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AGE-RELATED CHANGES AND SEASONAL VARIATIONS IN THE DAILY SPERM PRODUCTION OF THE STALLION

BY

MOSTAFA M. ABOU-AHMED

Department of Theriogenology, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt.

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INTRODUCTION

Although a variety of methods are available for quantifying rates of spermatozoal production, each of which entails certain assumptions and/or limitations (Berndtson, 1977; Berndtson et al., 1983). However, estimates of sperm production rates by quantitative histological analysis are imperative for clear and full evaluation of the effects of environments, drugs or other agents upon the gametogenic function of the testis (Amann, 1970).

In stallions, distinct effects of age and season on many aspects of reproduction have been documented (See Johnson and Neaves 1981). However, the corresponding influences of age and season on the quantitative expression of spermatogenesis have received limited attention. Such information may be useful in predicting a stallion's reproductive capacity and in establishing managerial practices which maximize reproductive efficiency in stallions.

Therefore, the present investigation was intended to: 1-determine the stallion's daily sperm production by a quantitative histological technique; and, 2-evaluate the extent of the changes due to age and season on daily sperm production of Arab and native horses in Egypt.

MATERIAL AND METHODS

Animals and thier assignment to groups:

The current study was carried out on 56 testes obtained from 28 Arab and native stallions along a complete annual cycle. No information was available on their reproductive history or breeding activity before castration. These animals were surgically castrated at the Department of Surgery, Faculty of Veterinary Medicine, Giza and the Veterinary Hospital of the Armed Forces, Cairo. The stallions were clinically sound and their testes were normal as determined by morphological and microscopi-

cal examinations. The age of each stallion was estimated by examination of the teeth and ranged from 3 to 18 years.

Age-related data were assigned to four groups: group I (3-5 yrs, n = 4), group II (6-8 yrs, n = 8), group III (9-13 yrs, n = 9) and group IV (14-18 yrs, n = 7 stallions). Concerning the influence of season, two seasonal periods were designated: breeding season (December-May, n=15) and non-breeding season (June-November, n=13 stallions).

Immediately after castration, the testes from each stallion were weighed. The tunica albuginea was removed and weighed, and the parenchyma weight was determined as the difference. Small pieces of testicular tissue were fixed in Bouin's solution and subsequently used for histological evaluations.

Histological analysis:

The fixed testicular tisssues were processed by the usual histological technique. Paraffin sections of 5-7 μ thickness were made, stained with Periodic acid-Schiff's reagent and counterstained with hematoxylin.

Determination of the daily sperm production from quantitative histological data required the use of a technique proposed by **Amann and Almquist (1962)**. The amount of shrinkage associated with histological processing was determine using Archimedes, principle. Th density of testicular tissue resulte from dividing the testis weight b the testis volume. The percentag of seminiferous tubules in the testi was determined by **Chalkley' technique (1943)**. Stage I of th seminiferous epithelium cycle wa characterized and determined in tu bules from the complete disappear ence of luminal spermatozoa to the onset of elongation of spermatic nuclei **(Swierstra et al., 1974)**. Th spermatid nuclei present in essen tially round stage I seminiferou tubule cross-section was enumerat ed in 50 tubules per stallion. The resulting crude counts were cor rected to true counts, where true count= crude count X (section thickness) ÷ section thickness + nuclear diameter **(Berndtson, 1977)**. The area of stage I seminiferous tubule cross-section was calculated from the diameter of tubule cross-section evaluated using an ocular micrometer. The duration (12.2 days) of one cycle of the seminiferous epithelium of the stallion **(Swierstra et al., 1974)** has been used for calculation of DSP in the present study. The results were averaged to provide an estimate for each stallion.

Statistical analysis:

The effect of consecutive ages were tested by the one-way analysis of variance. The Student's "t"

test was used for statistical interperetation of the seasonal data. The interactions amongst the studied parameters were estimated by correlation coefficients. All statistical methods were done according to **Snedecor and Cochran (1976).**

RESULT

As no significant differences were found between the parameters studied for the right and left testes or between Arab and native horses, the results were tabulated irrespective of testis side and breed.

