SEASONAL AND MONTHLY VARIATIONS IN SEMEN CHARACTERISTICS OF EGYPTIAN BUFFALO BULLS

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ABSTRACT

Monthly semen ejaculates were collected from four Egyptian buffalo bulls for 12 months in order to study their semen characteristics and the influence of season and monthly climatic conditions on the investigated characters. The relationships between the characters were also investigated. Results indicated that buffalo bull libido was increased (P<0.05) during the winter season as compared to other seasons. A positive (P<0.05) correlation was found between temperature humidity index and reaction time. Variations in semen ejaculate volume between different seasons and months were not significant. Sperm progressive motility percentage was high (P<0.05) during summer and autumn seasons and during June, October and November months. Percentages of live sperm were lower (P<0.05) during spring season than during the other three seasons. The percentages of abnormal spermatozoa were lower (P<0.05) during autumn and winter seasons than during the other two seasons. Semen initial fructose concentration (mg/100ml) was highest during winter and lowest during summer compared with other seasons. Sperm progressive motility(%) had a positive (P<0.01) correlation with live sperm, but a negative correlation (P<0.01) with dead sperm percentages. Sperm concentration had a significant (P<0.05) negative correlation with percentage of sperm abnormalities.

Keywords: Buffalo bulls, semen quality, reaction time, monthly and seasonal variations.

INTRODUCTION

The water buffaloes are generally considered seasonal breeders, although there is little evidence to indicate whether the seasonal breeding behavior is due to a genetic characteristic of the species or the result of climatic and/or nutritional stresses. This in fact goes to female and male buffaloes although it was clearly shown in female than male ones (Tahir *et al.*, 1981). Seasonal effects on semen quality have been observed in several species including rams (Mickelsen *et al.*, 1981), Boer and Angora goats and rams (Greyling and Grobbelaar, 1983), and Sahiwal and Brahamn crossbred bulls (Wildeus *et al.*, 1984). However, contradictory results were observed between different investigations concerning the effects of different seasons on semen quantity and quality. Most previous investigations indicated slightly higher semen quantity and quality during hot and humid periods of the year than during the colder months (Wierzbowski *et al.*, 1980), although other contradicting observations were reported (Mudgal, 1979). The present study was designed to evaluate the effect of seasonal and monthly subtropical climatic conditions on semen characteristics in Egyptian buffalo bulls.

MATERIALS AND METHODS

Animals and their management

Four Egyptian buffalo bulls (*Bos bubalis*) maintained at the Artificial Insemination Center of the Animal Production Farm, Faculty of Agriculture, Alexandria University, El-Shatby, were used in this study. The animals were apparently healthy and were used for routine semen collection. Bulls were between three to five years of age with average weights between 500 to 600 kg. They were housed and tied under shade in open yard and were fed individually. Throughout the experimental period, bulls were supplied with the normal farm ration supplemented daily with 5 kg of concentrate mixture of at least 61% TDN and 14% CP. Water was available to all animals at all times.

Climatic data

Depending upon the climatic conditions, the experimental period was divided into four seasons, winter (December to February), spring (March to May), summer (June to August) and autumn (September to November). Average daily dry bulb temperature (tdb), dew point temperature (tdp), percentage of relative humidity (RH), rain fall and daylight were recorded monthly. Temperature humidity index (THI) was calculated according to the following equation proposed by Johnson *et al.* (1961):

THI = tdb + 0.36 + tdp + 41.2°C

Where (tdb) is dry bulb temperature, and (tdp) is dew point temperature.

Semen collection and evaluation

Semen was collected monthly from each bull throughout the 12-month experimental period. Ejaculates were obtained in the morning between 08:00 and 10:00h by the use of an artificial vagina. Sexual drive of bulls was recorded monthly by using reaction time criteria. Reaction time is considered as the time elapsed from introducing the bull to the teaser and exteriortion of penis. The volume of each ejaculate was recorded using a graduated collection tube. Seminal plasma samples were obtained immediately after collection for determination of initial fructose concentration (mg/100 ml) immediately after

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collection, according to Mann (1948). Ejaculates were placed in a water bath at 38°C until semen evaluation. Forward motility (%) of spermatozoa was estimated at x400 magnification using light microscope equipped with a warm stage. Sperm concentration of each ejaculate was counted by means of a hemocytometer. The percentages of live and abnormal spermatozoa were assessed according to Blom (1983).

