Study on Dimensional Characteristics of Different Breeds of Bull Spermatozoa

M.J.U. Sarder

Department of Animal Husbandry and Veterinary Science, Faculty of Agriculture, University of Rajshahi,
Rajshahi-6205, Rajshahi, Bangladesh

Abstract: The study was conducted on the basis of semen sample collected from CCBSDF, Savar, Dhaka, RDCIF, Rajabarihat, Rajshahi and District AI Centre, Rajshahi over a period 1998 to 2003 to evaluate the effect of breed on dimensional characteristics of spermatozoa as well as correlation among the biometrical characteristics. The dimensional characteristics of spermatozoa were Head Length (HL), Head Breadth (HB), Head Shape (HS), Mid-piece Length (ML), Tail Length (TL) and total Sperm Length (TSL). A total of 915 spermatozoa from 71 bulls of six genetic groups were included in the study. The average for head length (HL), head breadth (HB), head shape (HS), mid-piece length (ML), tail length (TL) and total sperm length (TSL) were $9.164\pm0.53\mu$, $4.703\pm0.38\mu$, 1.958 ± 0.16, $13.367\pm1.18\mu$, $46.653\pm1.93\mu$ and $69.184\pm2.40\mu$, respectively of 71 bulls. Genetic groups of 100%Friesian, 100%Sahiwal, 75%Friesian×25%Local, 50%Sahiwal×50%Friesian, 50%Friesian×50%Local and 100 Local had significant ($p<0.05$) effect on all the dimensional characteristics of spermatozoa. The highest HL (9.371μ) was obtained for 100%Friesian with the lowest (8.759μ) in 100%Local breed. Significantly highest HB (4.843μ) was found in 50%SL×50%F with the lowest (4.539μ) in 100%Local breed. The HS, ML, TL and TSL were found significantly highest in 100%Friesian breed (2.021, 13.627μ, 47.591μ and 70.591μ, respectively) than 100%Local breed. The breeds of pure (exotic), cross-bred and pure local had significant ($p<0.05$) effect on dimensional characteristic of spermatozoa. Grossly, pure and cross-bred had significant ($p<0.05$) effect on dimensional characteristics except on head length. The head length of bull spermatozoa had significant positive correlation with HB, HS, TL, and TSL. Negative correlation was found also between ML, HS, and TL. Significant ($p<0.05$) positive correlation were also found between ML and TSL ($r=.510$; $P<0.05$) as well as between TL and TSL ($r=.836$; $P<0.05$). Results of the present study recommended that the genetic groups of 100%Friesian and 50%Sahiwal×50%Friesian had better performance of the dimensional characteristics of spermatozoa as well as Pure (exotic) and Pure breed had improved dimensional characteristics of bull spermatozoa.

Key words: Bull, breed, spermatozoa, dimensional characteristics and correlation

INTRODUCTION

It has been well established that the dimensional characteristics of spermatozoa is controlled by genes; as a results, different strains of laboratory animals and breeds of farm animals have shown significant differences in the dimensional characteristics of spermatozoa[1,4]. Breed differences in the characteristics of bull spermatozoa have also been reported by Mukherjee and Singh [3] and also several studies were carried out for charcterising the biometry of spermatozoa in zebu breed and crossbred cattle bulls other than Brown Swiss origin [5,6]. Mukherjee and Singh [7] reported breed differences in head length, maximum head width and mid-piece length in bull spermatozoa.Venkataswami and Vedanayagams[8] recorded breed differences in mid-piece length and tail length in cattle. Different workers have studied the biometrical values of spermatozoa in different animals.

Lagerlof [9], Rao [10], William and Savage [11] have shown that the biometrical data of spermatozoa head length variation were indicative of bull fertility. Measurement of spermatozoa varies from species to species and breed to breed within species[12].The characteristics of spermatozoa may vary due to several factors such as genetic, nutrition, climatic condition, disease, interval between collections of semen etc. However, in Bangladesh no such work has been performed yet. Therefore, the present study was designed to determine the biometry as well as to find out the effect of breed on dimensional characteristics of bull spermatozoa.

