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Metabolic Activity and Fructolysis in Buffalo Semen

S.T. Ibrahim

Faculty of Agric., Mansura University, Egypt.

SEMEN samples were collected twice monthly from four fertile buffalo bulls in Wadi El-Natron Farm for a period of 6 months. Each semen sample was examined for pH, sperm concentration, motility and initial fructose content. Fructolysis was estimated by determintation of the fructose content in combined semen samples incubated under 5°, 25°, 37° for various intervals. The amount of fructose utilized by 10^9 sperms during 3 and 12 hr was calculated for all experimental conditions.

Seminal fructose content decreased progressively in semen samples incubated at 37°, more than samples kept at 25° (room temperature) or in a refrigerator (5°). The amount of fructose utilized after 12 hr of incubation was higher than that after 3 hr at all incubation temperatures. It was suggested that metabolic activity of buffalo semen was a simple laboratory method for estimating fertility, depends upon the length of incubation period as well as the incubation temperature. Seminal pH, sperm motility, sperm concentration, initial fructose content and temperature were the important factors affecting Fructolysis.

During the last few years, there has been an increasing interest in the study of bull semen particularly in relation to its biochemistry. This has been largely due to the expanding practice of artificial insemination in cattle and to the growing recognition of dairy cattle infertility.

Fructose is the normal nutrient for sperm and the sole physiological substrats for lactic acid formation in semen. Spermatozoa depend on carbohydrate metabolism as their chief source of energy.

This metabolic activity of spermatozoa can be measured by the direct estimation of the rate of fructolysis (Mann, 1954). Index of fructolysis is used for measuring fructose ulilization by spermatozoa and it is defined as the number of milligrams of fructose utilized by 10^9 sperm in 3 hr (Gassner *et al.*, 1952) or one hour at 37° (Mann, 1954). In normal bull semen, the rate of fructolysis is approximately 1.4 - 2.0 mg / hr (Mann, 1954).

Erb *et al.* (1956) indicated that fructose utilization by 10^9 sperm per unit time is a satisfactory laboratory method for estimating fertility of bulls.

Mixner *et al.* (1957), suggested a method for determining the rate of fructolytic activity in sperm / one min and which is referred to as fructolysis coefficient (Ku).

$$Ku = \frac{2.303 \left(\log \frac{U_1}{U_2} \right)}{F_2 - F_1}$$

Where U_1 = milligrams of fructose utilized by 10^9 sperm in the first period.

U_2 = milligrams of fructose utilized by 10^9 sperm in the second period.

F_1 = time of initial sampling.

F_2 = time of second sampling etc. and $F_2 - F_1 = F_3 - F_2$.

The same investigators reported another method (maximum fructose utililation, MFU) in which the initial amount of fructose utilized by 10^9 sperm in one min. at zero time was measured as an index of metabolic acitivity as follows :

$$\text{MFU mg} = \text{anti log} \frac{\left(2 \log U_1 - \log \frac{U_1 + U_2}{2}\right)}{F_2 - F_1}$$

The aim of this work is to study the rate of fructolysis in buffalo semen as a simple measure for the evaluation of metabolic activity of sperm.

Material and Methods

Four fertile buffalo bulls ranging in age from 3 - 5 years were used. The experimental animals were maintained on the Farm of Wadi-El-Natron for a period of 6 months starting from January to June. The animals were fed clover, rice bran and pelleted feed mixture. The semen samples were collected by the use of an artificial vagina twice monthly for the first and second ejaculates according to the method of Herman and Ragsdale (1960). Each semen sample was examined for pH, motility and sperm concentration as indicated by Ibrahim (1969). Fructolysis was estimated by determining the fructose content in combined semen samples kept under room temperature 25°, 37° and 5° for various intervals. Initial fructose content was determined after 15 min of semen collection by the colorimetric method of Roe (1934). Repeated fructose estimations were made after 3 and 12 hr from the initial one. The fructose utilization (U) was determined by calculating the amount of fuctose utilized by 10^9 sperms during the first three hr and during 12 hr after the initial fructose estimation (Gassnrer *et al.*, 1952 and Mann, 1954).

Results and Discussion

The results are given in Tables 1 and 2. These data indicated that the incubation of freshly ejaculated buffalo semen at 37° was accompanied by a rapid decrease in the amount of fructose econtent than samples kept at room temperature (25°)or those left in a refrigerator (5°). Furctose utilization(U) was estimated by calculating the amount of fructose utilized by 10^9 sperms during the first 3 hr and during 12 hr after the initial determination of fructose. The average fructose utilization values in semen incubated for 3 and 12 hr were 0.925, 1.484; 1.931, 2.988 and 2.541. 4.219 at 5°, 25° and 37° for high fertile bulls No. 1 and 2, respectively (Table1).

