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Dimensional characteristics of spermatozoa of Nili-Ravi buffalo bulls: seasonal and climatic influences

Sajid HAMEED[1,*], Saima MASOOD[1], Hafsa ZANEB[1], Muhammad Sarwar KHAN[2], Muhammad YOUNIS[3], Muhammad AVAIS[2], Muti Ur Rehman KHAN[4]

[1]Department of Anatomy and Histology, University of Veterinary and Animal Science, Lahore, Pakistan
[2]Department of Clinical Medicine and Surgery, University of Veterinary and Animal Science, Lahore, Pakistan
[3]Semen Production Unit Qadirabad, Sahiwal, Livestock and Dairy Development Department, Punjab, Pakistan
[4]Department of Pathology, University of Veterinary and Animal Science, Lahore, Pakistan

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Abstract: This study was conducted in 2 different climatic zones of Punjab Province of Pakistan, the Sahiwal and Bahawalpur divisions, simultaneously for the period of 1 year divided into seasons. Seven adult Nili-Ravi buffalo breeding bulls from each division were used. Dimensional characteristics of spermatozoa were determined. Season had a nonsignificant effect on the sperm head length in both divisions and a nonsignificant difference was also observed between bulls of the Sahiwal and Bahawalpur divisions. The buffalo bulls of both divisions exhibited significantly increased sperm head breadth in autumn, whereas a nonsignificant difference was observed between divisions. Sperm head shape values were significantly different among the seasons in both the divisions whereas they were nonsignificantly different between the two divisions. Midpiece length value was significantly higher in winter than spring in both divisions and also significantly different between the divisions. Sperm tail length was significantly increased in autumn in Sahiwal and in autumn and spring in Bahawalpur. It was significantly longer in Sahiwal than Bahawalpur. The pattern of seasonal and climatic influence on total sperm length was similar to that of sperm tail length. In conclusion, autumn and spring seasons and the climate of the Sahiwal division have additive effects on sperm dimensions, except midpiece length.

Key words: Buffalo, Nili-Ravi, sperm, dimensional characteristics, season, climate

1. Introduction

Livestock is the major sector of agriculture that contributes 55.9% to agricultural GDP and 11.8% to the national GDP with a share of over 12% of the export earnings in Pakistan (1). Among livestock, buffalo (*Bubalus bubalis*) is a major milch animal and contributes about 61.66% of milk needs in Pakistan. According to the Economic Survey of Pakistan of 2013–2014, there are 34.6 million heads of buffalo in the country. Out of the total world buffalo population, 96% are in Asia and are almost exclusively owned by small landless farmers owing 2 or 3 animals/family. Buffalo is known as the world's second most important milch animal because it shares more than 95% of the milk produced in South Asia (2). Pakistan is home to the best dairy buffalo breeds of the world, the Nili, Ravi, Nili-Ravi, and Kundi breeds (3–5). Among these, the Nili-Ravi buffalo breed is the mainstay of the dairy industry in Pakistan. The home tract of the Nili-Ravi buffalo is in the canal-irrigated areas of central Punjab (6).

Testes are the main organ of the male reproductive system and testicular parenchyma is mainly composed of seminiferous tubules, where spermatozoa are produced, that maintain generation. For male sexuality and secondary male sex characteristics, testosterone hormone is produced in Leydig cells present in the testes (7).

In summer, the normal physiological functions in the body of the animal are affected, which causes a disturbance in the heat regulation system of the body. Increased environmental temperature during summer in tropical regions is an important limitation in animals' performance and the effects of high environmental temperature are aggravated when associated with increased humidity in the environment (8). The performance of the buffalo is reduced due to decreased intake of feed, metabolism disturbance, and disturbance in the secretion of the hormones and reactions taking place in the body as a result of heat stress. Buffalo bulls are comparatively more susceptible to heat stress than the females due to their poor heat regulation mechanism (9).

* Correspondence: sajidkumboh@yahoo.com

It is known that seasonality exists in buffalo reproduction (10) and therefore in the summer season the sexual performance of the animal is decreased (11). The effect of season on reproductive behavior of buffalo bulls is both direct and indirect. The direct effect of season is through macro- and microclimatic factors, like rainfall, humidity, temperature, and photoperiod. On the other hand, indirectly it acts by affecting vegetation, forage quality, and soil–plant–animal interactions. The magnitude of variations differs among breeds, locations, prevailing climatic conditions, feeding, and general management (12). Morphological modifications occurring in the testes in response to the different factors brought about by seasonal variations modulate the hormonal fluctuations, which in turn influence spermatogenesis (13).