MATERIALS AND METHODS

The present study has been dealing with the breed variation in dimensional characteristics of bull spermatozoa of different breed of bulls used for AI

Corresponding Author: M.J.U. Sarder, Department of Animal Husbandry and Veterinary Science, Faculty of Agriculture, University of Rajshahi, Rajshahi-6205, Rajshahi, Bangladesh

programme. The study was conducted in three Artificial Insemination (AI) Centres /Stations and at the Department of Genetics and Breeding, Rajshahi University during the period from 1998 to 2003. The AI Centres/Stations were Central Cattle Breeding Station and Dairy Farm (CCBSDF), Savar, Dhaka, District Artificial Insemination Centre (DAIC), Rajshahi and Rajshahi Dairy and Cattle Improvement Farm (RDCIF), Rajabarihat, Rajshahi. The basic materials for the present study comprised seventy-one bulls belonged to six genetic groups. Out of 71 bulls, 50 bulls from CCBSDF, Savar, Dhaka, 7 from RDCIF, Rajabarihat, Rajshahi and 14 bulls from DAIC, Rajshahi. For the study, 915 spermatozoa from 71 bulls belonging to (six) genetic groups namely, 100%Friesian(100%F, n=11), 100%Sawhiwal (100%SL, n=11),75%Friesian×25% Local(75%F×25%L, n=14), 50%Sahiwal× 50%Friesian(50%SL×50%F, n=11), 50%Friesian×50%Local(50%F×50%L, n=14) and 100%Local(100%L, n=10) from the three AI Centres, in Bangladesh. Six morphological characteristics of 915 spermatozoa were measured on 71 AI bulls. The effect of breed on dimensional characteristics of bull spermatozoa was studied. From each bull, at least 3 semen samples were collected by an artificial vagina at interval of 4 to 10 days between collection at the three AI centres or stations. Feeding and management system in three (3) AI centres were more or less uniform throughout the year. All three (3) AI centers of bulls were in similar housing and managemental conditions. The bulls were housed individually in pens with sufficient cross ventilation and protected from hot-sun and heavy rainfall to avoid abrupt fluctuation of their body temperature during the whole period of observation. The bulls were exercised once in a week in the form of running on the field. No bull was affected with remarkable diseases. The bulls of three centres/stations were regarded as clinically healthy and free from any significant abnormalities. The bulls received routine vaccination against Anthrax, HS, Blackleg, FMD and Anthelmentics like Fascioliasis and Round worm. Bulls were tested for fertility before putting them in the breeding herd.

The management systems of these bulls were maintained as recommended by CCBSDF, Savar, Dhaka. So, the management systems of three AI Centres were more or less same. So, the researcher has combined the study among the bulls in 3 AI Centres. Different types of breeds of bulls used for AI programme in Bangladesh. The experimental AI bulls were divided into 6 groups according to their genetic composition. Group1:100% Friesian(100%F), Group2: 100% Sahiwal(100% SL), Group3: ≥75% Friesian×≤25 %Local (≥75% F×≤25 % L), Group4: ≥50% Sahiwal×≤50% Friesian(50%SL×50%F) Group5:50%Friesian×50%Local(50%F×50%L) and

Group6: 100% Local(100%L).The bulls were also divided into three groups on the basis of exotic and non-exotic groups, viz. Group I: Pure (exotic) breed, Group II: Cross-bred bulls and Groups III: Local bulls. The bulls were grossly divided into two groups on the basis of breed types. Groups 1: Pure breed (Friesian, Sahiwal and Local of pure bulls) and Group2: Cross-breed (L×F, SL×F, L×F×F)

Collection of semen and processing: The bulls were trained to ejaculate in artificial vagina (AV) at homosexual mount using at three AI centres. The semen was obtained in a graduated tube previously assembled with the vaginal cone. The graduated collecting tube was separated from the cone, covered with a plastic cap and labeled. Two types of semen are produced in our country. One is chilled semen for short period preservation produced at District AI centre, Rajshahi and second is Deep frozen semen for long period preservation produced mostly at Central Cattle Breeding Station and Dairy Farm (CCBSDF), Savar, Dhaka and Rajshahi Dairy and Improvement Farm(RDCIF), Rajabarihat, Rajshahi.

