

Seasonal variations in stallion sperm features and their relationship with fertility of the breeding stock

Variações sazonais nas características do sêmen de garanhões e sua relação com a fertilidade do plantel

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SUMMARY

Seasonal influence upon spermatozoal concentration, progressive motility and percentage of morphologic abnormalities in the semen of horses was studied, and the fertility of the breeding stock during the corresponding breeding season was assessed. The semen of 7 stallions was collected monthly during 12 months for microscopic examination, and the matings were made by natural service. The highest spermatozoal concentration was recorded during autumn and the lowest, during spring. Along the months, the highest records were those in July and the lowest, in June, but no significant statistical differences were found for the seasonal and monthly variations. The highest rate of progressive motility was observed during summer and the lowest, during spring. Among the months, July presented the highest rate and April the lowest. Differences were significant between months ($p < 0,05$) and between seasons ($p < 0,10$). The highest rate of sperm morphologic abnormalities was recorded in summer ($p < 0,05$). January presented the highest rates of abnormal cells and March the lowest, but the differences among months were not statistically significant. During the breeding season, the fertility of the stock measured by pregnancy rate per cycle increased in a linear manner from 29% in October to 95% in February, not showing any apparent relationship with the seminal characteristics studied.

UNITERMS: Semen; Seasonality; Fertility; Equidae

INTRODUCTION

Many physiological and ambient factors affect some features of stallion semen, among which the photoperiod is one. In equine species, reproductive seasonality is not as conspicuous in males as in females, but an endogenous circannual rhythm in male is recognized, upon which photoperiod acts as a synchronizer^{8,9} through melatonin^{1,43}. Indeed, many authors have reported modifications in the reproductive activity of stallions connected with the period of the year^{2,3,4,12,19,21,25,26,28,29,30,33,34,36,37,38}, but results are conflicting.

Microscopic examination of semen is a method commonly employed for evaluating the fertility of stallions^{6,7,10,17,20,28,35,40,42}. The relationship between sperm features and fertility of stallions has deserved much attention^{5,7,10,11,16,17,39,42}, but little is referred to the relationship between sperms characteristics of stallions and their influence on fertility of the breeding stock as a whole.

In the present study we have examined seasonal influence upon the most common features used for evaluation of semen quality: spermatozoal concentration, progressive motility rate and percent of sperm morphologic abnormalities. We have also attempted to verify if a variation in these parameters affect the

fertility of the stock during the breeding season.

MATERIAL AND METHODS

Collection and analysis of semen: seven stallions were studied in this experiment and were maintained at the Equine Experiment Station of Colina, in the State of São Paulo, Brazil (20°43'05"S and 48°32'38"W). Three of the stallions belonged to Brasileira-de-Hipismo breed and 4 to Breton Postier, and were aged between 4 and 8 years except for one, which was 20 years old. The animals were kept in 24 h-turns in a stable and pasture, and had free access to hay and ration of concentrates. During the breeding season stallions mated regularly, according to the farm schedule. Single ejaculates of semen were collected from each stallion at the end of each month for microscopic examination, from March 1990 to February 1991, using an artificial vagina.

Spermatozoal progressive motility was evaluated immediately after semen collection, by placing one drop of semen between a microscope slide and a glass cover previously warmed at 37°C and observed at 100x to 400x magnification with a light microscope. Spermatozoal concentration was determined using a haemocytometric counting chamber and spermatozoal morpho-

logy was observed using phase-contrast microscopy at 1000x magnification²⁰. Any alterations in the head, middle piece or tail of the spermatozoon were considered as morphologic abnormalities.