The pattern of changes in testicular and parenchymal weight due to age are shown in Table (1). Age variations in testicular and parenchymal weights were significant ($P < 0.05$). On the other hand, age and each of testis weight and parenchymal weight were positively correlated ($P < 0.01$) up to 8 years old stallions, but a reverse correlations were noted with the advancement of age (Table 2).

The mean density of testicular tissues was 1.040 (range 1.034 to 1.048). Neither age nor season significantly influence the densities of tissues either within stallions or among the testes of stallions within groups (Tables 1 & 3).

Tunica albuginea weight and percent value showed a consistent and gradual increase over the whole age range studied. Although, tunica albuginea weight was not significantly influenced by age, the percent value was significanly ($P < 0.05$) affected. Moreover, age and tunica albuginea percent were correlated (Table 2).

The amount of shrinkage due to histological processing was 42% (range 39 to 45%) with no significant age and/or seasonal variations in the amount of shrinkage within stallions or among the testes of stallions within gorups.

A gradual increase in the percent of the seminiferous tubules in the testis was reported up to 13 years old, followed by a slight drop thereafter (Table 1). Age variations in the percentage of seminiferous tubules did not reach the level of statistical significance. Also, correlation coefficients between age and seminiferous tubules percent was below the level of significance (Table 2).

The corrected number of spermatids per stage I tubule cross section was 50.03 at 3 to 5 years, attained a maximum of 71.40 in group III and declined (54.69) thereafter. Such variations being highly ($P < 0.01$) significant. Moreover, age and number of spermatids per stage I tubule were highly correlated up to 13 years old stallions (Table 2).

Diameter of seminiferous tbules

Table (1) : Age-related changes in daily sperm production of Arab and native horses (± SD) .

Parameter	Group I (3-5 yrs.)	Group II (6-8 yrs.)	Group III (9-13 yrs.)	Group IV (14-18 yrs.)
Number of stallions	4	8	9	7
Testicular weight (g)	166.65 ±19.00	185.55 ±20.74	176.47 ±16.05	151.34* ±19.04
Testicular density	1.041 ±0.004	1.044 ±0.002	1.039 ±0.003	1.036 ±0.003
Parenchymal weight (g)	149.50 ±17.57	166.81 ±19.47	157.10 ±13.69	131.66 ±17.26
Tunica albuginea weight (g)	17.15 ±2.49	18.74 ±2.18	19.40 ±2.62	19.89 ±2.81
Tunica albuginea (%)	10.34 ±1.21	10.16 ±1.06	10.97 ±0.64	13.16* ±1.10
Seminiferous tubules (%)	61.83 ±7.91	63.01 ±5.23	64.39 ±5.59	58.98 ±3.41
Corrected number of spermatids / stage I tubule cross-section	50.03 ±3.43	56.61 ±4.54	71.40 ±4.95	54.69** ±5.44
Diameter of seminiferous tubules/ stage I (µm)	204.33 ±6.43	209,29 ±11.27	221.26 ±10.50	200.87* ±9.28
Daily sperm production (DSP):				
DSP/testis ($x10^9$)	3.75 ±0.80	4.56 ±0.86	5.30 ±0.67	3.38** ±0.81
DSP/stallion ($x10^9$)	7.50 ±1.60	9.11 ±1.72	10.60 ±1.35	6.76** ±1.63
DSP/g parenchyma ($x10^6$)	24.74 ±3.40	27.14 ±2.52	33.67 ±2.30	25.38** ±3.61

** Significant at 1% level & * Significant at 5% level

per stage I tubule displayed a consistent pattern of increase from gorup I through group III (Table 1). Age influenced ($P < 0.05$) and partially correlated with the diameter of seminiferous tubules (Tables 1 & 2).

