SOME PHYSICAL AND CHEMICAL PROPERTIES OF BUFFALO SEMEN

By

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SUMMARY

Semen from four buffalo bulls was used in a study of the physical properties and the determination of fructose, inorganic phosphorus, calcium, and total nitrogen of buffalo semen. The averages for ejaculate volume, initial motility, pH, and semen concentration were 3.50, 2.35 ml.; 3.14, 3.19; 6.90, 6.92; and 1193.44×10⁶, 1179.98×10⁵ per ml., in the first and second ejaculates, respectively. The percentage of abnormal spermatozoa was 14.41 and the mean specific gravity was 1.0293. No significant variations in smen volume, initial motility, pH, and sperm concentartion, were found between the first and second ejaculates, however, significant variations were observed between bulls in semen volume, initial motility and pH. Initial motility and pH of smen were negatively correlated.

The mean initial fructose values in buffalo smen were 468.75 and 364.45 mg. per 100 ml. for the first and second ejaculates, respectively. No significant variations were found neither between ejaculates nor between bulls, in fructose content of semen. A high level of fructose in semen did not always coincide with neither the high sperm concentration nor the high degree of sperm motility.

The average concentrations (mg./100 ml.) of inorganic phosphorus, calcium and total nitrogen in buffalo semen were 12.83, 15.42; 59.66 56.99; and 648.50, 626.50 for the first and second ejaculates, respectively. The differences in semen content of inorganic phosphorus and calcium were significant only between bulls and not between ejaculates.

INTRODUCTION

The lowered fertility levels of buffalo cows and heifers in the U.A.R. could be attributed partly to the lower fertility of buffalo bulls, and the need for specific standards for buffalo semen evaluation requires a thorough investigation. Such qualities of semen as color, volume per ejaculate, initial motility, pH, sperm concentration, percentage of abnormal spermatosoa and specific gravity were considered satisfactory for the evaluation of semen.

During the past two decades, there has been an increasing interest in the study of the chemical composition of bull semen due to the expanding practice of artificial insemination and to the growing recognition of dairy cattle infertility. The relationship between the chemical constituents of bull semen

and fertility has been shown by several investigators (Lardy and Phillips, 1943; Bonadonna et al, 1954; Bishop and Salisbury, 1955; White, 1956; and Cragle and Salisbury, 1959). Fructose in buffalo semen has been reported of a higher concentration than that in Indian buffaloes (Oloufa et al, 1959 and Roy et al, 1960). Values reported for inorganic Phosphorus, calcium and total nitrogen differed markedly between the Egyptian and Indian buffaloes and also within the same type. The present study, there fore, dealt also with the chemical composition of semen from Egyptian buffloes.

MATERIALS AND METHODS

Semen samples were collected from four breeding buffalo bulls kept at the Agricultural Experiment Station of Ain Shams College of Agriculture. Bulls were used for semen collection once or twice per week throughout the period of study that lasted from April 1960 till December 1962. All collections were made by the artificial vagina. Two rapid successive ejaculations were collected and each was treated separately. A total of 211 ejaculates were treated in this sudy. From the calibrated collecting tube, volume per ejaculate was determined just after collection. Color and density were determined visually. The determination of pH value was carried out by the application of fractionized pH comparative paper. Motility was determined under the low power of microscope and grades from 0 to 5 were given according to motility of spermatozoa. The determination of motility was carried out within not more than 10 minutes after collection and using a thermostatically controled warm cabinet (37 C) inside which the microscope was placed. Neubaur hemocytometer was used for counting of sperms and the percentsge of abnormal spermatozoa was determined in a stained film using 0.5%. alcoholic eosin. The weight of 10 ml. of semen in a volumetric flask was determined and the specific gravity was calculated.