Fertile sperms are produced as a result of the normal process of mitosis and meiosis of germ cells and appropriate functioning of germ cells and Sertoli cell (14). Although sperm dimensions of all species are under genetic control (15), in tropical bulls, the seasons influence the dimensional characteristics of spermatozoa (16,17). Very limited literature is available regarding seasonal influence on sperm dimensions in bulls. Change in environmental temperature influences the sperm head dimension and relative humidity also appears to influence the dimensions of sperm in buffalo bulls (18). The effect of relative humidity on the sperm dimensions has also been studied in Hariana bulls (17). However, no report is available on comparative climatic effects on dimensional characteristics of spermatozoa of buffalo breeding bulls when kept in different climatic zones. This paper describes the effect of seasonal and climatic variations on dimensional characteristics of spermatozoa in Nili-Ravi buffalo breeding bulls in the Sahiwal and Bahawalpur divisions of Pakistan.

2. Materials and methods

2.1. Study location and period

The study was conducted in two different climatic zones of Punjab Province of Pakistan, the Sahiwal and Bahawalpur divisions, simultaneously for the period of 1 year. Sahiwal is located at latitudes 30°N and 31.15°N and longitudes 73°E and 74°E, and at an altitude of 564 feet above sea level. It is a flat plane and an irrigated area of central Punjab with very fertile soil. Bahawalpur is located at latitudes 27°N and 29°N and longitudes 69°E and 75°E, and at an altitude of 380 feet above sea level. It is part of Southern Punjab and lies in the Cholistan desert areas of Punjab Province.

The climate of the two divisions is different. In the Sahiwal division, the average annual temperature is 31 °C and rainfall is 183 mm. The average humidity level of the area is 76%. The climate of the Bahawalpur division is hotter and arid, with average annual temperature and rainfall of 33 °C and 78 mm, respectively. The average humidity of the Bahawalpur division is 72% (http://www.pmd.gov.pk/).

The one year (2013–2014) was divided into four seasons, autumn (16 September to 14 November), winter (15 November to 15 February), spring (16 February to 30 April), and summer (1 May to 15 September). The summer season was further divided into dry summer (1 May to 30 June) and humid summer (1 July to 15 September) (19).

2.2. Experimental animals and semen collection

Fourteen adult Nili-Ravi buffalo breeding bulls (n = 7 from Semen Production Unit Qadirabad, Sahiwal division; n = 7 from Semen Production Unit Karaniwala, Bahawalpur division), aged 5–8 years, with clinically normal reproductive tract, were included in this study. Semen from experimental animals was collected fortnightly during early morning (before sunrise) with the help of an artificial vagina (IMV, France) connected with a rubber cone and graduated glass collection tube at a temperature of 42 °C, using an intact bull as a teaser. On each collection day, 2 ejaculates were collected from each breeding bull (20). After collection, semen was placed in a water bath maintained at 37 °C (21).

2.3. Initial semen analysis

The initial semen analysis was performed soon after collection. Among the semen parameters, volume and color were directly determined from the collection vials, and color of the semen was scored on a scale of 0 to 2 (20,22). A digital pH meter was used for semen pH (23). A phase contrast microscope with a heating stage was used to determine mass motility and individual sperm motility. Mass motility was scored according to the wave's pattern on a scale of 0 to 5 (24), whereas individual sperm motility was based on percentage of spermatozoa having normal forward progressive movement (25). Sperm concentration was measured using a photometer (Bovine photometer no. 1119, IMV, France) at 560 nm wavelength.