Preparation of slides for sperm measurements: The procedure described by Venkataswami and Vedanayagm [8] for cattle was used to determine the biometrical values of bull spermatozoa with slight modification during the dilution of semen and the technique of preparation of smear. The semen was diluted with sodium citrate dihydrate solution (2.94%) at the rate of 1:40 immediately after collection and was kept in water bath at 30-35°C. A small drop of diluted semen was taken at one end of a clean, gress-free microscopic glass slide and the smear was made with the help of another glass slide. The slides were coded to vail their identity. The smear, so prepared, was allowed to dry in air at room temperature under a ceiling fan. After air-drying the smear was fixed with equal volume of ethar and ethyl alcohol. The smear was then flooded with staining solution containing 2 part of 5% aquous solution of Aniline blue, 1 part of 5% aquous solution of Eosin B and 1 part of 1% aquous solution of Phenol and kept for 5-10 minutes and 40-60°C in a thermostatically controled incubator. The staining fluid was then washed off from the slides thoroughly and gently with distilled water. The slides were then dried in air and examined under oil immersion objectives. For mensuration characteristics of 3-5 normal spermatozoa per slide were selected at random. The six dimensional characteristics viz. Head length (HL), Head breadth (HB), Head Shape (HS), Mid-piece Length (ML), Tail Length (TL) and Total Sperm Length (TSL) of different breeds, age, body weight and scrotal circumference of bulls spermatozoa were taken for the study.

Table 1: Mean±SD with head length, head breadth, head shape, mid-piece length, tail length and total length of spermatozoa in micron of different genetic groups of bulls used for AI Programme in Bangladesh

Genetic groups	Biometrics of bull spermatozoa					
	Head length (HL) unit =μ	Head breadth (HB) unit =μ	Head shape (HS)	Mid-piece length (ML) unit =μ	Tail length (TL) unit =μ	Total sperm length (TSL) unit =μ
100% Friesian (F)	9.371± 0.450 [a] n=143	4.658± 0.332 [b] n=143	2.021± 0.172 [a] n=143	13.627± 1.036 [a] n=143	47.591± 1.246 [a] n=143	70.591± 1.486 [a] n=143
100% Sahiwal (SL)	9.172± 0.546 [b] n=170	4.694± 0.396 [b] n=170	1.965± 0.181 n=170	13.628± 1.379 [ab] n=170	46.645± 2.576 [bc] n=170	69.445± 3.172 [b] n=170
100% Local (L)	8.759± 0.568 [c] n=96	4.539± 0.321 [c] n=96	1.937± 0.166 [b] n=96	13.324± 1.136 [b] n=96	45.978± 1.684 [d] n=96	68.062± 2.201 [d] n=96
75% Friesian ×25%Local	9.139± 0.566 [b] n=190	4.696± 0.364 [b] n=190	1.954± 0.164 [b] n=190	13.304± 1.186 [b] n=190	46.496± 1.915 [bc] n=190	68.940± 2.346 [bc] n=190
50% Sahiwal ×50%Friesian	9.354± 0.433 [a] n=136	4.843± 0.392 [a] n=136	1.941± 0.147 [b] n=136	12.969± 0.857 [c] n=136	46.931± 1.601 [b] n=136	69.254± 1.817 [b] n=136
50% Friesian ×50% Local	9.092± 0.458 [b] n=180	4.734± 0.399 [b] n=180	1.930± 0.150 [b] n=180	13.303± 1.245 [b] n=180	46.229± 1.749 [cd] n=180	68.625± 2.174 [c] n=180
Total	9.164± 0.534 n=915	4.703± 0.380 n=915	1.958± 0.166 n=915	13.367± 1.185 n=915	46.653± 1.932 n=915	69.184± 2.405 n=915

n= Number of observation; a, b, c and d, values are same column statistically significant each other (p<0.05%) by DMRT.

Measurement of spermatozoa: The sperm was measured with a measuring scale graduated in micrometer under magnification of 100 objectives. Reading on the length of spermatozoa was taken by placing the sperm in parallel with the micro-meter as shown in the Fig.1.
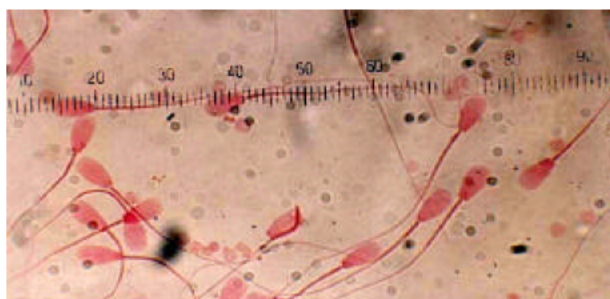