Evaluation of fertility: During the breeding season of 1990/1991 (from September to February), the team of stallions mated with 110 mares by natural service, each stallion mating for a maximum of three times per week. Mares were barren, maiden, or foaling mares and were considered fit for reproduction by routine, clinical examination and by analysis of their reproductive records. Mares were teased daily. As soon as a mare showed oestrous, the follicular maturation was monitored by ovary palpation *per rectum*. Based on the size and degree of fluctuation of the follicle, the intensity of oestrous symptoms and the length of previously recorded oestrous periods, the anticipated day of ovulation was predicted. Every mare was mated once or twice before ovulation and once again after it. Pregnancy status was determined by uterine palpation *per rectum* 30 days after the last mating.

Estimation of fertility of the breeding stock was assessed by the *pregnancy rate per cycle*, employing the equation used in the study of Jasko *et al.*¹⁷ (1990) and in other studies:

$$\text{pregnancy rate per cycle} = \frac{\text{total n}^\circ \text{ of pregnancies achieved}}{100 \text{ total n}^\circ \text{ of cycles mated}}$$

Statistical Analysis: Due to operational difficulties, some results were lost: 5 from concentration measurements; 5 from motility assessment; and 4 from morphologic abnormalities evaluation, all of which were recovered according to the method described by Winer¹⁴ (1971). One animal, however, could not be

included in the statistical analysis due to the loss of too many analyses of the samples obtained from it. Population means during the experimental period were compared by ANOVA for longitudinal data²⁷. Two types of analyses were performed: in the first, data obtained in the various months were compared, while the second compared the means of different seasons. The mean value obtained for the months of March, April and May is presented as autumn mean. In the same manner, results obtained in June, July and August are presented as the winter mean; September, October and November correspond to the spring mean and that of summer includes the data obtained in December, January and February. Motility and abnormality variables, expressed as percentage, were transformed into the arc-sine of the square-root²⁷.

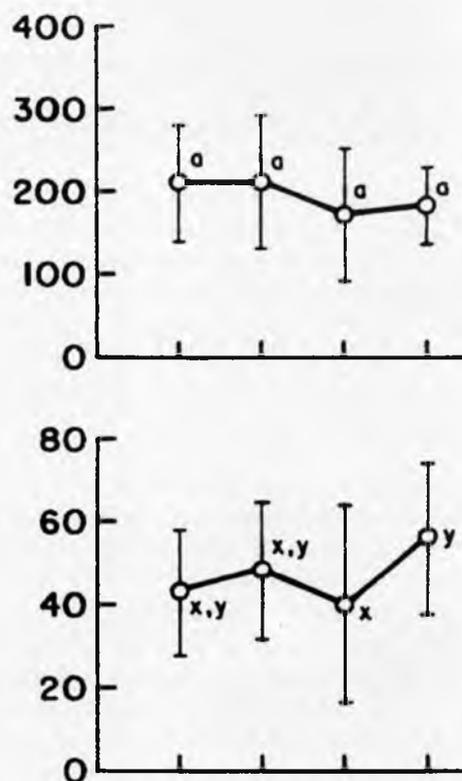
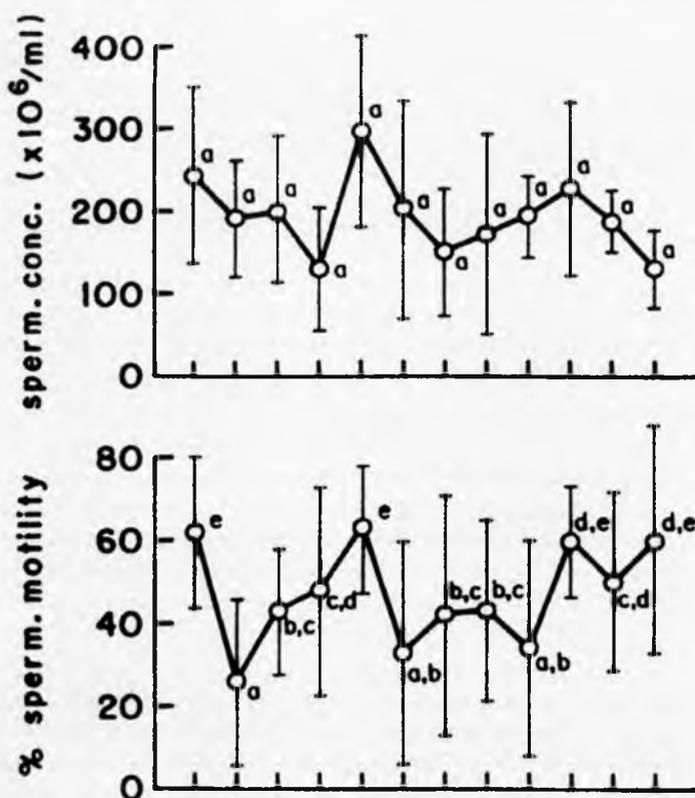
Adequacy of the covariance matrix of the data to the analysis was verified by the sphericity test¹⁸. When it failed, the Greenhouse-Geisser correction was used¹⁴. A residual analysis was conducted in order to verify any discrepancy of the normality of errors supposition.