The corresponding values for normal fertile bulls No. 3 and 4 were 0.616, 1.171; 1.386, 2.857 and 1.875, 3.362 (Table 2). It is evident therefore that the quantity of fructose utilized after 12 hr of incubation was higher than after 3 hr at all incubation temperature. However, the rate of fructolysis of high fertile bulls (Table 1) was increased than that of normal fertile bulls (Table 2). The results obtained indicate that the fructolytic metabolic activity is a suitable laboratory method for estimating fertility, depends upon the length of incubation period and the incubation temperature. The present studies were in agreement with those reported by Mixner *et al.* (1957); Hackmann (1963) and Georgiev *et al.* (1975) on bull semen.

Many factors seem to affect the fructolytic activity of buffalo semen. It was observed that seminal pH, sperm motility, sperm concentration, temperature and initial fructose level were the major effective factors on the rate of fructose utilization. Higher fructolytic activities were observed in those semen samples of high initial fructose content, high initial motility and lower initial pH as compared with other samples. freund *et al.* (1959), and Georgiev *et al.* (1975) reported similar results in bullsemen. It can be suggested that lower concentration of fructose in semen may depress the viability of spermatozoa.

Gassner *et al.* (1952), showed that adding fructose to fructose - poor ejaculates may prolong the potency and longivity of semen samples. However, when fructose was added to those samples containing initially an adequate amount of fructose and showing quite satisfactory fructolysis index, the ability of spermatozoa to utilize added fructose was decreased (Hackmann, 1963).

The results presented in Tables 1 and 2 showed that high levels of fructose in semen were not associated always with neither high sperm concentration nor a high degree of sperm motility. Gassner *et al.* (1952) stated that fructolysis was absent in spite of the high fructose concentration in bull semen samples showing azoospermia or necrospermia. Moreover, they indicated a close relationship between fructolysis and fertility. Semen of low quality from subfertile and infertile bulls showed a reduced rate of fructolysis. The fructolysis index was found to be a good accurate measure for indicating semen quality fertility and estimating breeding efficiency.

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TABLE 1. Fructolysis in buffalo semen at intervals of 3,12 hr and under different temperatures, each value is the average of bulls No. 1 and 2.[1]

Initial pH	Initial motility	Sperm concentration x 10^9	Fructose concentration mg/100 ml whole semen [2]											
			Initial fructose	5°				25°				37°			
				3hr	3hr(U)*	12hr	12hr (U)	3hr	3hr(U)	12hr	12hr (U)	3hr	3hr(U)	12hr	12hr (U)
6.62	4.3	135	695.0	520.0	1.30	457.5	1.76	450.5	1.81	392.0	2.24	395.0	2.22	120	4.26
6.75	3.8	172	520.0	407.5	0.98	351.3	0.98	270.0	1.45	70.0	2.62	188.8	1.93	45	2.76
6.94	3.8	102	457.5	438.6	0.19	407.5	0.49	326.2	1.29	132.5	3.19	270.0	1.84	40	4.09
7.10	2.9	162	732.5	613.8	0.73	432.5	1.85	476.2	1.58	226.3	3.12	395.0	2.08	44	4.25
6.90	3.7	154	420.0	270.0	0.97	245.0	1.14	195.0	1.46	170.0	1.62	170.0	1.62	20	2.60
6.82	4.0	120	832.5	625.0	1.73	540.0	2.44	470.0	3.02	426.3	3.39	395.0	3.65	295	4.48
7.00	3.7	96	676.2	570.0	1.11	534.0	1.48	520.0	1.63	470.0	2.15	330.0	3.61	32	6.71
6.65	3.5	84	470.0	350.0	1.43	265.0	2.44	132.5	4.02	20.0	5.36	95.0	4.46	20	5.36
7.02	3.5	130	608.0	525.0	0.64	422.0	1.43	402.2	1.58	175.0	3.33	336.4	2.09	42	4.35
7.08	3.9	138	495.0	425.5	0.50	380.0	0.83	292.5	1.47	100.0	2.86	231.8	1.91	35	3.33
Average					0.925		1.484		1.931		2.988		2.541		4.219

[1] Highly fertile buffalo bulls as indicated from the farm records.
[2] Analysis on 24 combined samples of the first and second ejaculates.
* (U) = Fructose utilized by 10^9 sperm (utilization).