Statistical analyses

Statistical analyses to assess the effects of the different seasons and months of the year on different attributes were conducted. A simple correlation coefficient was calculated between different meteorologyical factors and among semen characteristics. All statistical analyses were performed according to the method described by Snedecor and Cochran (1969). The percentage values were transformed using arcsine transformation before carrying out the analyses of variance.

RESULTS AND DISCUSSION

Meteorological variables

Average values and ranges of meteorological variables were recorded for different seasons and are shown in Table (I). Variations from month to month are presented in Figure (I). Variations in temperature humidity index (THI) which is a reflection of seasonal daily ambient temperature and dew point temperature throughout the experimental year were low during winter months and reached maximum values during summer, while relative humidity (RH) was more or less the same. Values of RH during spring and autumn months were intermediate between those of the winter and summer seasons. Autumn and winter seasons are considered the rainy seasons in Egypt.

Reaction time

The overall least square mean of reaction time of the studied Egyptian buffalo bulls was 70.8 ±8.74 seconds (Table 2) which is lower than the 98.3 sec reported by El-Azab (1980) and 109 sec by Mohamed (1981) and higher the 48.1 and 56.2 sec reported by Oloufa *et al.* (1959) and El-Hariri (1973)respectively, working on Egyptian buffalo bulls. Variations in reaction time from season to season were significant ($P<0.01$). The shortest reaction time ($P<0.05$) was observed during winter, increased throughout spring and reached the highest values during summer and autumn seasons (Table 2). Reaction time was shortest during January and February, and reached the highest value during August (Fig. 2).

These results indicate that bulls' libido was decreased (long reaction time) when THI was increased. This is supported by the significant ($P<0.01$) positive correlations between reaction time and both ambient temperature and THI noted in the present study (Table 3). Misra and Sen Gupta (1965) reported that heat stress caused rapid loss of libido in buffalo bulls.

Ejaculate volume

The overall least square mean of semen ejaculate volume was 3. 69±0.16 ml (Table 2) and this was higher than values reported by Osman (1988), Chaudhary and Gangwar (1977), Mathias and Yusuf (1985) and Rahman *et al.* (1991) working on Egyptian and Indian buffalo bulls, but it was lower than those reported by Tomar *et al.* (1966) and was almost equal to values reported by Mohamed (1981), Singh *et al.* (1983), Sekharan and Rao (1986), Rattan (1988) and Tomar and Singh (1996). Variations in ejaculate volume form season to season were not significant (Table 2). Also, ejaculate volume had no significant correlations with any of the meteorological variables (Table 3). However, volume was highest during summer season and throughout May, June and July months (Fig. 2) when ambient temperature, RH and THI and day light were high. Semen volume which represents the secretory activity of the accessory sex glands is primarily controlled by hormonal as well as by environmental factors such as climatic factors and nutrition.

Tomer *et al.* (1966) and Sinha and Prasad (1966) found no seasonal or monthly variation effects on the quantity of semen in different breeds of buffalo bulls which agree with the present results. However, Oloufa *et al.* (1959) and Sen Gupta *et al.* (1963) reported significant seasonal differences in the ejaculate volume in contrast to the present results. The variability of these results may be due to several factors such as age of the animal, breed of the animals, level of nutrition, management system and climatic conditions.

Progressive sperm motility (%)

The overall mean of sperm progressive motility % was 57.8±0.96% (Table 2), which appears to be similar to that found by Kanakaraj *et al.* (1984) working on Murrah buffaloes (58.4 %), but it was lower than those reported by El-Hariri (1973), El-Azab (1980) and Mohamed (1981) working on Egyptian buffalo bulls.