The method of Roe (1934), for the determination of blood and urine fructose was used with the following slight modifications: Standard solutions containing 1.000, 0.500, 0.250, and 0.125 ml. of the original furctose solution, containing one percent (per weight) fructose, each was diluted in 50 ml. volumetric flask to obtain concentrations of fructose of 0.200, 0.100, 0.050 and 0.025 mg. per ml., respectively. One ml. of semen was diluted with 4 ml. of distilled water and one part of this diluted semen + 7 parts distilled water + 1 part of 10% Zn SO₄.7 $\rm H_2O+$ 1 part of 2% NaOH; all were added together and mixed thoroughly, then filterated. The total volume was 10 ml., containing 0.2 ml. of semen. Two ml. of filtrate, were transferred into 15 ml. test tube, then 2 ml. resorcinol 1% and 6 ml. 30% HC1 were added. The total volume became 10 ml. containing 0.04ml. semen. Two ml. of each standard fructose solutions were treated in the same way. Fluids were strongly mixed and heated st 80°C. for 8 minutes in a water bath, then cooled again using tap water. Test tubes were compared in a HK2 photo-electric colorimeter using s green filter (wave length 4920-5500 A°) to determine light sbsorption value. Readings were obtained for both fructose standards containing 0.005, 0.010, 0.020 and 0.040 mg. fructose per ml. and semen dilution samples containing 0.004 semen per 1 ml.

A standard curve was drawn in which the vertical line represented the colorimeter reading, and the horizontal line represented the concentration of fructose per ml. of the standard solution. This standard curve was used for the determination of fructose concentration per 0.004 ml. of semen. Fructose values obtained by the use of this standard curve were then multiplied by 25000 in order to obtain the fructose concentration per 100 ml. of semen.

Fructolysis was estimated by determining the fructose content in semen samples kept under room temperature, 37°C., and 5°C. for various intervals. Initial fructose content was determined in all cases 15 minutes after collection. Repeated fructose estimations were made after 6 hours and after 24 hours from the initial one. The fructose utilization (U) was determined by claculating the amount of fructose utilized by 10° sperms during the first six hours and during 24 hours after the initial fructose determination.

The method of Fiske and Subbarow (1925), for the determination of inorganic phosphorus in serum and plasma, was used in the determination of inorganic phosphorus in semen. A slight modification was made due to the high concentration of inorganic phosphorus in buffalo semen. The dilution of semen 1:4 with distilled water, was considered better for obtaining a clear blue color for calorimeter reading. Calcium was determined by the method of Clark and Collip (1925). Total nitrogen was estimated by Kjeldahl distillation method.

RESULTS AND DISCUSSION

Physical properties:

The normal color of buffalo semen is milky white. Color is generally affected by sperm concentration, and samples with high sperm concentration were deeper in color. As shown in table 1, the mean volume of the first ejaculate was greater than the second one, yet this difference was not statistically significant. These results are in accordance with these shown by Prabhu and Bhattacharya (1951), in Indian buffaloes. The variations among buffalo bulls in semen volume were significant (P<0.01). Volume of semen is affected by the secretory activity of the accessory glands, especially the prostate and seminal vesicle. Perry (1960), stated that the seminal vesicle varied in size between bulls and that more than half of the ejaculate was produced from these glands.

The mean volume of buffalo semen observed in this study (3.50±0.1729) did not vary markedly from the values obtained by other workers (Mahmoud, 1952; Sayed and Oloufa, 1956; Oloufa and Sayed, 1956; Hafez and Darwish, 1956; Madatov, 1956; and Oloufa et al, 1959). Semen volume per ejaculate in cattle is apparently larger than in the buffalo as observed by the previous workers and by Mann (1954) and Perry (1960). The difference in semen volume between cattle and buffaloes may be characteristic of this species. It should be noted, however, that the testes and epididymal weight and size in buffaloes are smaller than in cattle which could also contribute to the smaller volume of the ejaculate (El-Sheikh, 1963).

