphometry of crossbred and Murrah buffalo bulls. *International Journal of Agricultural Science and Veterinary Medicine*, 2(3), 149-155.
14. Saini, J. (2015). Comparative morphometry histomorphological study of spermatozoa of cattle and buffalo. NDRI, MVSc Thesis. 1-80.
15. Singh, R.K., Chadudhary, B.D. (2014). Classificatory analysis, *Biometrical methods in quantitative genetic analysis*, 12, 224-229.
16. Sunderaraman, M.N., Kalatharan. (2007). Analyses of morphological and morphometrical deviation of bull spermatozoa by CASA. *Asian Journal of Andrology*. 4, 196-204.
17. Thompson, L.A., Brook, P.F., Warren, M.A., Barratt, C.L.R., Cooke, I.D. (1994). A morphometric comparison of the nuclear morphology of fresh and frozen thawed human zona- bound and unbound sperm. *Journal of Andrology*, 15, 337-342.
18. Walters, A., Eyestone, W., Saacke, R., Pearson, R., Gwazdauskas, F. (2005). Bovine embryo development after IVF with spermatozoa having abnormal morphology. *Theriogenology*, 63(7), 1925-1937.
19. Yathish, H.M., Kumar, S., Chaudhary, R., Mishra, C., Sivakumar, A., Kumar, A., Chauhan, A., Ghosh, S.K. and Mitra, A. (2018). Nucleotide variability of protamine genes influencing bull sperm motility variables. *Animal Reproduction Science*. 193: 126-139.

DOI 10.5455/ijlr.20190803060237