Similar to the results of testis weight, percentage of seminiferous tubules, corrected number of spermatids and diameter of seminifer-

Table (2) : Correlation coefficients amongst the studied parameters .

Parameters	Testis wt.	Parench. wt.	T.alb. (%)	SNT (%)	No.of spermatid	Diameter of SNT	DSP/ testis	DSP/ g.
Age								
≤8 yr.[a]	0.434*	0.427*	-0.472**	0.099	0.433*	0.229	0.400*	0.344
≤13 yr.b	0.091	0.054	0.134	0.228	0.839**	0.554**	0.614**	0.747**
overall[c]	-0.412*	-0.469**	0.643**	0.234	-0.032	-0.106	-0.259*	-0.097
DSP/g								
8 yr.	0.800**	0.804**	-0.380*	0.580**	0.815**	0.091	0.958*	
13 yr.	0.305*	0.277	0.193	0.524**	0.916**	0.438**	0.912**	
overall	0.475**	0.473**	-0.281*	0.602**	0.932**	0.532**	0.926**	
DSP/testis								
8 yr.	0.930**	0.933**	-0.415*	0.649**	0.800**	0.285		
13 yr.	0.661**	0.639**	0.071	0.692**	0.775**	0.536**		
overall	0.797**	0.795**	-0.441**	0.764**	0.819**	0.630**		
Diameter of SNT								
8 yr.	0.479**	0.468**	-0.061	0.245	0.360			
13 yr.	0.438**	0.402**	0.241	0.505**	0.522**			
overall	0.559**	0.545**	-0.209	0.562**	0.604**			
No. of spermatid								
8 yr.	0.657**	0.678**	-0.493**	0.134				
13 yr.	0.144	0.119	0.194	0.227				
overall	0.344**	0.348**	-0.270*	0.338**				
SNT(%)								
8 yr.	0.727**	0.695**	-0.026					
13 yr.	0.662**	0.629**	0.161					
overall	0.797**	0.701**	-0.230					
T.alb.(%)								
8 yr.	-0.368	-0.446*						
13 yr.	-0.169	-0.267						
overall	-0.507**	-0.539**						
DSP/g								
8 yr.	0.997**							
13 yr.	0.996**							
overall	0.994**							

** Significant at 1% level. & * Significant at 5% level

$a_n = 12$ $b_n = 21$ $c_n = 28$

Table (3) : Seasonal variations in daily sperm production of Arab and native horses (± SD) .

Parameter	Non-breeding season	Breeding season	Overall mean
Number of stallions	13	15	28
Age (Years)	9.46 ±4.48	10.13 ±3.31	9.80 ±4.23
Testicular weight (g)	158.85 ± 19.57	182.24** ± 17.45	171.38 ± 22.00
Testicular density	1.041 ±0.003	1.039 ±0.004	1.040 ±0.004
Parenchymal weight (g)	141.05 ±18.83	162.29** ±16.39	152.42 ±20.87
Tunica albuginea weight (g)	18.34 ±1.52	19.70 ±2.75	19.01 ±2.55
Tunica albuginea (%)	11.54 ±1.60	10.82 ±1.37	11.20 ±1.51
Seminiferous tubules (%)	60.00 ±5.26	64.25 ±7.05	62.35 ±6.26
Corrected number of spermatids/stage I tubule crosssection	54.98 ±7.32	64.51** ±8.14	60.10 ±9.28
Diameter of seminiferous tubules/stage I (µm)	201.31 ±6.69	218.10** ±11.14	210.32 ±12.77
Daily sperm production (DSP):			
DSP/testis ($x10^9$)	3.66 ±0.85	5.01** ±0.89	4.39 ±1.12
DSP/Stallion ($x10^9$)	7.33 ±1.70	10.03** ±1.78	8.77 ±2.25
DSP/g parenchyma ($x10^6$)	25.45 ±4.78	30.84* ±4.25	28.45 ±4.78

