

## Some Physical and Biochemical Attributes of Buffalo Semen

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**I**N 72 semen samples from two mature buffalo bulls, physical and some biochemical attributes were determined. The mean values were 3.6 ml for ejaculate volume, 63.2% for sperm motility, 0.93 ( $\times 10^9$ /ml) for sperm concentration, 79.6% for live cells in fresh semen, 15.3% for abnormal cells in fresh semen, 54.8% for live cells immediately after semen exposure to cold shock and 17.7% for abnormal cells immediately after cold shock. The mean values of protein (g/100 ml), Calcium (mg/100 ml) and phosphorus (mg/100 ml) in both seminal plasma and spermatozoa extract were 2.2, 0.56, 42.0, 13.2, 11.25 and 1.6, respectively. Correlation coefficients among various physical attributes of semen, as well as between physical attributes and the biochemical assays were determined.

In the current practice of artificial insemination, the use of physical assays of semen quality is not completely satisfactory (Lindford *et al.*, 1976). Accordingly, Garner (1984) suggested that laboratory tests of seminal quality should be based on physical and biochemical measurements.

The present work was planned to appraise buffalo semen using both physical and biochemical assays. The relationships between the characters studied were also investigated.

### Material and Methods

Semen samples were collected twice weekly in two successive ejaculates for 9 weeks from two buffalo bulls 5-7 years old using an artificial vagina. The bulls were located at the Agricultural Experimental Farm, Faculty of Agriculture, Zagazig University, Egypt.

Immediately after collection, semen was maintained at 30° C until evaluation which was carried out after no longer than 10-20 min delay. The physical characteristics assayed in the fresh semen were: ejaculate volume, percentage of motile sperm, concentration and percentages of live and abnormal spermatozoa. The latter two characteristics were also determined after subjecting semen to cold shock as described by SenGupta *et al.* (1963).

Calcium, phosphorus and total protein were assayed in seminal plasma and in spermatozoa extract. Preparation of seminal plasma and extract was carried out according to Hadarag *et al* (1976). The concentrations of inorganic phosphorus, calcium and protein in spermatozoa extract and seminal plasma were measured using the methods detailed by BioMerieux\*.

Statistical analysis was carried out according to Snedecor and Cochran (1982). Percentage values were transformed to Arc-Sin values before they were statistically analysed.

## Results and Discussion

### *Physical assays*

Mean values of physical characteristics of semen and their relationships are presented in Table 1. In this respect, the present findings were in agreement with the results obtained by Sayed (1958), Younes (1975) and Rattan *et al.* (1980). The results of the correlations showed that the motility and live cells percentages decreased significantly with the increase of ejaculate volume.

The results revealed highly significant positive correlations between sperm motility and each of sperm concentration, percentage of live cells in fresh semen and percentage of live cells of cold shocked semen. Likewise, correlation coefficients were significant between sperm concentration and each of the percentage of live cells ( $P < 0.01$ ) and abnormal cells ( $P < 0.05$ ) in fresh semen but were not significant for sperm concentration and each of the characteristics of cold shocked semen indicating that these criteria of spermatozoal resistance are independent of sperm concentration in fresh semen samples.

From the results in Table 1, highly significant negative correlation coefficients were observed between percentages of live cells in fresh semen samples and percentages of each of abnormal cells in fresh and cold shocked samples. Likewise, a similar trend was found for the percentage of abnormal cells in fresh semen and the percentage of live cells estimated immediately after cold shock. Consequently, it is suggested that the increase in the proportion of abnormal sperm in the initial samples of semen, or resulting from semen processing and handling, might increase spermatozoal susceptibility to metabolic destruction.

### *Biochemical assay*

Table 2 shows that the protein content of seminal plasma was about four times as much as that of sperm extract. This might be due to the leakage of intracellular sperm proteins as a result of the increasing permeability of the sperm cell membranes which arose during centrifugation (Mann, 1964).

\* Bio-Merieux 69260 Charbonnières les Bains-France.

TABLE 1. Physical attributes of buffalo semen and their relationships.

Semen traits	Mean ±S.E. (N: 72)	Correlations							
		Motility	Concentration	Live cells	Abnormal cells	Live cells (a)	Live cells (b)	Abnormal cells (a)	Abnormal cells (b)
<i>In fresh Semen :</i>									
Volume (ml)	3.6±1.8	-0.40**	0.004	-0.49**	0.22	-0.71**	-0.96**	-0.07	-0.38*
Motility (%)	63.2±0.01		0.77**	0.84**	-0.28	0.99**	0.99**	0.01	-0.31*
Concentration (x 10 <sup>6</sup> /ml)	0.93±0.07			0.63**	0.35*	0.04	0.11	0.11	0.16
Live cells (%)	79.6±0.02				0.69**	0.43**	-0.09	-0.61**	-0.45*
Abnormal cells (%)	15.3±0.01					-0.60**	0.13	-0.21	0.23
<i>After cold shock :</i>									
Live cells (%) <sup>a</sup>	54.8±0.06								
Live cells (%) <sup>b</sup>	44.1±0.12								
Abnormal cells (%) <sup>a</sup>	17.7±0.03								
Abnormal cells (%) <sup>b</sup>	18.9±0.02								

a) Immediately after cold shock.