For multiple comparison of mean values of populations presenting different values of variance the Tukey method was applied²⁷.

Statistical calculations were performed by BMDP-2V, a program described in Jenrich *et al.*¹⁸ (1990).

RESULTS AND DISCUSSION

The studies on seminal features referred to in the literature include a wide range of different conditions related to animals or experimental conditions such as age, breed, feeding, management,



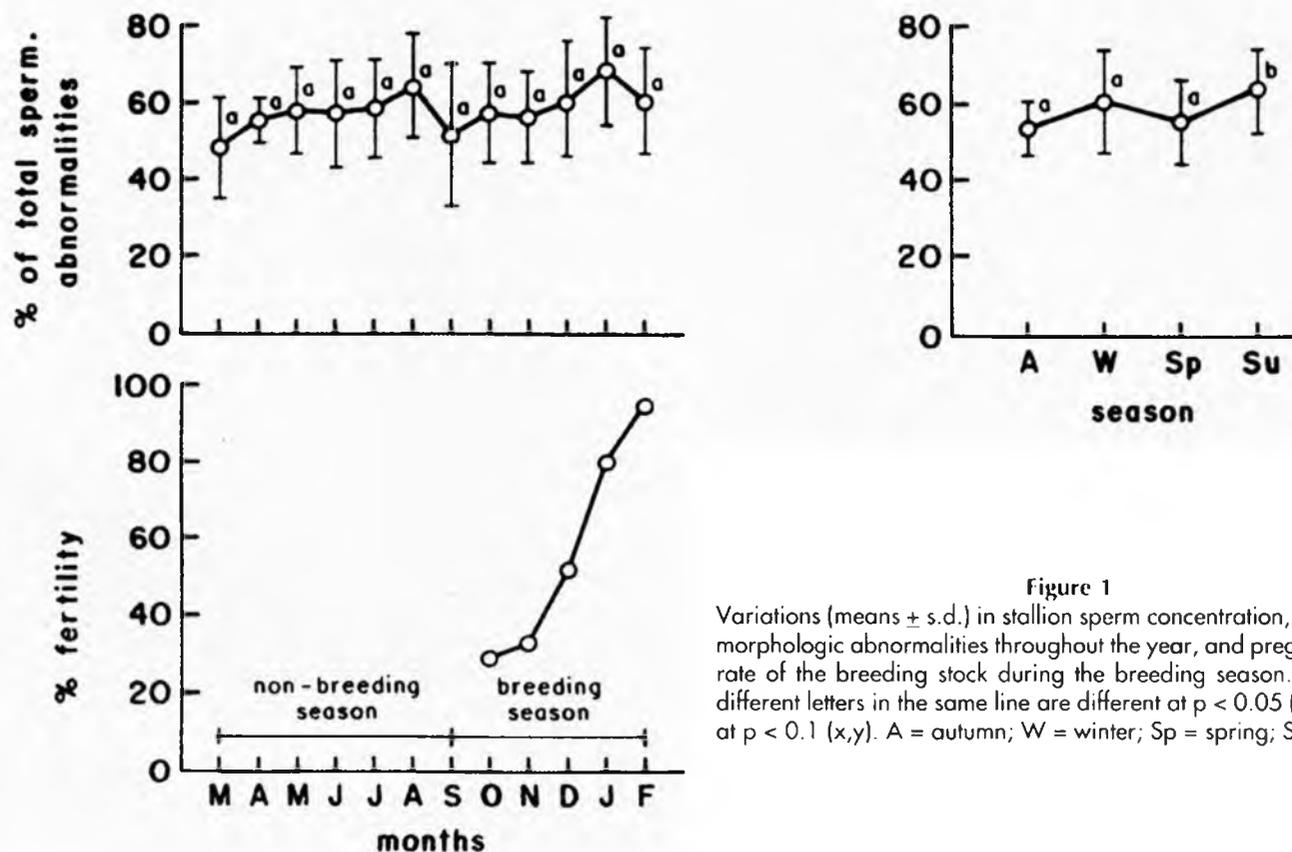


Figure 1
Variations (means \pm s.d.) in stallion sperm concentration, motility, and morphologic abnormalities throughout the year, and pregnancy/cycle rate of the breeding stock during the breeding season. Means with different letters in the same line are different at $p < 0.05$ (a,b,c,d,e) or at $p < 0.1$ (x,y). A = autumn; W = winter; Sp = spring; Su = summer.

Table 1
Concentration, motility, and morphologic abnormalities in stallion sperm collected monthly throughout the year (means \pm s.d.). Colina - SP, Mar. 1990 to Feb. 1991

sperm feature	Mar	Apr	Mai	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
concentr.	239,9 ^a	187,6 ^a	196,3 ^a	127,9 ^a	294,0 ^a	200,8 ^a	150,0 ^a	168,7 ^a	194,5 ^a	223,4 ^a	186,5 ^a	131,7 ^a