** Significant at 1% level. & * Significant at 5% level

ous tubules per stage I, daily sperm production (DSP) per testis, DSP per stallion and DSP per gram parenchyma increased from 3.75 x 10^9, 7.50 x 10^9 and 24.74 x 10^6 (group I) to 4.56 x 10^9, 9.11 x 10^9 and 27.14 x 10^6, respectively (group II). Nevertheless, in age group III, inspite of the slight drop in testis weight, DSP per testis, DSP per stallion and DSP per gram continued to increase where maximum values of 5.30 x 10^9, 10.60 x

and 33.67 x 10^6 were achieved. age group IV, the decline in rates corresponds to a similar in the other criteria studied le 1). Age exerted a highly (P 01) significant influence on per testis, DSP per gram. ecover, age and each of DSP testis and DSP per gram were ially correlated (Table 2).

Testicular weight, parenchyma ght, number of spermatids per ge I tubule cross-section and P per gram accounted for 64%, , 67% and 86% of the varia- in the DSP per testis, respec- ly. Also, the number of sper- tids per stage I tubule accounted 87% of the variation in DSP per m parenchyma. The coefficient correlations were high enough to ow accurate prediction of the lat- from the former.

As regard seasonal influence, tis and parenchyma weights owed a substantial differences tween the breeding and non- eding season (182. 24 vs 158.85 P < 0.01) and (162. 29 vs 141.05 P < 0.01), respectively.

Although a slight increase in tu- ca albuginea weight was noted in e breeding season, differences in ther weight or percent contribu- on were not significantly influ- ced by season (Table 3).

The percentage of seminiferous tubules in the testis of stallions castrated in the breeding season was slightly higher than (64.25 vs 60%) those in the non-breeding season. Seasonal variation in the percentage of seminiferous tubules was not significant. On the other hand, season exerted a highly significant effect on the diameter of seminiferous tubules (201.31 vs 218.10 µm; $P < 0.01$) and the corrected number of spermatids (54.98 vs 64. 51; $P < 0.01$) per stage I, where about 8% increase for the former and 15% for the latter criterion were recorded in the breeding season (Table 3).

The production of sperm (per testis/day or stallion/day) was significantly lowest (3.66 x 10^9 vs 5.01 x 10^9 and 7.33 x 10^9 vs 10.03 x 10^9; $P < 0.01$) in the non-breeding season, averaging only 73% of the DSP in the breeding season. Whereas, the seasonal influence on the DSP per gram parenchyma was at a lower level ($P < 0.05$) of significance.

DISCUSSION

Estimates of testicular density, tunica albuginea weight and percent, and amount of shrinkage due to histological processing are crucial, when histological methods are practiced for determination of daily spermatozoal production. In the present study, the mean density of

testicular tissues reported for Arab and native horses was similar to the 1.042 (Hemeida et al., 1980) for the same breeds, 1.041 (Gebauer, et al., 1974) for the same species and 1.038 to 1.041 reported for the testes of the bull, boar and rabbit (Swierstra, 1966, 1986; Amann, 1970). The mean weight and percent contribution of tunica albuginea (19.01 g and 11.20%) were very close to 19.18 g and 12.01% (Hemeida et al., 1980) and 20 g and 12.3% (Swierstra et al. 1974). The increase in tunica albuginea weight and percent value with advancement of age could be related to the progressive testicular fibrosis, which is a histological feature of aging (Humphrey and Ladds, 1975; Hemeida et al., 1980). In agreement with Gebauer et al. (1974), the amount of shrinkage due to histological processing was more consistent among the stallion testes than that (41 to 58%) reported for bull (Swierstra, 1966) and 45 to 55% for boar testes (Swierstra, 1968).

Testicular weight of Arab and native horses (171.38g) was close to 170 g (Amann et al ., 1979), 167g (Hemeida et al., 1980), 163g (Swierstra et al., 1974); higher than 125 to 149 g (Berndtson et al. 1983) and lower than 213 and 216 g (Gebauer et al., 1974). Large inherent variability in testicular size (Berndtson et al., 1983), breed, rearing system and varied ages could account for such discrepa cy. The results of age-relate changes in tesricular weight are i partial agreement with that repor ed by Johnson and Neaves (198 Berndtson et al. (1983), an Johnson and Thompson (1983 who reported that testicular weig increased with age.