2.4. Measurement of sperm dimensions

Dimensional characteristics of spermatozoa from all the collected semen samples were studied. The slides for the sperm measurements were prepared with eosin-nigrosin stain (26). Stain having osmotic pressure of 290 mOsm/kg was prepared by dissolving 1% eosin B (w/v) (Art. 1343, Merck, Germany) and 5% nigrosin (w/v) (Art. 10172, Merck, Germany) into 3% trisodium citrate dihydrate solution (27). A small drop of semen was placed on a prewarmed glass slide and mixed with a relatively larger drop of eosin-nigrosin stain by an applicator stick. Instantly, a thin and uniform smear was made using a thin edge of coverslip and air-dried. Three slides were prepared from each semen sample and four unstained and morphologically normal spermatozoa from each slide were selected for sperm measurements. Six spermatozoa dimensional characteristics (head length, head breadth, head shape, midpiece length, tail length, and total sperm length) were studied. Head shape was determined by dividing the head length by head breath (28). All the measurements were performed using PixelPro software.

2.5. Statistical analysis

The data on seasonal variations within one zone were analyzed using one-way analysis of variance (ANOVA), whereas to compare seasons between two zones an independent Student t-test was applied. The differences between the seasons/factors were compared by the Duncan multiple range test. $P < 0.05$ was considered as statistically significant. Statistical analysis was conducted with SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Initial semen parameters

Data on initial semen parameters are given in Table 1. Ejaculatory volume, individual sperm motility, and sperm concentration were higher in buffalo bulls of Sahiwal than Bahawalpur division. Lower semen pH was recorded in buffalo bulls of Sahiwal compared to Bahawalpur division.

3.2. Dimensional characteristics of spermatozoa

3.2.1. Sperm head length

Values of sperm head length were observed to be 7.60 ± 0.01 µm and 7.58 ± 0.01 µm in buffalo bulls of the Sahiwal and Bahawalpur divisions, respectively. Season had a nonsignificant effect ($P > 0.05$) on sperm head length in both divisions. In the Sahiwal division maximum head length was observed in autumn, followed by spring and dry summer. Minimum head length was noted in winter and humid summer. In the Bahawalpur division maximum head length was observed in autumn and spring, followed in order by dry summer, winter, and humid summer. A nonsignificant difference ($P > 0.05$) in sperm head length was observed between buffalo bulls of the Sahiwal and Bahawalpur divisions. Overall sperm head length was also nonsignificantly different ($P > 0.05$) in buffalo bulls of the Sahiwal and Bahawalpur divisions (Table 2).

3.2.2. Sperm head breadth

Sperm head breadth values were 4.80 ± 0.02 µm and 4.74 ± 0.02 µm in buffalo bulls of Sahiwal and Bahawalpur, respectively. The buffalo bulls of Sahiwal exhibited significantly increased ($P < 0.05$) sperm head breadth in

Table 1. Minimum and maximum values of various semen parameters of Nili-Ravi buffalo bulls in two different climatic zones.

Divisions	Minimum	Maximum
Ejaculatory volume (mL)		
Sahiwal	1.50	9.50
Bahawalpur	1.00	6.50
Color (score 0–2)		
Sahiwal	0	2
Bahawalpur	0	2
pH		
Sahiwal	5.67	7.00
Bahawalpur	5.98	7.09
Mass motility (score 0–5)		
Sahiwal	1	3.50
Bahawalpur	0	4.50
Individual sperm motility (%)		
Sahiwal	40	85
Bahawalpur	0	75
Sperm concentration (millions/mL)		
Sahiwal	526	2035
Bahawalpur	401	1777

Table 2. Dimensional characteristics of spermatozoa of Nili-Ravi buffalo bulls in Sahiwal and Bahawalpur divisions during different seasons.†