TABLE 2. Fructolysis in buffalo semen at intervals of 3,12 hr and under different temperatures, each value is the average of bulls No. 3 and 4.[1]

Initial pH	Initial motility	Sperm concentration x 10^9	Fructose concentration mg/100 ml whole semen[2]											
			Initial fructose	5°				25°				37°			
				3hr	3hr(U)*	12hr	12hr (U)	3hr	3hr(U)	12hr	12hr (U)	3hr	3hr(U)	12hr	12hr (U)
6.65	4.2	193	770.0	678.2	0.48	522.0	1.28	522.0	1.28	270.0	2.59	395.0	1.94	76.2	3.59
6.60	3.9	125	803.8	713.5	0.72	678.8	1.00	612.0	1.53	222.5	4.65	386.0	3.34	32.5	3.83
7.01	3.0	266	722.5	621.2	0.38	485.0	0.89	515.5	0.78	190.0	2.00	474.5	0.93	30.0	3.60
6.90	4.0	171	627.5	471.4	0.91	427.5	1.17	427.5	1.17	246.2	2.23	390.0	1.39	44.0	3.41
6.82	4.5	107	470.0	390.0	0.75	220.0	2.34	312.0	1.48	045.0	3.97	255.0	2.01	35.5	4.06
7.02	3.9	134	458.0	400.0	0.43	365.0	0.69	270.0	1.40	095.0	2.71	245.0	1.59	62.5	2.95
6.95	4.1	215	688.4	595.0	0.43	470.4	1.01	465.8	1.04	106.5	2.71	350.4	1.57	68.4	2.88
6.64	4.3	142	762.6	580.5	1.28	512.0	1.76	320.0	3.12	174.4	4.14	244.2	3.65	55.0	4.98
7.08	3.9	150	270.0	212.4	0.38	176.0	0.63	082.5	1.25	045.0	1.50	62.0	1.39	15.0	1.70
7.05	2.8	258	696.4	592.5	0.40	454.0	0.94	488.0	0.81	162.0	2.07	454.5	0.94	20.5	2.62
Average					0.616		1.171		1.386		2.857		1.875		3.362

1. Normal fertile buffalo bulls as indicated from the Farm records.
2. Analysis on 24 combined samples of the first and second ejaculates.
* (U) = Fructose utilized by 10- sperm (utilization).

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النشاط الحيوى والتمثيل الفركتوزى فى السائل المنوى لذكور الجاموس

دكتور سامى طلعت ابراهيم ابو طالب

كلية الزراعة ، جامعة المنصورة ، مصر

جمعت عينات السائل المنوى كل أسبوعين لأربعة فحول جاموس خصبة من المزرعة الحكومية للانتاج الحيوانى فى وادى النطرون على مدى ستة شهور . فحصت كل عينةمن السائل المنوى للمكونات التالية : تركيز أيون الايدروجين، تركيز الحيوانات المنوية ، حركة الحيوانات المنوية ، تركيز سكر الفركتوز فى البداية .

قدرت عملية التمثيل الفركتوزى على أساس تقدير محتوى العينة من الفركتوز أثناء تحضينها على ٥°م ، ٢٥°م ، ٣٧°م لفترات مختلفة . حسبت كمية سكر الفركتوز المستهلكة بواسطة ١٠ حيوان منوى خلال فترات ٣ ساعات ، ١٢ ساعة فى ظروف درجات الحرارة المختلفة للتحضين .

وجد أن سكر الفركتوز فى بلازما السائل المنوى يتناقص تدريجيا فى العينات المحضنة على ٣٧°م أكثر بكثير من تلك المحضنة على ٢٥°م (حرارة الغرفة) أو تلك المحضنة فى الثلاجة (٥°م) . وكانت كمية الفركتوز المستهلكية بعد ١٢ ساعة من التحضين أعلى من تلك المحضنة لمدة ٣ ساعات تحت ظروف درجات الحرارة المختلفة . ويمكن القول أن النشاط الحيوى لعملية التمثيل الفركتوزى فى السائل المنوى تعتبر وسيلة عملية مناسبة لقياس الخصوبة وتعتمد أساسا على طول فترة التحضين كما تعتمد فى نفس الوقت على درجة الحرارة المناسبة أثناء التحضين . ويعتبر تركيز أيون الأيدروجين وحركة الحيوانات المنوية وتركيزها ومحتوى العينة من سكر الفركتور عند بداية جمعها من أهم العوامل المؤثرة على عملية التمثيل الفركتوزى فى السائل المنوى لذكور الجاموس .