The present findings confirm the results of Swierstra et a (1974) that the seminiferous t bules made up to 61.3 % of th stallion testis ; 58 to 61 .6 % r ported by Berndtson et al., (198 with no significant differences b twewn the age groups studied. corroboration with Johnson an Neaves (1981), the diameter of t seminiferous tubules was signi cantly higher in mature than youn horses. Slightly higher values 212 to 242 µm (Johnson an Neaves, 1981) and lower values 156 µm (Swierstra et al ., 197 and 115 to 158 µm (Berndtson al., 1983) were reported.

The corrected number of spe matids per stage l tubule displaye a similar pattern with the adanc ment of age as did the DSP/testi DSP/stallion and DSP/g. The ove all mean DSP/testis and DSP sta lion determined in the curre study, coincided with the 4.0x10 testis (range 2.5 to 5.4 x 10^9) and mean of 8.0x10^9 /stallion estimat by quantitative histological analy sis (Gebauer et al., 1974), 3.9

.6x 10^9 / testis estimated by ho-
ogenate method (El-Wishy et al.,
980) and higher than 1.27 to
.18x10^9 / testis reported by John-
on and Neaves (1981). The mean
alue for DSP/g parenchyma of
Arab and native stallions was close
o the 26.72x10^6 (Hemeida et al.,
980) using a divisor time of 5.1
lays and higher than those (21.0 to
1.5x10^6) reported by Gebauer et
l. (1974) for Quarter and Thor-
oughbred horses which have larger
estes (213 and 216 g). The lower
igure given by Amann et al.
1979) was due to the use of a
onger divisor time of 6.0 days.
Moreover, difference due to breed,
selection, rearing system, age and
nutritional regimen may be con-
tributing factors.

In the present study, the in-
crease in DSP efficiency with sub-
sequent leveling- off up to 13 years
old and the decrease is spermato-
genic activity of the testes in older
horses is consistent with age-
related studies in stallions. The
peak values for DSP rates repoted
herein coincided with a similar
peak in the gonadal and epididymal
sperm reserve (El-Wishy et al.,
1980), the extragonadal sperm re-
serve (Amann et al., 1979) and the
number of ejaculated spermatozoa
(Pickett et al., 1979, El-Baghdady
et al., 1990). With the approach of
senescence, the testis of the stallion
diminish their capacity to produce
spermatozoa as indicated by the
drastic drop in DSP rates reported
for older horses. It is possible that
age-related changes in Leydig cell
population (Johnson and Neaves,,
1981) may be involved. Also, a
progressive reduction in Sertoli
cell functions and/or regression in
their numbers (Jones and Berndt-
son, 1986) occurred in the stallion
testes with senescence. The dele-
terious effect of the aged basement
membrane and tunica propria of
the seminiferous tubules (which
separate the germinal epithelium
from blood supply due to fibrosis
and thickening) on the spermato-
genic processes (Paniagua et al.,
1987) might be implicated. Finally,
testosterone is required for the
completion of meiosis during sper-
matogenesis (Steinberger, 1971),
the apparent reduction in its level
(both peripherally and intratesticu-
larly) at older ages (Gusmao et al.,
1988; Berndtson and Jones,
1989) may increase the rate of cell
loss during the postprophase divi-
sion which ultimately result in re-
duced rate of daily sperm produc-
tion (Johnson and Thompson,
1983).

Although Arab and native hors-
es are capable of breeding all the
year round, the testes in the breed-
ing season (December-May) are
more adapted for high levels of
sperm production. However, testes
from horses in the non-breeding
season (June-November) did con-
tinue to produce spermatozoa al-
biet at a lower rate. In the current
study, some significant seasonal

changes were detected and these may have implications for the breeding potential of stallions.