($\times 10^6$ /ml)	+103,1	+71,5	+83,8	+77,5	+117,6	+131,2	+77,6	+119,3	+49,5	+104,2	+33,3	+47,2
	autumn: 207,9 ^a \pm 71,0			winter: 207,6 ^a \pm 80,5			spring: 171,1 ^a \pm 80,6			summer: 180,5 ^a \pm 42,6		
motility (%)	61,7 ^e	25,8 ^a	43,3 ^{bc}	48,3 ^{cd}	63,3 ^e	33,3 ^{ab}	41,6 ^{bc}	43,3 ^{bc}	34,2 ^{ab}	59,8 ^{de}	49,2 ^{cd}	59,2 ^{de}
	$\pm 18,6$	$\pm 20,9$	$\pm 14,9$	$\pm 25,4$	$\pm 17,0$	$\pm 26,9$	$\pm 29,0$	$\pm 21,3$	$\pm 26,2$	$\pm 12,9$	$\pm 20,9$	$\pm 26,8$
	autumn: 43,6 ^{xy} \pm 14,4			winter: 48,3 ^{xy} \pm 17,5			spring: 39,7 ^x \pm 24,3			summer: 56,0 ^y \pm 17,9		
cell abnorm.	47,6 ^a	55,5 ^a	57,2 ^a	57,3 ^a	58,4 ^a	64,2 ^a	51,3 ^a	57,2 ^a	56,4 ^a	59,4 ^a	68,8 ^a	60,2 ^a
	$\pm 13,2$	$\pm 6,2$	$\pm 11,5$	$\pm 13,9$	$\pm 13,6$	$\pm 12,7$	$\pm 18,3$	$\pm 12,4$	$\pm 12,6$	$\pm 14,5$	$\pm 14,3$	$\pm 13,3$
	autumn: 53,4 ^a \pm 6,7			winter: 59,9 ^a \pm 13,0			spring: 55,0 ^a \pm 11,4			summer: 62,8 ^b \pm 11,6		

Means with different letters in the same line are different at $p < 0.05$ (a,b,c,d,e) or at $p < 0.1$ (x,y).

sexual status, intensity of male sexual stimulation, frequency of matings or of semen collection. This makes it difficult to better compare those data among authors and with those in the present work. Nevertheless, an attempt will be made.