The percentage of progressive sperm motility varied significantly ($P<0.05$) among seasons (Table 2). The highest ($P<0.05$) motility values were noted during summer and autumn seasons. Motility was also highest during November and lowest during March (Fig. 2). Thus, it can be seen that as ambient temperature and THI increased sperm progressive motility increased, although no significant correlations were detected between sperm motility and any of the meteorological variables, except for rainfall ($P<0.01$, Table 3). Earlier reports, however, gave conflicting results regarding the effect of seasonal variations on the percentages of sperm progressive motility. Gill

et al. (1974) and Sidhu and Gill (1994) reported significant enhancement in buffalo bull initial sperm motility during autumn months, which agree in part with the present results. Other studies, however, failed to detect any significant seasonal variations in buffalo sperm motility (Agarwal *et al.*, 1991, and El-Fouly *et al.*, 1993).

Sperm concentration

The overall mean sperm concentration in Egyptian buffalo bull semen was $502.9\pm18.8\times10^6$/ml (Table 2). This value was much lower than those reported by Abdou *et al.* (1979) , El-Azab (1980) and Mohamed (1981) working on Egyptian buffaloes, and by Singh *et al.* (1983) working on Murrah buffaloes. Results indicated that sperm cell concentration varied significantly ($P<0.01$) among different seasons of the experimental year, and tended to be highest ($P<0.05$) during winter and spring seasons and lowest during the summer season (Table 2). The concentration was highest during the period from December to May then decreased sharply till August and increased again from August. to October then decreased sharply in November (Fig. 2). During summer months (June–July) the high ambient temperature, THI and relative humidity seem to contradict with the spermatogenic activity of the bull, which started to recover during the autumn season (September – October) followed by a complete recovery during winter and spring seasons. Significant ($P<0.01$) negative correlations were found between sperm concentration and ambient temperature, relative humidity, THI and daylight (Table 3).

The present results are not in agreement with those of Tomer *et al.* (1966), Ibrahim (1969) and El-Wishy (1978) working on Indian, Egyptian and Iraqi buffaloes who reported insignificant seasonal and monthly variations in sperm cell concentration. Highest values of sperm concentration in winter and spring seasons are in agreement with those previously reported by El-Azab (1980) and Mohamed (1981).

Live and abnormal spermatozoa

The overall least square means of live and abnormal spermatozoa percentages were 62.1 ± 0.95, and 11.67 ± 0.64%, respectively (Table 2). These values were lower than those previously reported by Sen Gupta *et al.* (1963), Tomer *et al.* (1966), El-Sheikh and Mohamed (1967), El-Azab (1980), Mohamed (1981), and Singh *et al.* (1983) working on Egyptian and Indian buffalo bulls.

Significant seasonal and monthly variations in live ($P<0.05$) and abnormal spermatozoa ($P<0.01$) were noted in the present study. The lowest ($P<0.05$) live sperm percentages was observed during the spring season (Table 2), followed by winter, summer and autumn seasons. Values of live sperm were lowest in March then increased up to November (Fig. 3). Percentages of abnormal sperm were significantly highest ($P<0.05$) during spring and summer seasons (Table 2). Highest abnormal spermatozoa % were noted from June-July and August (Fig. 3).

Salem *et al.* (1973) recorded lowest live sperm percentage and greatest sperm abnormality during the spring season which is partly in accordance with the present results. Abnormal sperm percentages were low from December up to June but were highest during July and August months, then exhibited low values. This is in accordance with those reported by Tomer *et al.* (1966) working on Harriana and Murrah buffalo bulls. The increased percentages of abnormal sperm during summer months when ambient temperature and THI were high can be supported by the significant ($P<0.01$) positive correlation noted in the present study between these variables (Table 3). Percentage of live sperm had no significant correlation with any of the meteorological variables except the rainfall ($P<0.01$). Abnormal sperm cells correlated negatively ($P<0.01$) with rainfall and positively with daylight (Table 2).

Semen initial fructose

Least square overall means of initial fructose concentration in Egyptian buffalo bull semen was 642.6 ± 32.4 mg/100 ml (Table 2). Previous studies reported more or equal values for Egyptian buffalo bulls (Oloufa *et al.*, 1959), and for Murrah buffalo bulls (Banerjee and Ganguli, 1973). El-Sheikh and Mahmoud (1967), Ibrahim (1969) and El-Azab (1980) reported low values ranging from 355.0 to 591.5 mg%. On the other hand, Mohamed (1981) reported a much higher value of 739.8 mg% as compared with the present and previous results. Seasonal variations in semen initial fructose concentration were significant ($P<0.01$). The highest concentration was observed during winter (in December), and was followed by autumn (October and November). During summer months when ambient temperature and THI increased, initial fructose concentration decreased (Fig. 3). This was reflected in a significantly ($P<0.01$) negative correlations between semen initial fructose and ambient temperature, THI and daylight throughout the experimental period (Table 3).