Testicular weight, parenchymal weight, diameter of seminiferous tubules, corrected number of spermatids per stage I tubule, DSP/testis and DSP/stallion increased by 13%, 14%, 8%, 15%, 27% and 27%, respectively in the breeding season. The current results confirms the observations of **Berndtson et al. (1983) and Johnson and Thompson (1983)** on the seasonality of equine spermatogenesis and the seasonal nature of spermatozoa production by a quantitative histological technique. Furthermore, seasonal changes in testicular weight and DSP rates reported in this study, might be driven seasonal fluctuations in concentrations of LH **(Thompson et al., 1977)** and testosterone **(Berndtson et al., 1974)** in the blood or intratesticular **(Berndtson et al., 1983; Berndtson and Jones, 1989)**. These seasonal changes possibly could be induced by photoperiod, which drives seasonal changes in hormone (FSH, LH and testosterone) concentrations **(Clay et al., 1988)**. Moreover, the increase in the testicular weight and DSP rates reported in the breeding season could be explained by an elevated population of A spermatogonium **(Johnson, 1985; Johnson and Tatum, 1989)**, the number of Sertoli cell/testis **(Johnson and Nguyen, 1986)** and Leydig cells **(Johnson and Thompson, 1986)**. An elevated Sertoli cell population in the breeding season would accommodate a larger number of germinal cells than would normally be supported during the non-breeding season, when sperm out put and sperm production continued at lower rate **(Thompson et al. 1977; Johnson and Thompson 1983)**.

Based upon the present results the onset of breeding season in Arab and native horses was associated with a singificant increase in testicular weight, parenchymal weight, diameter of seminiferous tubules, corrected number of spermatids per stage I tubule cross section and DSP rates. Therefore stallions are capable of producting significantly more spermatozoa (2.70×10^9 additional sperm stallion/day) during the breeding season (December-May) and could supply sufficient numbers of sperm for insemination of more mares at this time. Presumbly, it is reasonable that stallions of 6 to 13 years old are adapted for greater sperm production potential as well that could either younger or older horses.

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SUMMARY

Fifty-six testes were collected from 28 Arab and native stallions (aged 3 to 18 years) along a complete annual cycle to determine the influence of age and season on the daily sperm production (DSP) by a quantitive histological technique. Overall mean values for testicular measurements, percentage of seminiferous tubules (SNT%), corrected number of spermatids and diameter of SNT/stage I cross-section were given in the text. Estimates of 4.39 x 10^9 sperm/testis/day, 8.77 x 10^9 sperm/stallion/day and 28.45 x 10^6 sperm/g parenchyma were noted. highly significant ($P < 0.01$) increase with age in these traits was evident up to 13 years old stallions, where peak values of 5.30 x 10^9, 10.60 x 10^9 and 33.67 x 10^6 for the foregoing parameters were achieved. On the other hand, testicular weight, parenchymal weight, number of spermatids per stage I tubule cross-section and DSP/g accounted for 64%, 63%, 67% and 86% of the variation in the DSP/testis, respectively. Age-related changes in the above mentioned parameters were also scrutinized.

The onset of breeding season (December-May) in Arab and native horses was associated with a significant increase in testicular weight (13%), parenchymal weight (14%), diameter of SNT/stage I cross-section (8%), corrected number of spermatids/stage I tubule cross-section (15.%), DSP/testis (27%) and DSP/stallion (27%). The production of sperm (per testis/day or stallion/day) was lowest (3.66 x 10^9 vs 5.01 x 10^9 and 7.33 x 10^9 vs 10.03 x 10^9; $P<0.01$) in the non-breeding season, averaging only 73% of the DSP in the breeding season. Therefore, stallions are capable of producing significantly more spermatozoa (2.70 x 10^9 additional sperm/stallion/day) in the breeding season and could supply sufficient numbers of sperm for insemination of more mares at this time.

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