Fig. 1: A normal spermatozoa parallel with the Ocular meter scale for measurement under the light microscope at 1000x

Actual length of the scale of micrometer = 1 cm.
1 cm has been divided into 100 divisions
1 small division = 1/100 cm=0.01 cm=0.1 mm
1 mm =1000μ
0.1mm =100μ
i.e. 1 small division = 100 μ
Multiplication factors

- When observed under 10x objective,70 unit=(0.01 mm) 100 unit 1 =1.42 =14.2 μ
- When observed under 45x objective, 60 unit (eye piece) =(0.01 mm) 20 unit (stage micrometer) 1 unit = 0.33 = 3.3μ
- When observed under 100x objective, 28 unit (eye piece) = (0.001) 4 unit in stage micrometer1unit = 0.14 = 1.42 μ i.e. 1 small division =1.42 small multiplication factor when magnification was 1000x.

Procedure: A total number of 915, almost straight, spermatozoa with clear outline were selected at random and measurements of HL, HB, HS, ML, TL and TSL were recorded with the help of an ocular micrometer. The head shape (HP) was determined by dividing the head length (HL) by its breadth.

Statistical analysis: Duncan's multiple range test was used to test the significance among the genetic groups according to the method described by Steel and Torrie (1980).

RESULTS AND DISCUSSION

A total 915 spermatozoa from 71 bulls belongs six genetic groups from three AI Centres, to evaluate the dimensional characteristics of the bull spermatozoa. The dimensional/biometrics considered were the average Head Length(HL), Head Breadth(HB), Head Shape (HS), Mid-piece Length (ML), Tail Length(TL) and Total Sperm Length (TSL). The averages for Head Lenght (HL), Head Breadth (HB), Head Shape (HS), Mid-piece Length (ML), Tail Length(TL) and Total Sperm Length (TSL) were 9.164

Table 2: Analysis of variance for the biometrics of spermatozoa in different genetic groups of bulls used for AI in Bangladesh

Dimensional traits of sperm	Sources of variation	D.F	Sum of square	Mean of square	F-value
Head length (HL) (μ)	Between genetic groups	5	27.80	5.56	21.63
	Within genetic groups	909	233.66	0.25	
	Total	914	261.47		
Head breadth (HB) (μ)	Between genetic groups	5	5.72	1.14	8.22
	Within genetic groups	909	126.555	0.14	
	Total	914	132.281		
Head shape (HS)	Between genetic groups	5	0.80	0.16	5.94
	Within genetic groups	909	24.52	2.697E-02	
	Total	914	25.32		
Mid-piece length (ML) (μ)	Between genetic groups	5	44.45	8.90	6.52
	Within genetic groups	909	1238.41	1.36	
	Total	914	1282.86		
Tail length (TL) (μ)	Between genetic groups	5	217.32	43.46	12.36
	Within genetic groups	909	3191.96	3.51	
	Total	914	3409.28		
Total sperm length (TSL) (μ)	Between genetic groups	5	483.41	96.68	18.30
	Within genetic groups	909	4797.51	5.28	
	Total	914	5280.92		

Table 3: Dimensional characteristics of spermatozoa in between the Pure (exotic), Cross-bred and Local breed of AI bulls used in Bangladesh

	Breed			
Sperm parameters	Pure (exotic) n=327	Cross-bred n=492	Local breed n=96	Overall n=915
Head length (HL) (μ)	9.256±0.510 [a]	9.182±0.508 [a]	8.759±0.568 [b]	9.164±0.534
Head breadth(HB) (μ)	4.676±0.369 [b]	4.753±0.387 [a]	4.539±0.321 [c]	4.703±0.380
Head shape(HS)	1.990±0.178 [a]	1.941±0.155 [b]	1.937±0.166	1.958±0.166
Mid-piece length (ML) (μ)	13.604±1.227 [a]	13.218±1.142 [b]	13.324±1.136 [a]	13.367±1.185
Tail length (TL) (μ)	47.072±2.105 [a]	46.506±1.798 [b]	45.978±1.682 [c]	46.653±1.932
Total sperm length (TSL) (μ)	69.933±2.581 [a]	68.907±2.171 [b]	68.062±2.201 [c]	69.184±2.405

N= Number of observation, The values are Mean±SD, a,b,c Mean±SD with different superscript letters in the same row differs significantly with each others($p<0.05$) by DMRT.