Correlations among seminal characteristics

The reaction time correlated positively ($P<0.05$) with sperm progressive motility, and negatively ($P<0.05$) with semen initial fructose (Table 4). Ejaculate volume did not correlate with any other semen characteristics. These results are not in accordance with those of Wells *et al.* (1972) who reported positive correlations between semen volume and concentration and live spermatozoa percentages. Sperm motility showed a positive ($P<0.01$) correlation with live sperm. Dhami and Kodagali (1988) working on Surti buffalo bulls reported similar correlations for motility with live and dead spermatozoa. Sperm concentration showed significant ($P<0.05$) negative correlation with percentage of sperm

abnormalities. This is contradictory to the finding of Tomar and Singh (1996) who reported a positive correlation ($P<0.05$) between sperm concentration and sperm abnormalities. On the other hand, Dhami and Kodagali (1988) gave similar results to our finding. Live sperm percentage was negatively ($P<0.01$) correlated with dead sperm percentage. Abnormal spermatozoa percentage correlated ($P<0.01$) negatively with initial fructose concentration.

Results of this study indicate that semen volume was not influenced by season. On the other hand, during winter season although sperm motility decreased, buffalo bulls' libido increased, semen sperm concentration and initial fructose concentration increased and abnormal cells decreased. These results suggest that buffalo bulls' semen can be collected during winter season, diluted and freezed to be used throughout the rest of the year.

Table 1. Average values and ranges of meteorological variables for different seasons of the year.

	Seasons			
	Winter (Dec.- Feb.)	**Spring (Mar.-May)**	**Summer (June-Aug.)**	**Autumn (Sep.-Nov.)**
Temperature, tdb (°C)	14.6 (13.8-15.5)[b]	19.6 (16.6-23.6)	26.4 (25.1-27.7)	23.4 (20.0-26.3)
Relative humidity, RH (%)	67.9 (66.3-69.0)	64 (63.5-64.3)	69.3 (67.6-71.3)	67.2 (65.7-68.6)
Dew point, tdp (°C)	8.80 (7.5-10.30)	12.3 (9.7-15.3)	20.3 (19.0-21.1)	16.7 (13.0-19.8)
Temperature humidity index (THI)[a] (°C)	58.9 (57.7-60.4)	65.2 (61.3-70.0)	74.9 (73.2-76.5)	70.6 (65.9-74.6)
Rainfall, RF (mm/day)	0.18 (0.06-0.36)	0.03 (0.00-0.06)	0 (0.00-0.00)	0.86 (0.00-2.20)
Day light, DL (h)	10.5 (10.1-11.0)	12.7 (11.8-13.6)	13.7 (13.2-14.0)	11.4 (10.6-12.3)

a : THI = tdb + 0.36 tdp + 41.2°C.

b : Values in parenthesis represent the range of the variable.

Table 2. Seasonal variations in reaction time and semen characteristics of Egyptian buffalo bulls (Least square means ± SE).

Item	Winter season	Spring season	Summer season	Autumn season	Overall mean
Reaction time (sec.)**	22.3±4.39^{c}	58.3±10.89^{b}	103.3±22.5^{a}	99.2±14.33^{a}	70.8±8.74
Ejaculate volume (ml)	3.70±0.36	3.64±0.41	4.02±0.26	3.40±0.18	3.69±0.16
Sperm progressive motility (%)*	56.3±1.86^{b}	54.2±2.2^{b}	59.6±1.40^{b}	61.3±1.56^{a}	57.8±0.96^{b}
Sperm concentration (x10^{6}/ml)**	573.4±23.6^{a}	576.8±24.3^{a}	365.0±26.8^{c}	496.0±39.1^{b}	502.9±18.8
Live sperm (%)*	62.3±1.83^{a}	57.2±2.34^{b}	63.7±0.9^{a}	65.4±1.65^{a}	62.1±0.95
Abnormal sperm (%)**	9.41±0.09^{c}	12.7±0.8^{b}	15.9±1.11^{a}	8.66±1.05^{c}	11.67±0.64
Semen initial fructose (mg/100ml)**	758.0±35.3^{a}	617.4±76.0^{ab}	527.1±48.7^{b}	674.0±80.5^{ab}	642.6±32.4