The variations between ejaculates in the initial motility of spermatozoa were not significant. Hafez and Darwich (1956), showed that the more frequent collection caused a significant reduction in semen motility. This is dependent upon the time interval between ejaculates. Variations between bulls in initial motility of spermatozoa were significant. Spermatozoa with poor motility are considered incapable of fertilization since they fail to reach the ovum in good numbers (Anderson, 1945). Motile spermatozoa, however, are not always fertile (El-Sheikh and Casida, 1954).

Buffalo semen is slightly acid (table 1), and the mean pH obtained in the present study (6.9) was similar to that obtained by Mahmoud (1949), Sayed and Oloufa (1956) and Oloufa et al (1959). Differences in pH values of semen were not significant between bulls. A significant negative correlation was found between pH of semen and initial motility (P<0.01). Highly motile sperms decreased the pH of smen more rapidly than sperms with lower motility. Thus the determination of pH may be considered an indication of semen quality. Anderson, (1942), stated that the more acid the bull semen was at collection, the better was its retained motility on storage.

Variations in sperm concentration of buffalo semen were observed, yet the differences lacked statistical significance both between bulls and between ejaculates. Prabhu and Bhattacharya (1951), found significant differences between the first and second ejaculates of buffalo semen in sperm concentration. However, Vidric and Davidovic (1960), found no variations in the concentration of spermatozoa between the two successive ejaculates in cattle bulls. Hafez and Darwich (1956), and Oloufa and Sayed (1956), found less sperm Concentration in buffalo semen than what was obtained in the present sutdy (645 imes 106 and 939 imes 106 per ml.) respectively. The latter investigators used 3 years old buffalo bulls in their experiment and smaller number of samples. When Sayed and Oloufa (1957), collected buffalo semen once every week, they obtained a similar mean value for sperm concentration as that obtained in the present study. Variations in sperm concentration are always expected since the spermatogenic activity of the testes is subject to considerable physiological fluctuations of hormonal origin and influenced by factors such as light, temperature, season, state of nutrition etc. (Mann 1954).

The average percentage of abnormal spermatozoa was 14.41 ± 1.16 (table 1). This value is higher than that estimated by Kushwaha *et al.* (1955), Prabhu and Bhattacharya (1951), Medatov (1956), in Indian buffaloes and Oloufa and Sayed (1956) and Oloufa *et al.* (1959), in Egyptian buffaloes.

The mean value for the specific gravity of buffalo semen was 1.0293. A mean value of 1.0194 was obtained by Mahmoud (1952). The mean value of specific gravity of semen from cattle bulls is slightly higher than that from buflalo bulls. Anderson, (1946) and Mann, (1954), found that the specific gravity of bull semen was 1.033-1.035. Samples containing high sperm concentration had higher values for specific gravity than samples of lower concentration. The fluctuations in specific gravity of semen were due to the variable ratio of spermatozoa to seminal plasma.

TABLE I.—Mean, standard deviation, and coefficient of variability for the first, second, and combined ejaculates

Observation	First ejaculate					
	No. of Samples	Mean	σ	C.V. %		
Volume ml	92	3.50 ± 0.1729	1.66	47.43		
Motility	77	3.14 ± 0.0760	0.87	21.27		
pH	80	6.90±0.0596	0.14	19.71		
\times 1.000.000)	50	1193.44 ± 90.0127	636.39	53.32		
% atnormals	-	200	-	-		

Observation	Second ejaculate					
Observation	No. of Samples	Mean	σ	C.V. %		
Volume ml	87	3.25 ± 0.1755	1.64	50.37		
Motility	75	3.19 ± 0.0934	0.81	25.36		
pH	76	6.92 ± 0.0128	0.11	16.19		
\times 1,000,000)	45	1179.98±55.6766	373.59	31.66		
% abnormals			-	-		

Observation	Combined ejaculates					
Observation	No. of Samples	Mean	. σ	C.V. %		
Volume ml	17	6.39 ± 0.4660	1.92	30.05		
Motility	17	3.70 ± 0.0975	0.40	10.85		
pH	17	6.85 ± 0.0136	0.06	0.82		
\times 1,000,000)	17	1450.29 ± 11.0971	457.20	31.52		
% abnormals	17	14.41 ± 1.1650	4.80	33.31		

[&]quot; Of two successive ejaculates together.