Table 4: Analysis of variance for the biometrics of spermatozoa of different breeds of bulls used for AI in Bangladesh

Sperm morphology	Sources of variation	D.F	Sum of square	Mean of square	F-value
Head length (HL) (μ)	Between breeds	2	18.66	9.33	35.05
	Within breeds	912	242.80	0.26	
	Total	914	261.47		
Head breadth (HB) (μ)	Between breeds	2	4.04	2.02	14.37
	Within breeds	912	128.23	0.14	
	Total	914	132.28		
Head shape (HS)	Between breeds	2	0.52	0.26	9.54
	Within breeds	912	24.80	2.720E-02	
	Total	914	25.32		
Mid-piece length (ML) (μ)	Between breeds	2	29.37	14.69	10.67
	Within breeds	912	1253.48	1.37	
	Total	914	1282.86		
Tail length (TL) (μ)					
	Between breeds	2	111.56	55.78	15.41
	Within breeds	912	3297.72	3.62	
	Total	914	3409.28		
Total sperm length (TSL) (μ)	Between breeds	2	341.06	170.53	31.45
	Within breeds	912	4939.86	5.42	
	Total	914	5280.92		

±0.53μ, 4.703±0.38μ, 1.958±0.16, 13.367±1.18μ, 46.653±1.93μ and 69.184±2.40μ respectively, for 71 bulls from 6 breeds (Table1).

Effect of breed: The effect of breed on Head Length (HL), Head Breadth (HB), Head Shape (HS), Mid-piece Length (ML), Tail Length (TL) and Total Sperm Length (TSL). The averages for Head Length (HL), Head Breadth (HB), Head Shape (HS), Mid-piece Length (ML), Tail Length(TL) and Total Sperm Length (TSL) as influenced by genetic groups of AI bull is presented in the Table 1, 3 and 5. Genetic groups had significant ($p<0.05$) effect on all the dimensional characteristics of spermatozoa (Table-1 and Table 2) except head length in case between the pure and cross-bred bulls. The highest HL was obtained for 100% Friesian with 9.371μ and then for 50% Sahiwal×50% Friesian with 9.354 μ followed by 100% SL with 9.172μ. The minimum head length was for 100%Local with 8.759μ

Table 5: Mean±SD with comparison of dimensional characteristics of spermatozoa   considering the Pure and Cross-bred AI bulls

Sperm parameters	Pure breed (100%F, 100% SL and 100% Local)			Cross-bred (Friesian cross with Local and SL)		
	N	Mean	SD	N	Mean	SD
Head length (HL) (µ)	423	9.143	0.563	492	9.182	0.508
Head breadth (HB) (µ)	423	4.645 [b]	0.363	492	4.453 [a]	0.387
Head shape (HS)	423	1.978 [a]	0.176	492	1.941 [b]	0.155
Mid-piece length (ML) (µ)	423	13.540 [a]	1.211	492	13.218 [b]	1.142
Tail length (TL) (µ)	423	46.823 [a]	2.066	492	46.506 [b]	1.798
Total sperm length (TSL) (µ)	423	69.507 [a]	2.617	492	68.907 [b]	2.171

N= Number of observation. SD= Standards of Deviation. F= Friesian. SL= Sahiwal. a, b values are statistically significant to each others (p<0.05) by DMRT.

Table 6: Analysis of variance for the biometrics of spermatozoa between the pure and cross-bred bulls used for AI in Bangladesh

Biometrics of spermatozoa	Sources of variation	D.F	Sum of square	Mean of square	F-value
Head length (HL) (µ)	Between breeds	1	0.35	0.35	1.22
	Within breeds	913	261.12	0.28	
	Total	914	261.47		
Head breadth (HB) (µ)	Between breeds	1	2.65	2.65	18.71
	Within breeds	913	129.62	0.14	
	Total	914	132.28		
Head shape (HS)	Between breeds	1	0.31	0.31	11.43
	Within breeds	913	25.01	2.739E-02	
	Total	914	25.32		
Mid-piece length (ML) (µ)	Between breeds	1	23.59	23.59	17.08
	Within breeds	913	1259.26	1.38	
	Total	914	1282.86		
Tail length (TL) (µ)	Between breeds	1	22.80	22.80	6.14
	Within breeds	913	3386.48	3.71	
	Total	914	3409.28		
Total sperm length (TSL) (µ)	Between breeds	1	81.67	81.67	14.32
	Within breeds	913	5199.25	5.70	
Total 914			5280.92		