* ($P < 0.05$), **($P<0.01$)

$^{a-c}$ Values within seasons bearing different letters differ significantly ($P<0.05$).

Table 3. Simple correlation coefficients between meteorological variables, reaction time and semen characteristics.

	Mean temperature	RH[a]	THI[b]	Rainfall	Daylight
Reaction time	0.533**	0.094	0.551**	0.105	0.285*
Ejaculate volume	0.124	0.154	0.126	–0.227	0.194
Sperm motility	0.202	0.145	0.204	0.402**	–0.014
Sperm concentration	–0.467**	–0.339*	*–0.483**	–0.056	–0.378**
Live sperm	0.162	0.246	0.168	0.280*	–0.068
Abnormal sperm	0.437**	0.101	0.436**	–470**	0.648**
Semen initial fructose	–0.393**	–0.026	–0.387**	0.214	–0.437**

* (P<0.05), ** (P<0.01)
[a] RH : Relative Humidity
[b] THI : Temperature Humidity Index.

Table 4. Simple correlation coefficients among semen characteristics.

	Ejaculate volume	Motility	Sperm conc.	Live sperm	Abnormal sperm	Initial Fructose
Reaction time	–0.103	0.282*	–0.188	0.197	0.243	–0.297*
Ejaculate volume		–0.008	–0.152	0.086	0.164	0.087
Sperm motility			–0.113	0.885**	–0.015	–0.022
Sperm concentration				–0.089	–0.288*	0.246
Live sperm					–0.04	–0.084
Abnormal sperm						–0.546**

