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Original article

Is spermiogenesis common or rare in young male European bison aged 2 and 3 years?

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Abstract

The aim of the present study was to determine how often spermiogenesis occurs in young male European bison up to 3 years old. Research was performed on sections of the testes and epididymes collected from 51 male bison aged 2-3 years. The animals were divided into 2 age groups: young males up to 2 years and young males up to 3 years old, with further separation into specimens with or without spermiogenesis. The animals were culled during the autumn-winter seasons in 1994-2008 (after rutting period) in the Bialowieża Primeval Forest. Spermiogenesis in the 2-year-old animals was a rare condition found in 16.7% of cases. However, at the age of 3 years more than half of the individuals examined (53.3%) had spermiogenesis. Young males up to 2 years old with spermiogenesis were characterized by a significantly higher body weight and their right testis also weighed more than the left testis of the animals without spermiogenesis, the difference being on the border of statistical significance.

There was no significant differences in the body mass and weight of the left testis between older animals, up to 3 years old, with or without spermiogenesis. However, young males up to 3 years old with spermiogenesis were characterized by a significantly higher weight of the right testis than those without spermiogenesis.

Key words: *Bison bonasus,* juveniles, frequency of **s**permiogenesis, body weight, testis weight

Introduction

The research performed on the postnatal development of spermiogenesis in male European bison *Bison bonasus* (L.) in 1969-1993 revealed no occurrence of spermiogenesis in the group of the youngest males up to 2 years of age (37 individuals) (Czykier et al. 1999). In the same study, of the males up to 3 years old (13 individuals) only 3 animals had spermiogenesis. To verify the results, a subsequent research was conducted in 1998-2003 on 45 males of European bison in the age ranges from 3 months to 3 years. Spermiogenesis was not found in calves up to one year old. However, it was observed in animals up to 2 years old, with the youngest case of a 15-month-old male (Czykier and Krasińska 2004). The occurrence of spermiogenesis in young male European bison up to 3 years old was also confirmed (Czykier and Krasińska 2004). Studies performed by other authors based on direct observations of males and analysis of pedigree data from European Bison Pedigree Book revealed successful matings by males aged 15-20 months in captivity (Zablocki 1949, Mohr 1952, Jaczewski 1958, Daleszczyk and Czykier 2010). Also Bomba (1995) demonstrated the presence

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of spermatozoa in an electron microscopic picture in one 1.5-year-old European bison male. Our findings (Czykier et al. 1999, Czykier and Krasińska 2004, Czykier 2010) suggest that the occurrence of spermiogenesis in young European bison up to 3 years old is a rare phenomenon.

The aim of the present study was to determine the frequency of occurrence of spermiogenesis in young male European bison in the age range of 18-36 months and to compare the weight of the body and testes between animals with and without spermiogenesis.

Materials and Methods

Animals

The histological material originated from the testes and epididymes of 51 male European bison aged 18-36 months. The material was collected from the individuals culled during the autumn-winter season in 1994-2008 (after rutting period) in the Bialowieża Primeval Forest (Krasiński and Raczyński 1967). Forty-three males at the age of 18-36 months were from a free-ranging population, and 8 animals aged 18-27 months were from captive breeding.

The animals were eliminated due to routine reduction in the population number. The choice was made for a variety of reasons i.e.: diseases, with a special emphasis on posthitis/balanoposthitis, poor condition, exterior defects, injuries of various origin, aggression towards people or senile age (Krasińska and Krasiński 2007). A relatively numerous material was obtained from diseased males with epididymal cysts (n=19) (Matuszewska and Sysa 2001, Matuszewska and Sysa 2002).

The age of free-ranging European bison males was estimated by Z.A. Krasiński and J. Dackiewicz from Bialowieza National Park (BNP) according to the sequence of eruption of primary teeth and exchange into secondary dentition (Węgrzyn and Serwatka 1984), and size and shape of horns (Krasiński et al. 1982). The age of animals from captive breeding was defined in the data from European Bison Pedigree Book. The culling took place in the morning hours.

Sections from the upper pole of the left and right testis, and fragments of the epididymal corpus were obtained separately for examination. The material was fixed in Bouins fluid; 6 µm paraffin sections were stained with hematoxylin and eosin (H+E). Microscopic preparations of the testes and the epididymes were subjected to histological examination using light microscopy (maximum magnification 1000x). The data concerning body weight (n=51), and weight of right testis (n=36) and left testis (n=34) of European bison males were provided by BNP. The animals involved in the study were divided into two age groups: young males up to 2 years old and young males up to 3 years old, with further separation into specimens with or without spermiogenesis. The criteria of the presence of spermiogenesis included the existence of spermatids in seminiferous epithelium of the testes or the presence of spermatozoa in the lumen of the seminiferous tubules of the testes and/or frequency of spermatozoa in the epididymal duct (Czykier and Krasińska 2004, Czykier 2008). Calves up to 1 year old were discarded from the analysis, because in none of the investigations the presence of spermiogenesis was observed in such young animals. The limiting age of 3 years was established since the previous histological research showed that at the age of 4 years all the animals tested had spermiogenesis in the seminiferous tubules of the testes (Czykier et al. 1999).

Statistical methods

The quantity variables were described by the mean value ± standard deviation (SD) analysis. Both parametric (t-Student test) and nonparametric tests (Mann-Whitney test) were used for analysis. The results statistically significant were accepted at the level of $p<0.05$. In evaluation, the statistics software SPSS 7.5 was used.