\* P&lt;0.05

\*\* P&lt;0.01

b. One hour after cold shock.

TABLE 2. Some biochemical attributes of buffalo semen and their correlations with physical traits.

Semen traits	Mean ± S.E.						Correlations <sup>@</sup>			
	Seminal plasma	Sperm extract	Volume (ml)	Motility (%)	Concentration	Live cell%	Live cell%		Abnormal cell%	
						F.S.	C.S.S.	F.S.	C.S.S.	
Protein (g/100 ml)	2.2±0.2	0.56±0.08	-0.15	-0.02	0.19	0.02	-0.16	-0.04	-0.14	
Calcium (mg/100 ml)	42.0±4.12	13.2±2.5	0.07	0.21	-0.23	0.20	0.23	-0.47**	0.15	
Phosphorus (g./100 ml)	11.25±0.12	1.64±0.35	0.04	-0.18	0.09	-0.26	-0.17	0.17	0.33*	

+ Fresh semen.

\* P &lt; 0.05

\*\* P &lt; 0.01

+ + Immediately after cold shock of semen.

<sup>@</sup> Between biochemical attributes in seminal plasma and physical attributes of the bull semen.

However, the protein content of seminal plasma found in the present study was lower than that obtained in bulls by Alexander *et al.* (1971), but was higher than that found in buffalo by El-Azab (1980) and in bulls by El-Keraby *et al.* (1980). From another point of view, Table 2 shows that none of the correlation coefficients between seminal protein and any of the seminal attributes were significant. However, Pedroso *et al.* (1978) reported that ejaculate volume was correlated significantly with protein content of seminal plasma.

The values of calcium (mg/100 ml) in seminal plasma and sperm cell extracts in the present study were  $42.0 \pm 4.12$  and  $13.2 \pm 2.5$ , respectively (Table 2). Similar findings were obtained by Rattan *et al.* (1980) in buffalo semen. In contrast El-Azab (1980) obtained higher concentrations of calcium in bull semen. The present results showed that buffalo semen contained higher amounts of calcium in seminal plasma than in the spermatic cells. This was in agreement with the findings of Mann (1964). The estimated correlation coefficients between calcium content of seminal plasma and each of the physical attributes of semen (Table 2) failed to reach statistical significance, except that between calcium and the percentage of abnormal cells in fresh semen samples, which was highly significant. This was not surprising since one of the important roles of calcium is to maintain the osmotic pressure of the extracellular media of spermatozoa within normal limits and if this is changed, the abnormalities increase.

The average values of phosphorus (mg/100 ml) in seminal plasma and spermatozoal extract were  $11.25 \pm 0.12$  and  $1.64 \pm 0.35$  respectively (Table 2). The phosphorus content of seminal plasma found in the present study was similar to that obtained by Sayed *et al.* (1960) and Singh *et al.* (1970). As for calcium, the phosphorus content of seminal plasma was higher than in the sperm extract. The correlation coefficient values were not significant between phosphorus and each of ejaculate volume, motility of spermatozoa, sperm concentration and live cell percentage in fresh and cold shocked semen but were significant ( $P < .05$ ) between phosphorus and abnormal cell percentage of cold shocked semen.

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## دراسة على بعض الصفات الطبيعية والكيمائية للسائل المنوي للجاموس

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أخذت عدد ٧٢ عينة سائل منوي من طلوقتين جاموسى لدراسة الصفات الطبيعية وبعض الخواص الكيمائية والعلاقة بينها . وكانت النتائج كالتالى :  
كان متوسط حجم القذف ، النسبة المئوية للحركة ، التركيز فى السنتيمتر المكعب ، النسبة المئوية للحيوانات المنوية والشاذة ٣٦٦ مل ، ٦٣٢٪ ، ٩٣ × ٧٩٦،٩١٠٪ ، و ١٥٣٪ على التوالي .  
بعد تعرض السائل المنوي لصدمة البرد مباشرة كانت النسبة المئوية للحيوانات المنوية الحية والشاذة ٥٤٨٪ و ١٧٧٪ على التوالي .  
قدر البروتين ( جم / ١٠٠ مل ) ، الكالسيوم ( مجم / ١٠٠ مل ) والفوسفور ( مجم / ١٠٠ مل ) فى كل من بلازما السائل المنوي ومستخلص خلايا السائل المنوي . كان متوسط محتوى بلازما السائل المنوي من البروتين الكالسيوم والفوسفور هو ٢٢ ، ٤٢ ١١٢٥ على التوالي بينما كان محتوى مستخلص الخلايا من البروتين ، الكالسيوم والفوسفور هو ٥٦ ، ١٣٢ و ١٦٤ على التوالي .

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