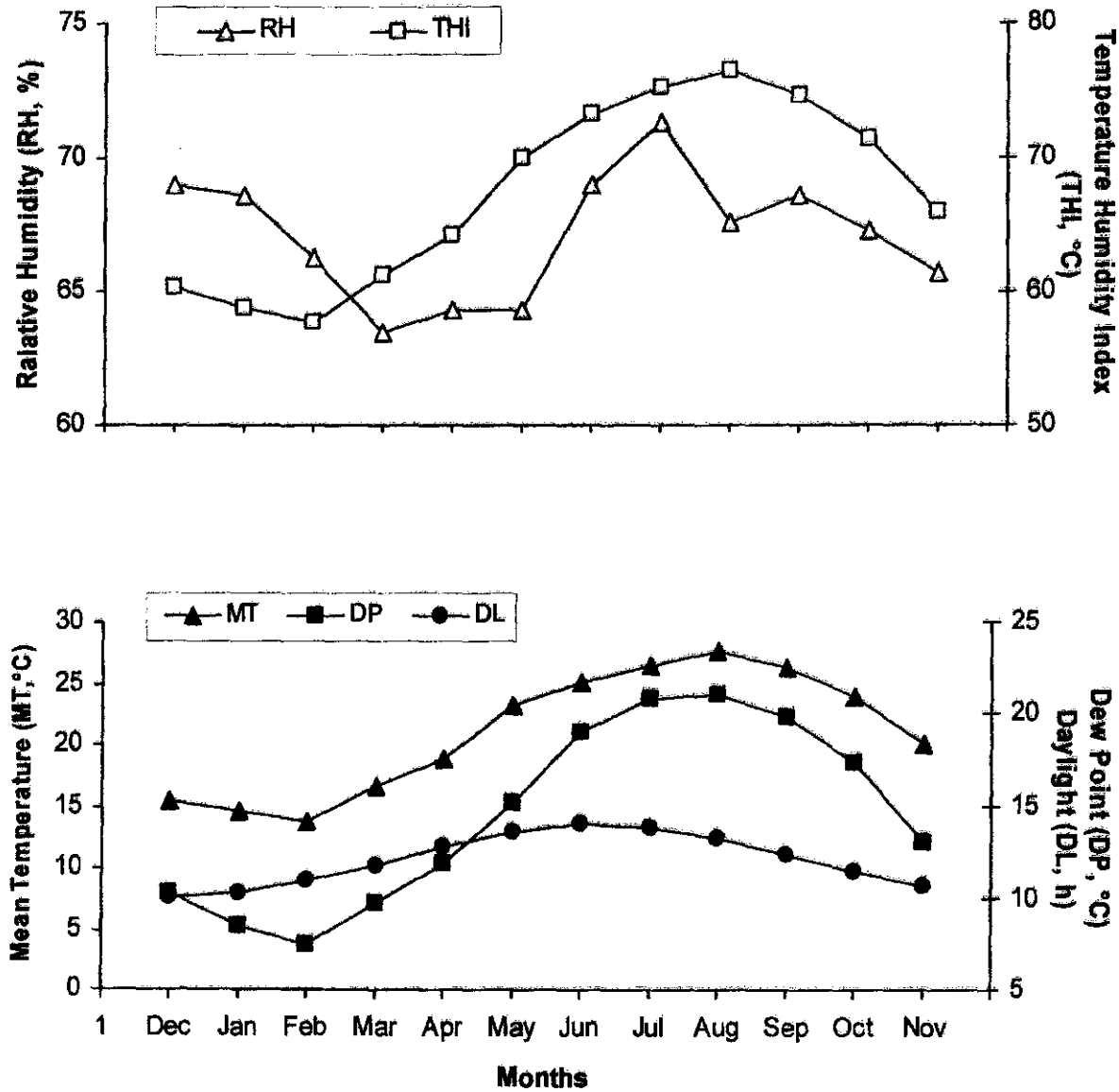

Fig. 1. Average values of relative humidity (RH), temperature humidity index (THI), mean temperature (MT), dew point (DP) temperature and day light (DL) throughout different months of the year.