The highest mean spermatozoa concentration was observed in Autumn and Winter (mean \pm s.d. = 207,9 \pm 71,0 $\times 10^6$ /ml and 207,6 \pm 80,5 $\times 10^6$ /ml, respectively), although the lowest value occurred in June (127,9 \pm 77,5 $\times 10^6$ /ml (Tab. 1 and Fig. 1). The lowest mean concentration corresponded to Spring (171,1 \pm 80,6 \times

10^6 /ml), although the highest value was recorded in July (294,0 \pm 117,6 $\times 10^6$ /ml). However, no significant statistical differences could be detected among months or among seasons, possibly due to the great variability observed among animals. Voss *et al.*³² (1981) had already verified a marked variation of the morphological characteristics of the semen among stallions and among ejaculates within stallions. Lower mean values during the reproductive season had already been recorded in the northern hemisphere by McLeod; McGee²⁴ (1950), who reported a lower concentration in

May (spring) and the highest in February (winter). Mann *et al.*²⁶ (1956) recorded lower values in August (summer) and Pickett²⁸ (1968), also referred to the lowest concentration in August, and the highest in November (autumn). The results presented here are in accordance to those of Skinner; Bowen³⁶ (1968) and those of Magistrini *et al.*²⁵ (1987), who recorded an increase of spermatozoa concentration in those months of a shorter photoperiod. A seasonal effect upon spermatozoa concentration was also observed by Dowsett; Pattie¹² (1987), who verified a higher concentration in autumn and a lower in spring.

Studying the influence of the season of the year and of the photoperiod upon some characteristics of the equine semen, Clay *et al.*⁸ (1987) observed that, apparently, semen volume is greater in Spring and Summer months. Being the semen volume related mainly to the volume of seminal plasma, the lower cell concentration observed in the present paper in Spring and Summer must be associated with a higher dilution of the cells in seminal fluid, and consequently did not show the fluctuations of spermatogenesis during the year. Notwithstanding, some studies showed an increase in both spermatozoa production and release in those seasons^{3,4,8,19}. Higher sperm and seminal fluid production in Spring and Summer are compatible with the elevation of FHS, LH and testosterone concentrations in serum and in testis, recorded in the same period of the year^{3,4,9,21,23}. The interpretation of these results is difficult, but it becomes more comprehensible if we consider a hierarchic response of the testes and of the accessory glands to those hormones. Berndtson *et al.*³ (1974) suggested that the peak of concentration of plasma testosterone recorded in May and June (spring) might be responsible for the increased spermatozoa production in July (summer), time elapsed between the hormonal stimulus and the maximal response of the testis.

The results obtained in the present work are different from those reported by Pickett *et al.*³¹ (1970), who recorded maximal spermatozoa concentration in Spring months and minimal in Autumn, although the fluctuations showed some discrepancy between the two ejaculates collected in a 4-hour interval. Our results are also different from those reported by Pickett *et al.*^{32,30} (1975, 1976), who found higher values in Spring and Summer and lower values in Winter. No correction was made in the present study for spermatozoal losses in the collection equipment, as reported by Pickett *et al.*³³ (1974).