Fructose content and fructolysis:

The average frucose concentration in the combined ejaculates of buffalo semen was higher than the averages for each ejaculate separately (table 2). Collection of combined ejaculates was made only once every week and collection twice per week was made when each ejaculate was analysed separately. Apparently more frequent collections caused a reduction in fructose content. These results agreed fairly well with those of Sayed and Oloufa (1957) who collected semen once and three times per week. The above investigators (1956), found that the average fructose content of buffalo semen ranged between 325 and 478 mg. per 100 ml. In another study Oloufa, et al (1959), obtained an average concentration, of fructose in buffalo semen, of 680.06 mg per 100 ml. Roy, et al (1960), found a lower fructose concentration in semen of Indian buffalo (355 mg./100 ml.). These differences in fructose content of buffalo semen may be due to such factors as individuality and breed, intervals between collections, method of fructose determination, time interval between collection and determination, temperature and sperm concentration. Ehlers, et al. (1953; Ehlers, et al (1958); Ullner and Schmitz, (1958); Freund, et al (1959 b); Erb, et al (1959); and Freund, et al (1959 a), found that the average fructose concentration in bull semen ranges between 424 and 552 mg. per 100 ml.

TABLE 2.—Mean, standard deviation and coefficient of variability of fructose concentration in buffalo semen

Observation	First ejaculate	Second ejaculate	Combined* ejaculates
Number af collections	21	16	17
Mean (mg./100 ml.)	468.75±54.18	364.45±37.75	591.54±39.81
Standard deviation	248.16	150.99	164.40
Coefficient of variability	52.94	41.43	27.79

^{*} Of two successive ejaculates.

No significant variations were found between ejaculates, in the level of fructose in buffalo semen which may be due, amongst other things, to the fairly regular intervals between collections and that no multiple ejaculates were collected. Mann (1948), however, found that the multiple collection of bull semen was not accompanied by a fall in fructose concentration. In 1954, Mann showed that in some bulls it is possible to recover up to 50 ml. of seminal vesicle secretion, which is enough to provide at least 12 fructose-rich ejaculates. The differences between buffalo bulls in fructose oncentration were not significant.

The high level of fructose in semen, is generally correlated with the better semen quality, yet it does not necessarily reflect the fertilizing capacity of spermatozoa. Mann, (1948), observed a normal level of fructose in bulls of low fertility and in case of azeospermy. Semen samples, therefore, could show a high fructose content but low sperm concentration. The source of fructose in semen is the seminal plasma and not the sperm cell (Mann, 1948). Therefore, there is no constant ratio between sperm density and fructose content. Higher values for fructose concentration in buffalo semen was observed in the present study in some samples with low sperm concentration. Erb, et al. (1956), stated that the greater concentration of spermatozoa in bull semen reduced plasma volume and thereby reduced the fructose content per ml. of semen. Mann (1948), Gassner, et al. (1952), and Perry (1960), found that fructose was not utilized by azeospermic or by necrospermic semen.

The incubation of freshly ejaculated buffalo semen at 37°C was accompanied by a more rapid decrease in the amount of fructose content than that obseved in samples kept at room temperature (28°C) or kept in a refrigerator at 5°C. Fructose utilization (U) was hinger after 24 hours of incubation than after 6 hours at all the temperatures of incubation (table 3). The results obtained indicate that the furctolytic rates depend upon the length of incubation period and the incubation temperature. Gassner, et al (1952), showed that adding fructose to fructose-poor ejaculates may prolong the potency and longivity of semen samples, however, adding fructose to samples initially containing an adequate amount of fructose and showing quite satisfactory fructolysis index, exhaustes the ability of spermatozoa to utilize the added fructose.