Divisions	Seasons					
	Autumn	Winter	Spring	Dry summer	Humid summer	Overall mean
Sperm head length (µm)						
Sahiwal	7.63 ± 0.02^{Aa}	7.58 ± 0.02^{Aa}	7.61 ± 0.02^{Aa}	7.59 ± 0.02^{Aa}	7.58 ± 0.03^{Aa}	7.60 ± 0.01^{A}
Bahawalpur	7.61 ± 0.02^{Aa}	7.56 ± 0.03^{Aa}	7.61 ± 0.02^{Aa}	7.57 ± 0.02^{Aa}	7.55 ± 0.02^{Aa}	7.58 ± 0.01^{A}
Sperm head breadth (µm)						
Sahiwal	4.91 ± 0.05^{Ab}	4.76 ± 0.05^{Aa}	4.79 ± 0.04^{Aab}	4.77 ± 0.04^{Aa}	4.75 ± 0.04^{Aa}	4.80 ± 0.02^{A}
Bahawalpur	4.85 ± 0.04^{Ab}	4.71 ± 0.05^{Aab}	4.78 ± 0.05^{Aab}	4.67 ± 0.05^{Aa}	4.70 ± 0.04^{Aa}	4.74 ± 0.02^{A}
Sperm head shape (head length/head breadth)						
Sahiwal	1.55 ± 0.01^{Aa}	1.59 ± 0.01^{Ab}	1.59 ± 0.01^{Ab}	1.59 ± 0.01^{Ab}	1.60 ± 0.01^{Ab}	1.59 ± 0.01^{A}
Bahawalpur	1.57 ± 0.01^{Aa}	1.61 ± 0.01^{Ab}	1.59 ± 0.01^{Aab}	1.62 ± 0.01^{Ab}	1.61 ± 0.01^{Ab}	1.60 ± 0.01^{A}
Sperm midpiece length (µm)						
Sahiwal	11.53 ± 0.04^{Aab}	11.61 ± 0.04^{Ab}	11.48 ± 0.04^{Aa}	11.56 ± 0.04^{Aab}	11.51 ± 0.03^{Aab}	11.54 ± 0.02^{A}
Bahawalpur	11.65 ± 0.04^{Bab}	11.71 ± 0.03^{Ab}	11.57 ± 0.04^{Aa}	11.62 ± 0.04^{Aab}	11.63 ± 0.04^{Aab}	11.64 ± 0.02^{B}
Sperm tail length (µm)						
Sahiwal	46.01 ± 0.93^{Ab}	42.82 ± 0.71^{Aa}	44.55 ± 0.81^{Aab}	42.62 ± 0.68^{Aa}	42.08 ± 0.93^{Aa}	43.62 ± 0.42^{A}
Bahawalpur	42.08 ± 0.97^{Bb}	39.06 ± 1.05^{Ba}	42.09 ± 0.92^{Ab}	38.09 ± 0.82^{Ba}	38.10 ± 0.95^{Ba}	39.88 ± 0.51^{B}
Total sperm length (µm)						
Sahiwal	65.17 ± 0.92^{Ab}	62.01 ± 0.69^{Aa}	63.64 ± 0.79^{Aab}	61.77 ± 0.67^{Aa}	61.17 ± 0.92^{Aa}	62.75 ± 0.42^{A}
Bahawalpur	61.34 ± 0.96^{Bb}	58.33 ± 1.04^{Ba}	61.27 ± 0.91^{Ab}	57.28 ± 0.80^{Ba}	57.28 ± 0.94^{Ba}	59.10 ± 0.50^{B}

†Values are mean ± SE.
Values in each row with different superscript lowercase letters differ significantly (P < 0.05).
Values in each column with different superscript uppercase letters differ significantly (P < 0.05).

autumn than winter, dry summer, and humid summer. Likewise, the buffalo bulls of Bahawalpur demonstrated significantly increased (P < 0.05) head breadth in autumn than dry summer and humid summer. Division-wise comparison of sperm head breadth showed a nonsignificant difference (P > 0.05) in buffalo bulls of Sahiwal and Bahawalpur in all the seasons (Table 2).

3.2.3. Sperm head shape

Sperm head shape values were 1.59 ± 0.01 and 1.60 ± 0.01 in buffalo bulls of Sahiwal and Bahawalpur, respectively. Sperm head shape value was significantly lower (P < 0.05) in autumn than other seasons in buffalo bulls of Sahiwal. Similarly, in Bahawalpur sperm head shape value was also significantly lower in autumn (P < 0.05) than all other seasons, except spring. The values of sperm head shape were nonsignificantly lower (P > 0.05) in buffalo bulls of Sahiwal than Bahawalpur in all the seasons except spring. Overall sperm head shape values were nonsignificantly different (P > 0.05) in Sahiwal and Bahawalpur (Table 2).