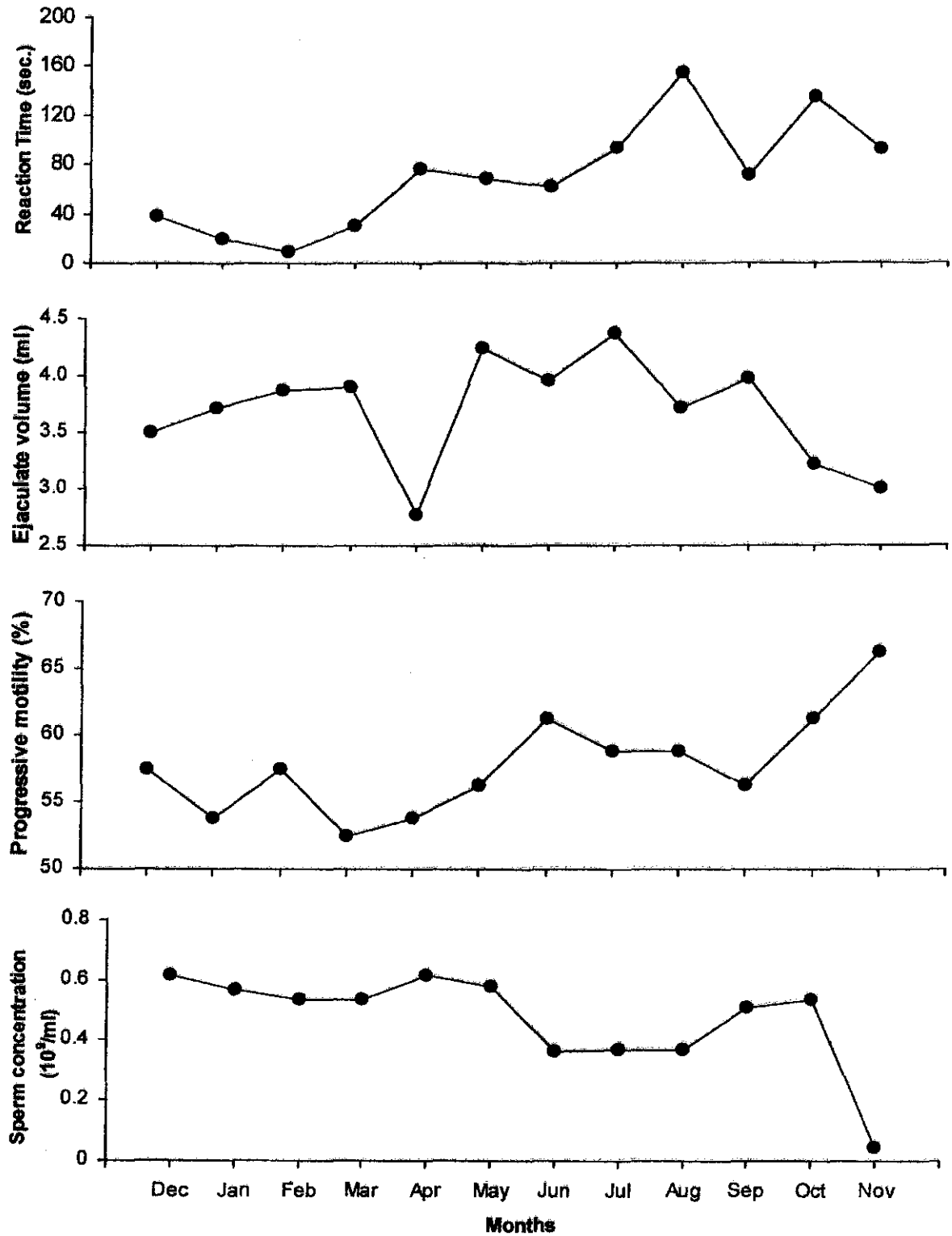

Fig. 2. Monthly variations in reaction time, ejaculate volume, sperm progressive motility and sperm concentration of buffalo bulls.