The high level of fructose in buffalo semen did not always coincide with higher sperm concentration or with a higher degree of sperm motility (table 3). Mann (1948), however, showed that semen samples of low fructose content were those from bulls with a poor fertility record. Other investigators (Rollison (1951); Anderson (1951); Gassner, et al. (1952); Schmidt and Steager (1957); and Mixner, et al. (1957), indicated a close relationship between fructolysis and fertility. It is generally believed that fructolysis index affords a more accurate measure for semen quality and for estimating the breeding efficiency of bulls.

Inorganic phosphorus in buffalo semen:

The averages for inorganic phosphorus concentration in buffalo semen were 12.83+1.88 and 15.42+2.18 mg./100 ml. in the first and second ejaculates respectively (table 4). Sayed and Oloufa (1957), Oloufa, et al. (1959), and Roy, et al. (1960), obtained mean values of inorganic phosphorus in buffalo semen lower than those obtained in the present study and ranging between 6.40 and 9.67 mg./100 ml. On the other hand Perry (1960), showed that the mean value of inorganic phosphorus in buffalo semen was 17 mg./100 ml. Mann (1954) and White (1958), found that the mean value of inorganic phosphorus in bull semen was 9 mg. 100 ml.

TABLE 3.—Fructose utilisation in buffalo semen at 37°C,

		12	Fructose concentration							
Initial motility	Sperm concent, × 10 ⁶	Initial content	37°C							
			6 hr.	24 hr.	Utilisation U					
					6 hr.	24 hr.				
Bull 219 :										
3.6	1460	250.000	050.000	000,000	0.1369	0.1712				
4.1	1380	743.750	225.000		0.3759	_				
3.9	2105	668,750		_						
3.8	1310	437.500	225,000	043.750	0.1622	0.3006				
4.3	1050	450.000	-		_					
3.8	1670	612.500	375.000	031.250	0.1422	0.3481				
2.8	2630	706.250	468.750	025.000	0.0903	0.2590				
3.7	1210	793.750	375.000	025.000	0.3461	0.6353				
4.0	1880	750.000	375.000	056.250	0.1995	0.3690				
Average		_			0.2076	0.3472				
	= 1	,	* 3			0.				
Bull 292:			2							
3.4	820	450.000	075.000	000.000	0,4573	0.5488				
3.6	930	656.250		_						
3.8	1170	812.500	375.000	275,000	0.5739	0.4594				
3.6	1500	400.000	150.000	000.000	0.1666	0.2666				
2.8	1580	712.500	375.000	025.000	0.2136	0.4351				
3.7	990	437.500	250.000	025.000	0.1894	0.4166				
3.7	1670	500.000	168,750	025.000	0.1984	0.2844				
4.1	1300	675.000	375 000	100.000	0.2308	0.4423				
Average	8	-			0.2614	0.4076				

ROOM TEMPERATURE AND 5°C AT 6 AND 24 HOURS INTERVAL

mg./100 ml. semen

	1	room tem	perature		5°C.				
	6 hr.	24 hr.	Utilisation (U)				Utilisation (U)		
			6 hr.	24 hr.	6 hr.	24 hr.	6 hr.	24 hr.	
	062.500	025,000	0.1291	0.1541					
	300.000	156.250	0.3216	0.4257			_		
	456.250	087.500	0.1009	0.2761					
	250.000	075.000	0.1431	0.2767			_	-	
	293.750	025.000	0.1488	0.4048	375.000	200.000	0.0714	-	
	412.500	231.250	0.1198	0.2283	456.250	412.500		0.2001	
	500.000	175.000	0.0784	0.2019	666.250	468.750		0.1100	
		206.250	0.1601	0.4855	693.750	668,750	0.0000	0.0008	
	500.000	250.000	0.1329	0.2659	668.750	500.000	0.0626	0.1000	
			0.1483	0.3021		000.000	0.0452	0.1329	
					1	1	0.0058	0.1369	
	-					1			
- 1	12.500	000.000	0 4770				-1		
				0.5488	-	-	_		
		0.0	0.000	0.2218	-		-	-	
				0.3472	_			_	
						225.000	0.1000	0.1166	
						12.500	0.0752	0.1899	
	1					87.500	0.0674	0.0505	
		- 1			750	- 1	0.0792	0.1227	
					00.000 4	37.500	0.1346	0.1827	
		0.	. 2083 0	. 3042			0.0792	0.1281	