3.2.4. Sperm midpiece length

Sperm midpiece length was 11.54 ± 0.02 µm and 11.64 ± 0.02 µm in buffalo bulls of Sahiwal and Bahawalpur, respectively. In Sahiwal the midpiece length value was significantly higher (P < 0.05) in winter than spring season. Likewise, in Bahawalpur midpiece length value was also observed to be significantly higher (P < 0.05) in winter than spring. Midpiece length value was nonsignificantly lower (P > 0.05) in Sahiwal than Bahawalpur during all seasons except autumn, when it was significantly lower (P < 0.05) in Sahiwal. Overall midpiece length value was significantly lower (P < 0.05) in Sahiwal (Table 2).

3.2.5. Sperm tail length

Sperm tail length values were 43.62 ± 0.42 µm and 39.88 ± 0.51 µm in buffalo bulls of Sahiwal and Bahawalpur, respectively. In buffalo bulls of Sahiwal sperm tail length was significantly increased ($P < 0.05$) in autumn as compared to winter, dry summer, and humid summer. Sperm tail length of buffalo bulls in Bahawalpur was significantly increased ($P < 0.05$) in autumn and spring compared to other seasons. Sperm tail length of buffalo bulls was significantly longer ($P < 0.05$) in Sahiwal compared to Bahawalpur in all the seasons except spring. Overall the sperm tail length was significantly longer ($P < 0.05$) in buffalo bulls of Sahiwal than Bahawalpur (Table 2).

3.2.6. Total sperm length

Total sperm length of 62.75 ± 0.42 µm and 59.10 ± 0.50 µm was observed in Sahiwal and Bahawalpur, respectively. The pattern of seasonal influence on total sperm length was similar to that of sperm tail length in both divisions. In buffalo bulls of Sahiwal the total sperm length was significantly increased ($P < 0.05$) in autumn compared to winter, dry summer, and humid summer. Total sperm length of buffalo bulls in Bahawalpur was significantly increased ($P < 0.05$) in autumn and spring compared to other seasons. Total sperm length of buffalo bulls was significantly longer ($P < 0.05$) in Sahiwal compared to Bahawalpur in all the seasons except spring. Overall the total sperm length was significantly longer ($P < 0.05$) in buffalo bulls of Sahiwal than Bahawalpur (Table 2).

4. Discussion

Reproductive performance of buffalo bulls is directly or indirectly affected by season and climate. Direct effects are due to climatic factors, like rainfall, humidity, temperature, and photoperiod, whereas indirect effects result from vegetation, fodder quality, and soil–plant–animal interactions. However, the magnitude of these effects are variable, depending upon breed, place, climatic differences, feeding practices, and general management procedures (29). In response to seasonal variations the testes undergo morphological modulations resulting in hormonal fluctuations and ultimately affecting spermatogenesis (13). Increased environmental temperature in summer has a detrimental effect on semen volume and sperm concentration. Likewise, extremes of heat or cold deteriorate sperm motility (30).

In the present study, values of sperm head length were 7.60 ± 0.01 µm and 7.58 ± 0.01 µm in buffalo bulls of the Sahiwal and Bahawalpur divisions, respectively. In a previous study the sperm head length in Murrah buffalo bulls was 7.59 ± 0.01 µm (31), which is in close agreement with the findings of the present study. In a previous study on dimensional characteristics of spermatozoa in different breeds of cattle bulls, mean sperm head length of 9.164 ± 0.53 µm was reported (28). The sperm head lengths of Sahiwal, Friesian, and crossbred (Friesian × Sahiwal) cow bulls are 8.85 ± 0.88 µm, 9.15 ± 0.19 µm, and 8.43 ± 0.53 µm, respectively (32). This indicates that sperm head length is variable in different species and in various breeds within the same species. In the present study, seasonal influence on sperm head length was nonsignificant and longer sperm heads were seen in autumn and spring compared to winter, dry summer, and humid summer in both of the divisions. Longer sperm heads during autumn and spring could be the result of enhanced spermatogenesis/increased seminiferous epithelial height, resulting in better quality of semen. In a previous study nonsignificant seasonal effect on sperm head length was observed in buffalo bulls (18). Similar to the results of the present study, previous researchers found longer sperm heads in autumn and spring than other seasons (18). Increased sperm head length in buffalo bulls in the Sahiwal division could be the result of overall better orchidometric values and better quality of semen than in Bahawalpur.