The highest rate of progressive motility recorded in the present work occurred in July (mean \pm s.d. = $63,3 \pm 17,0\%$), and the lowest, in April ($25,8 \pm 20,9\%$) (Tab.1 and Fig. 1), and the differences among months were statistically significant ($p < 0,05$). Among seasons, the highest rate was recorded in Summer, and the lowest, in Spring ($56,0 \pm 17,9$ and $39,7 \pm 24,3\%$, respectively, $p < 0,1$). Pickett²⁸ (1969) also mentioned seasonal variations in progressive motility, although he reported the highest rate in August, and the lowest, in January (corresponding respectively to February and July in the southern hemisphere). In agreement with the present results, Van Der Holst³⁸ (1975) and Magistrini *et al.*²⁵ (1987) also noticed an increase of motility during breeding season. Skinner Bowen³⁶ (1968) observed an association between sperm motility and photoperiod, but these authors associated the highest rates of motility with shorter photoperiod months, while the present work shows increased values in the summer. On the other hand, Pickett

et al.^{32,30} (1975, 1976) and Clay *et al.*⁸ (1987) did not evidence variation of motility in stallions submitted to different artificial photoperiods, nor in the different seasons of the year.

The lowest rate of abnormal spermatozoa was observed in March, and the highest in January (means \pm s.d. = $47,6 \pm 13,2\%$ and $68,8 \pm 14,3\%$, respectively), but the differences among the months were not statistically significant (Tab. 1 and Fig. 1). Among seasons, the highest percentage was recorded in Summer ($62,8 \pm 11,6\%$, $p < 0,05$), in contrast to the data of Van Der Holst³⁸ (1975), who reported a lower percentage during breeding season and in animals out of breeding season, but submitted to 24 h-light photoperiod.

The percentages of sperm morphologic abnormalities, which ranged between $47,6 \pm 13,2\%$ and $68,8 \pm 14,3\%$ (mean \pm s.d.) in the 12-month period studied, are higher than the values between 22% and 42% recorded by Van Der Holst³⁸ (1975). They are also higher than the mean value of 46,5% reported by Jasko *et al.*¹⁷ (1990) in two consecutive breeding seasons, and the variation between 20,6% and 21,9% reported by Von Frey *et al.*⁴¹ (1986) in the months preceding breeding season. Voss *et al.*⁴² (1981) reported less than 60% of normal cells in stallions followed for two consecutive years. Differences in breed of the animals, age and mating frequency relatively to other studies may be accounted for the relatively high percent of sperm morphologic abnormalities observed in this experiment.

It is known that getting the stallions to daily sperm output (DSO), by repeated daily ejaculation over several days^{13,14}, would improve accuracy of the results of semen analyses by removing individual variability. Thus, seasonal or monthly changes would perhaps be detected. This procedure, however, was not outlined in this experiment in order to avoid disturbances in the breeding farm routine.

Fertility of animals can be estimated by different criteria^{11,16,17,40,42}. In the present study, we aimed to estimate the fertility of the breeding stock during the breeding season, and, with this intent, we considered it adequate to use the per cycle pregnancy rate, according to Jasko *et al.*¹⁷ (1990). These authors used this rate to estimate the fertility of stallions for comparative purposes. In the present study, this rate was applied to estimate the fertility of the herd as a whole during the breeding season included in the year studied. Undoubtedly, reproductive problems associated with the mares contribute to the general fertility rate. Although the equation of the per cycle pregnancy rate does not put in evidence the reproductive performance of each mare, because it is applied collectively to a herd, the estimation of fertility that it offers satisfactorily shows the fertility of the stock in the considered breeding season.