TABLE 4.—Mean, standard deviation and coefficient of variability for inorganic phosphorus, calcium, and total Nitrogen in buffalo semen.

Observation	First ejaculate						
*	No. of samples	Mean	σ	C.V. %			
Inorganic phosphorus							
	12	12.830 ± 1.880	6.53	50.89			
Calcium	10	59.663±5.123	16.24	27.22			
Total Nitrogen	10	648.500±38.681	122.62	18.19			
	Second	ejaculate					
Inorganic Phosphorus	12	15.420±2.180	7.56	49.03			
Calcium	10	56.987 ± 4.817	15.27	26.79			
Total Nitrogen	10	626.500±92.974	294.73	47.04			
, Co	mbined e	ejuculates					
norganie Phosphorus	_	_					
alcium	_) - 1				
otal Nitrogen	6	797.333±53.183	130.30				

^{*} Of two successive ejaculates together.

Significant differences were found between bulls in the concentration of inorganic phosphorus in semen (P<0.01). Such differences may be due to variations in sperm concentration. Cragle and Muntz (1956), and Cragle, et al. (1958 b), showed that the concentration of phosphorus in the sperm cells was higher than that in the seminal plasma. The pressent data showed that the variations between ejaculates in organic phosphorus were not statistically significant.

Calcium concentration in buffalo semen:

The averages calcium concentration in ten collections obtained from two buffalo bulls were 59.663 + 5.123 and 56.987 ± 4.817 mg./100 ml. for the first and second ejaculate, respectively (table 4). The mean calcium concentration of the first ejaculate was insignificantly higher than in the second one. Anderson(1945), Mann(1954), White(1958), and Cragle ,et al.(1958 a) obtained mean values for calcium concentration in bull semen of 35.2, 34.0, 34.0, and 26.0 mg./100 ml., respectively. Roy, et al. (1960), and Perry (1960) mentioned values of 40.0 and 42.0 mg./100 ml. respectively for calcium concentration in Indian buffalo semen which is near to the value reported by Cragle, et al. (1958 b) for bull semen.

The results obtained indicated significant variations (P<.0.01) between buffalo bulls in calcium concentration in semen. Cragle, et al (1958 a), showed that there are differences in calcium concentration between washed spermatrozoa and seminal plasma of bulls. Rothschild and Barnes (1954), found no differences between bulls in the calcium concentration of the seminal plasma. Since the concentration of spermatozoa in different semen samples may vary widely, variations in calcium concentration in the whole semen are expected.

Such high levels of calcium in buffalo semen, may be the cause of its short period of livability. Lardy and Phillips (1943), described the deleterious effect of calcium upon respiration and glycolysis and its depressing effect on motility of bull spermatozoa. Blackshaw (1953), showed that calcium is undesirable in diluents due to its depressing effect on motility of bull spermatozoa. Further studies are needed to dedermine the effect of different calcium levels on the viability of spermatozoa in buffalo semen.