In the present study, sperm head breadth values were 4.80 ± 0.02 µm and 4.74 ± 0.02 µm in buffalo bulls of Sahiwal and Bahawalpur divisions, respectively. In earlier studies sperm head breadth was 5.11 ± 0.01 µm in crossbred and 4.91 ± 0.01 µm in Murrah buffalo bulls (31). In cattle sperm head breadth of 4.70 ± 0.38 µm was observed (28). In the present study, unlike sperm head length, head breadth was significantly influenced by seasons, which is supported by previous findings in buffalo bulls (18). Increased sperm head breadth observed in autumn and spring in the present study is also congruent with the results of an earlier study in buffalo bulls (18). Sperm dimensions of all species are under genetic control (16), but in tropical areas the seasons influence the dimensional characteristics of spermatozoa (16,17).

In the present study, head shape values were 1.59 ± 0.01 and 1.60 ± 0.01 in buffalo bulls of Sahiwal and Bahawalpur divisions, respectively. Similar findings were recorded in a previous study on buffalo bulls (18). Contrarily, in previous studies on cow bulls, an increased sperm head shape value was observed (26,28) that revealed the difference in sperm head shape between buffalo bull and cow bulls. In this study, sperm head shape value was decreased in autumn in both the divisions, which is supported by the results of earlier studies in buffalo bulls (18).

In the present study, sperm midpiece length was 11.54 ± 0.02 µm and 11.64 ± 0.02 µm in buffalo bulls of Sahiwal and Bahawalpur divisions, respectively. These results are congruent with previous findings in buffalo bulls (18). An increased sperm midpiece length (13.37 ± 1.18 µm) was present in cattle bulls (28). In another study, longer midpiece lengths in Sahiwal (13.72 ± 0.51 µm), Friesian (13.22 ± 0.57 µm), and crossbred (13.35 ± 0.56 µm) cow bulls were observed (32). In this study, the midpiece length of buffalo bulls in the Sahiwal division was significantly greater in winter than spring, while it was lowest in spring. Similar results were found in the Bahawalpur division. In an earlier study, nonsignificant seasonal effect on midpiece length was reported in buffalo bulls (18). Midpiece length was significantly different between buffalo bulls of Sahiwal and Bahawalpur divisions.

In contrary to published data on sperm tail length in crossbred and Murrah buffalo bulls (31), sperm tail length in the present study was shorter in buffalo bulls of both the Sahiwal and Bahawalpur divisions. In cattle bulls sperm tail length of 46.65 ± 1.93 µm was reported (28). Likewise, sperm tail length of 45.06 ± 3.69 µm, 45.35 ± 6.86 µm, and 43.13 ± 5.456 µm in Sahiwal, Friesian, and crossbred (Friesian × Sahiwal) cow bulls was documented, respectively (32). In the present study, seasonal effect on sperm tail length was significant in both of the divisions. The sperm tail length was increased in autumn in Sahiwal, while it was longer in autumn and spring in Bahawalpur. This revealed that milder seasons have augmented effect on tail length. Significantly increased sperm tail length in buffalo bulls of Sahiwal than Bahawalpur could be attributed to the favorable climate of Sahiwal.

Total sperm length of 62.75 ± 0.42 µm and 59.10 ± 0.50 µm was observed in Sahiwal and Bahawalpur divisions, respectively. In a previous study on different breeds of cattle, longer sperm length (69.18 ± 2.40 µm) was observed (28). Similar to tail length, the seasonal influence on total sperm length was observed to be significant in both the divisions with longer sperms in autumn in Sahiwal, while it was longer in autumn and spring in Bahawalpur as compared to other seasons. Significantly longer sperms were observed in Sahiwal as compared to Bahawalpur division. The justification of seasonal and climatic variations in total sperm length is the same as for tail length, because in mammals, the total sperm length variations are mostly due to the tail length variations and sperm length is mainly contributed by the tail (33). This means that milder seasons have an augmented effect on the total sperm length. Longer sperm observed in Sahiwal as compared to Bahawalpur could be attributed to the favorable climate of Sahiwal for spermatogenesis.

In conclusion, autumn and spring have additive effects on sperm dimensions, except for the midpiece length. Sperm head length, head breadth, tail length, and total sperm length are greater in Nili-Ravi buffalo breeding bulls in the Sahiwal division, whereas sperm head shape and sperm midpiece length are greater in bulls of the Bahawalpur division.

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