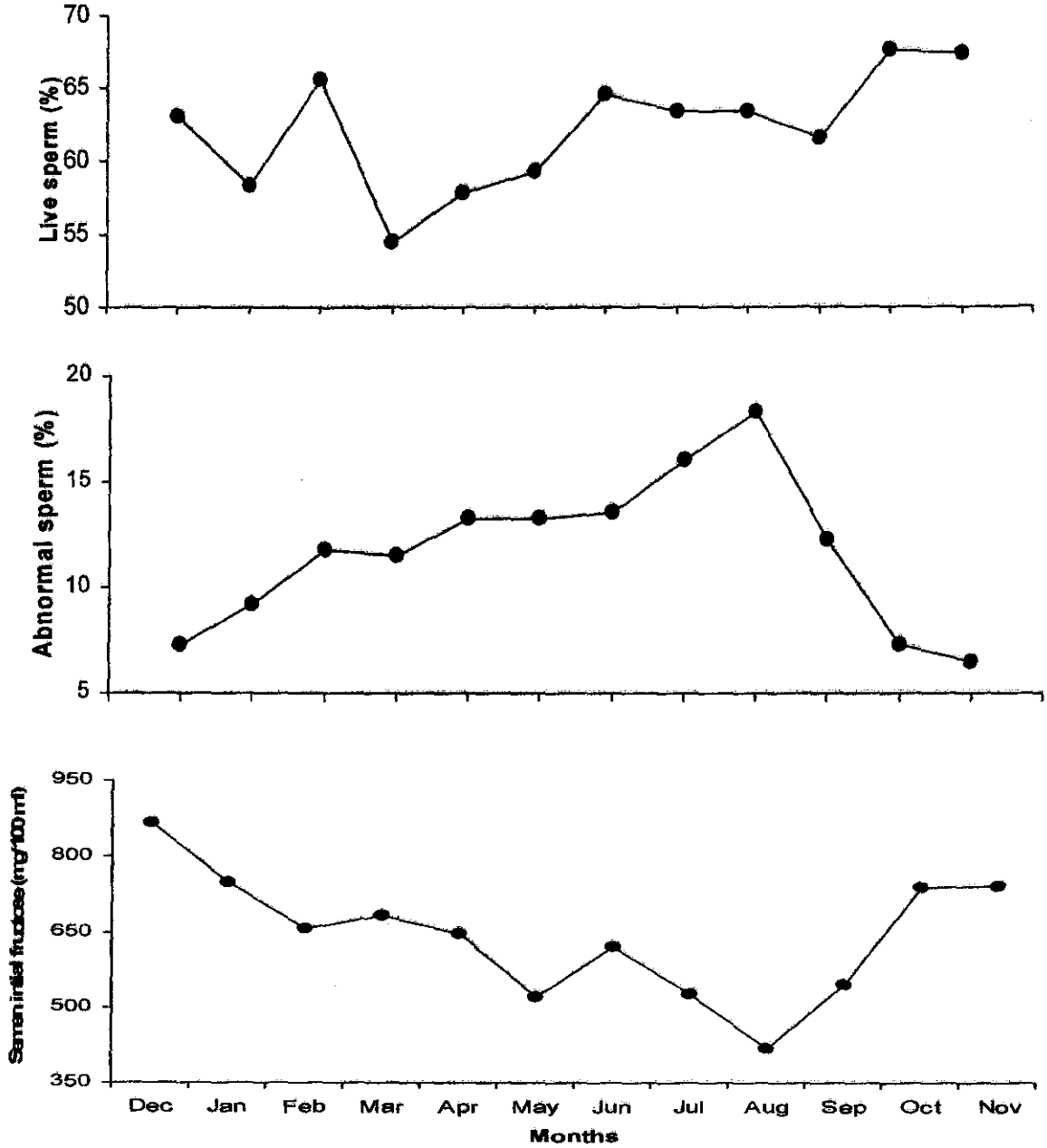

Fig. 3. Monthly variations in live sperm, abnormal sperm and semen initial fructose concentration of buffalo bulls.

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الملخص العربى

التغيرات الشهرية والموسمية في صفات السائل المنوي لطلائق الجاموس المصري

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تم جمع عينات السائل المنوي من ٤ طلائق جاموس مصري خلال ١٢ شهر وذلك لدراسة تأثير التغيرات الشهرية والموسمية علـــي صفات السائل المنوي .وتم دراسة العلاقة بين هذه الصفات مع بعضها البعض. أظهرت النتائج أن الرغبة الجنسية لطلائق الجاموس كانت أعلي معنوية (بإحتمال ٠,٠٥) أثناء موسم الشتاء مقارنا بالمواسم الأخري. وجد إرتباط معنوي موجب (بإحتمال ٠,٠٥) بين الرغبة الجنـــسية ودليـــل الرطوبة والحرارة. ووجد أن التغيرات في حجم القذفة لم يختلف معنويا بإختلاف شهور ومواسم السنة. الحركة التقدمية للحيوانات المنوية كانت معنوية (بإحتمال ٠,٠٥) أثناء مواسم الصيف والربيع وأثناء شهور أكتوبر ونوفمبر. وجد في فصل الربيع أن النسب المئوية للحيوانات المنويـــة الحية كانت أقل معنوية (بإحتمال ٠,٠٥) وعن المواسم الأخري. ولوحظ أن النسب المئوية للحيوانات المنوية الحية كانت أعلي في ذلك الوقـــت عن مواسم السنة الأخري. النسبة المئوية للحيوانات المنوية المشوهة كانت أقل معنوية (بإحتمال ٠,٠٥) أثناء موسمي الخريـــف والـــشتاء عـــن الموسمين الآخرين. ولوحظ أن تركيز الفركتوز كان مرتفعا خلال موسم الشتاء ومنخفض أثناء موسم الصيف بالمقارنة بالمواسم الأخري. وجـــد إرتباط معنوي موجب عالي (بإحتمال ٠,٠١) بين الحركة التقدمية للحيوانات المنوية والنسبة المئوية للحيوانات المنوية الحية. ولكن وجد إرتباط معنوي سالب عالي (بإحتمال ٠,٠١) بين النسبة المئوية للحيوانات المنوية الميتة وبين الحركة التقدمية للحيوانات المنوية. ووجد إرتباط معنـــوي سالب (بإحتمال ٠,٠٥) بين تركيز الحيوانات المنوية وبين النسبة المئوية للحيوانات المئوية المشوهة.