Total nitrogen in buffalo semen:

The average concentration of total nitrogsn in ten collections of the first ejaculates obtained from three buffalo bulls was 648.500± 38.681 mg. /100 ml. The mean in the second ejaculates was 626.500±92.974 mg./100 ml. A mean of 797.333 ± 53.183 mg. per 100 ml. for combined two successive ejaculates, was obtained in six collections using four buffalo bulls (table 4) The average total nitrogen obtained in the present study was higher than that obtained by Oloufa, et al (1959). A total protein value of 2.93 g. per 100 ml. represents 468 mg. of total nitrogen/100 ml. Total nitrogen concentration in semen is dependent upon the concentration of spermatozoa in semen samples. Oloufa, et al (1959), in their above study used buffalo bulls of lower sperm concentration than the bulls used in the present work.

The concentrations of total nitrogen in bull semen were 756 and 755 mg./100 ml. as reported by Mann(1954), and White (1958), respectively. It could be seen that buffalo bulls resemble cattle bulls in the total nitrogen content of semen, however, the mean sperm concentration in buffalo bulls is apparently higher than that in cattle.

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بعض الصفات الطبيعية والكيميائية للسائل المنوى في الجاموس

اللخص

استخدمت في هذا البحث أربعة عجول جاموس تابعة لمحطة البحوث الزراعية بكلية الزراعة جامعة عين شمس كان يجمع السائل المنوى منها لدراسة الخواص الطبيعية للسائل المنوى ولتقدير تركيز الفركتوز ومعدل استعمال الفركتوز ولتقدير الفسفور الغير عضوى والكالسيوم والنتروجين الكلى •

وكان متوسط حجم القذفة وحركة الحيوانات المنوية عند الجمع وتركيز ايونات الايدروجين وتركيز الحيوانات المنوية بالسائل المنوى كالآتى :

0.7 سم 7-31.7 – 9.7 – 9.7 – 9.7 (a) 1 سم 7 b) القذفة الأولى على التوالى أما في القذفة الثانية فكانت المتوسطات كالآتى على التوالى: 0.7 سم 0.7 – 0.7 – 0.7 – 0.7 – 0.7 – 0.7 بن المتوسط الكثافة النوعية 0.7 بن المتورنات المنوية الشاذة 0.7 المنائل المنوى وفي حركة الحيوانات المنوية وفي دقم تركيز أيونات الايدروجين وفي عدد الحيوانات المنوية في 1 سم 0.7 بين القذفتين تركيز أيونات الايدروجين وفي عدد الحيوانات المنوية في 1 سم 0.7 بين القذفتين ذات معنوية احصائيا ولكن الاختلافات بين الطلائق في حجم السائل المنوى وحركة الحيوانات المنوية وتركيز أيونات الايدروجين كانت معنوية كما وجد علاقة سالبة بين الحركة عند الجمع وتركيز أيونات الايدروجين 0.7

وكان متوسط احتواء السائل المنوى عند الجمع من الفركتوز هو ٥٧ر٨٦٥ مليجرام / ١٠٠ سم في القذفة الأولى و ١٥٠٥مليجرام / ١٠٠ سم في القذفة الأولى و ١٥٠٥مليجرام المدنق المنافق القذفة الثانية ولم تكن الاختلافات بين القذفتين وبين الطلائق في هذه الصفة بذات معنوية كما لم يقترن ارتفاع تركيز الفركتوز بالسائل المنوى ولا بدرجة عالية المنوى بزيادة في تركيز الحيوانات المنوية بالسائل المنوى ولا بدرجة عالية من الحركة .

وكان متوسط تركيز الفسفور الفير عضوى والكالسيوم والأزوت الكلى وكان متوسط تركيز الفسفور المير عضوى والكالسيوم والأزوت الكلى في السائل المنوى كالآتى 10.1 - 10.1 سم وذلك في القذفة الأولى أما في القذفة الثانية فكانت التركيزات كالآتى 10.1 - 10.1 بالمليجرام 10.1 - 10.1 سم على التوالى 10.1 - 10.1

كما أن الاختلافات فى تركيز السائل من الفسفور الفير عضوى والكالسيوم معنوية وذلك بين الطلائق وليس بين القذفتين الأولى والثانية .