and then for 50%F×50%L with 9.092µ. Significantly (p<0.05) highest head breadth was found in 50%SL×50%F with 4.843µ and lowest was found in 100% L (4.539µ). The HS, ML, TL and TSL were found significantly (p<0.05) highest in genetic group of 100% Friesian (2.021, 13.627µ, 47.591µ and 70.591µ respectively). The dimensional characteristics of bull spermatozoa were found significant (p<0.05) differences between the pure (exotic), cross-bred and local breed (Table 3 and 4). The pure and cross breed had significant (p<0.05) between the dimensional characteristics of spermatozoa (Table 5 and 6). Several workers have found significant differences of the biometrical characteristics between breeds of different species. Beatty and Napier [14] found breed differences on the biometrics of head length and maximum head width in rabbit spermatozoa. Mukhergee and Singh [7] also found breed differences in head length, maximum head width and mid-piece length in bull spermatozoa. Venkataswami and Vedanayagam [8] reported breed differences in mid-piece length and tail length in cattle. They observed that all the breeds the mean head length, head width, mid-piece length and tail length were resp. 9.45, 5.08, 14.19 and 47.54µ which is close agreement the present findings. Pandya et al.,[15] reported the averages for 3 dimensional characteristics of bull spermatozoa viz. head length, head breadth and mid-piece were found 9.6227±0.1386µ, 4.9602±0.0256µ and 14.4328±0.1205µ respectively. Raman

Kant and Chakravarty [16] stated that the least and highest means of head length, head width and mid-piece length of Karan Swiss bull spermatozoa were found to be 8.40±0.5µ and 8.89±0.03µ, 4.30±0.03µ and 4.81±0.03µ, 12.79±0.3µ and 19.36±0.5 µ. Bardoli and Sharma [12] also stated that measurement of spermatozoa varies from species to species and breed to breed within species.

Correlation coefficient between HL, HB, HS, ML, TL and TSL were calculated in different breed of AI bulls is presented in Table-7. Significantly (p<0.05) positive correlation was obtained between HL, HB, HS TL and TSL of various AI bulls except ML. Significantly (p<0.05) strong negative correlation was observed between HB and HS. Negative correlation was found also between ML and HS, TL and HS. Significant strong positive correlation was found between the ML and TSL (r=0.510; P<0.05) as well as between TL and TSL (r=0.836; P<0.05).

It is apparent from the coefficient-correlation (r) values shown in Table-7 that the HL, HB, HS, ML, TL had positive correlation with TSL and between HB and HS have highly negative correlation.

The results of the present study indicated that, the genetic group of 100% Friesian and 50%SL×50%F are superior for all the dimensional characteristics of spermatozoa compared to other breeds as well as Pure (exotic) and Pure breed had improved dimensional characteristics of bull spermatozoa.

Table 7: Correlation between head length, head breadth, head shape, mid-piece length, tail length and total sperm length of bull spermatozoa

Biometrics of bull spermatozoa	Head length (HL) unit =μ	Head breadth (HB)unit =μ	Head shape (HS)	Mid-piece length (ML) unit =μ	Tail length (TL)unit =μ	Total sperm length (TSL) unit =μ
Head length (HL) (μ)	1.000	0.285**	0.417**	0.048	0.127**	0.348**
Head breadth (HB) (μ)	0.285**	1.000	-.746**	0.097**	0.135**	0.219**
Head shape (HS)	0.417**	-0.746**	1.000	-0.056	-0.040	0.033
Mid-piece length (ML) (μ)	0.048	0.097**	-0.056	1.000	0.008	0.510**
Tail length (TL) (μ)	0.127**	0.135**	-0.040	0.008	1.000	0.836**
Total sperm length (TSL) (μ)	0.348**	0.219**	0.033z	.510**	0.836**	1.000

** Correlation is significantly at the 0.01 level (2-tailed); Number of observation =915.

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