All mated mares were previously submitted to routine clinical examination and their reproductive records taken into account, in order to be considered fit for reproduction. The scheme of mating only after ovulation was avoided because it requires frequent ovarian palpations²² and may impair mare fertility²⁹. In spite of the irregular oscillations of the seminal characteristics throughout the year and during the breeding season, the fertility of the herd increased progressively throughout the breeding months: 29%, 33%, 58%, 80% and 95%, respectively for October, November, December, January and February (Fig. 1). It is well known that

the incidence of ovulation in mares is minimal or absent during the Winter, transitionally increasing during Spring (September, October and November in the Southern hemisphere), maximal during Summer (December, January and February in the same hemisphere), and transitionally decreasing during Fall¹⁵. Consequently, the fertility of mares expressed as conception rates is low during the Winter and early Spring. The fertility line obtained in this study is regularly ascendant from October through February, in contrast with the irregular lines of the semen features observed during the same breeding months. This seems to suggest that the fertility status of a controlled breeding stock is strongly determined by the progressive increase in fertility of mares, being the semen features of the stallions an accessory factor, although recognized as an important one. In a statistical view, no correlation was verified between the percentage pregnancy/cycle and the semen features studied.

Several author also verified a disagreement between semen characteristics and fertility. Pickett *et al.*²⁹ (1974) failed to observe any modification in the pregnancy rate by using fresh semen of stallions containing such different spermatozoa count as 100×10^6 and 500×10^6 ml. Voss *et al.*⁴² (1981) reported that three stallions followed for two consecutive years showed acceptable fertility in spite of low rate (< 60%) of normal cells present in their semen. In one of the seasons, these authors observed that the highest pregnancy rate (83,3%) was achieved by one stallion that had the lowest proportion of normal spermatozoa (45,5%). Despite many reports in the literature attempt to correlate various

seminal characteristics with fertility^{5,7,10,11,16,17,20,29,35,38,39,40,42}, the data presented in this report shows that some features of this correlation still remain obscure.

Taking into account that researches have been led under considerably different conditions, producing at times conflicting results, studies should be carried out in carefully standardized conditions, in order to clarify the real influence of seasonality on the characteristics of equine semen and the extend to which variations alter the fertility of the breeding stock.

CONCLUSIONS

Under the conditions of the present experiment it can be concluded that:

1. Season of the year did not show statistical significant influence on the spermatic concentration in the semen of stallions, despite the fluctuations observed amongst the months.
2. Progressive motility rate varied significantly amongst the months, being highest in July and lowest in April; it was significantly higher in Winter and lower in Spring.
3. The proportion of abdominal spermatozoa did not vary amongst the months; nonetheless, in Summer it was significantly higher than in the other seasons.
4. The fertility of the breeding stock increased linearly in the months of the breeding season, not showing any correlation with the oscillations of the concentration, progressive motility rate and proportion of abnormal spermatozoa verified in the semen of stallions.

RESUMO

Foi estudada a influência da estação do ano sobre a concentração, a motilidade progressiva e a percentagem de anormalidades morfológicas em espermatozoides de garanhões, tendo-se registrado também a fertilidade do plantel durante a estação de monta inserida no período estudado. O sêmen de 7 garanhões foi colhido mensalmente, durante 12 meses, para os exames microscópicos e as coberturas foram efetuadas por monta natural. A concentração mais alta de espermatozoides foi observada no outono e no inverno e a mais baixa na primavera; dentre os meses, a maior concentração foi registrada em julho e a menor em junho, mas as diferenças entre as estações e entre os meses não foram estatisticamente significantes. A maior taxa de motilidade progressiva foi registrada no verão e a menor na primavera; dentre os meses, a taxa mais alta ocorreu em julho e a mais baixa em abril, tendo sido significativas as diferenças entre os meses ($P < 0,05\%$) e entre as estações ($P < 0,10$). A maior proporção de espermatozoides anormais foi registrada no verão ($P < 0,05$); dentre os meses, a maior proporção ocorreu em janeiro e a menor em março, mas as diferenças não foram estatisticamente significantes. Durante a estação de monta, a fertilidade do plantel, medida pela percentagem de prenhez por ciclo, apresentou uma elevação linear desde 29% em outubro até 95% em fevereiro, não apresentando nenhuma relação aparente com as características do sêmen estudadas.

UNITERMOS: Sêmen; Eqüidae; Estacionalidade; Fertilidade.

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