Results

Young males up to two years old

In this age class (n=36), only 6 European bison (16.7%) had spermiogenesis (Fig. 1), and only two of them were from captive breeding and four from a free-ranging herd. The mean value of the animals' weight was 277.50 kg (with spermiogenesis) and 221.17 kg (without spermiogenesis), the difference being statistically significant ($p<0.002$) (Table 1). The mean weight of the right testis with spermiogenesis was 65.93 g and was statistically significantly higher ($p<0.03$) than the mean weight of the right testis without spermiogenesis – 42.37g (Table 2). The mean weight of the left testis with spermiogenesis was 62.23g and was higher compared to that without spermiogenesis – 34.13 g, the difference being on the border of statistical significance ($p<0.05$) (Table 2). In this age class, 11 (30.7%) European bison males had epididymal cysts.

Young males up to three years old

In this age class (n=15), 8 European bison (53.3%) had spermiogenesis (Fig. 2), which in com-

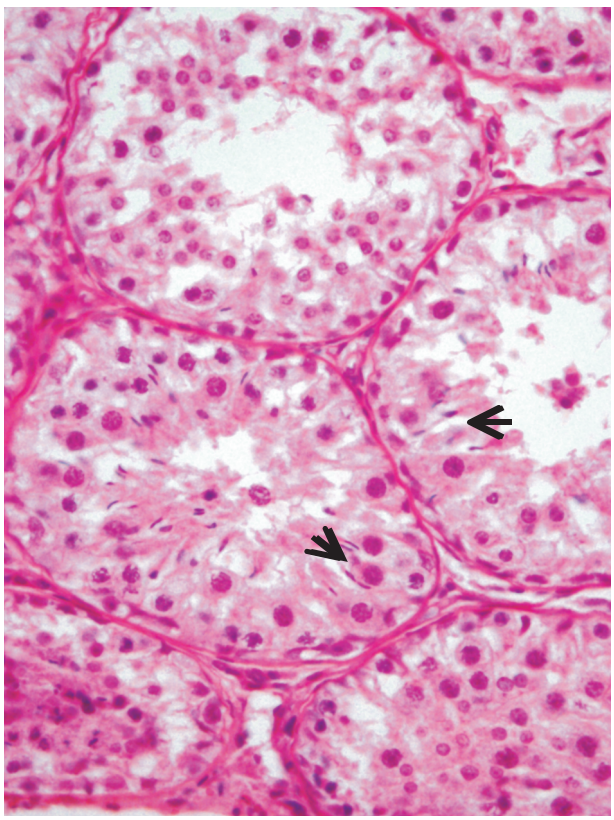

Fig. 1. Fragment of the testis in a 24-month-old male European bison. The seminiferous tubules show increased tortuosity, enlarged diameter and there is a considerably decreased amount of loose coonective tissue between them. The seminiferous tubules contain elongated late spermatids within the seminiferous epithelium (arrows). H+E. Magnification x 400.

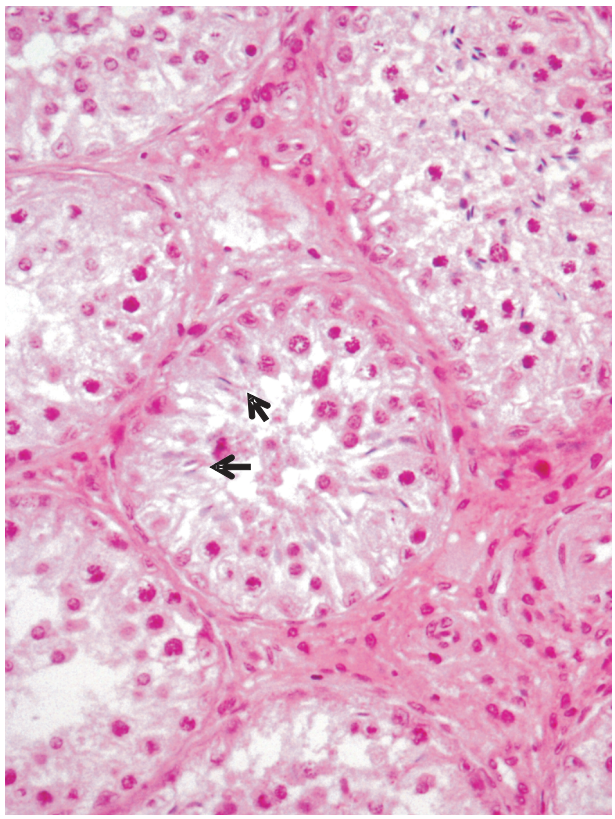

Fig. 2. Fragment of the testis in a 36-month-old male European bison. The seminiferous tubules contain elongated late spermatids (arrows). H+E. Magnification x 400.

Table 1. Body weight of all young males of European bison (I – young males up to 2 years old, II – young males up to 3 years old). A – young males with spermiogenesis, B – young males without spermiogenesis, ns – insignificant difference, *n* – number of individuals.

Age class		*n*	Body weight (kg)				
			Mean	SD	Min	Max	*p*
I	A	6	277.50	49.17	235.00	340.0	$p<0.002$
	B	30	221.17	33.95	160.00	310.0	
II	A	8	307.50	19.09	240.00	390.0	ns, $p=0.397$
	B	7	291.43	54.29	290.00	340.0	

parison to the frequency of spermiogenesis in young European bison males up to 2 years old was a statistically significant difference ($p<0.01$). Of these 8 males up to 3 years old, only two were from captive breeding and 6 were from a free-ranging herd. The animals up to 3 years old with spermiogenesis had mean body weight of 307.50 kg and those without spermiogenesis in the same age group reached 291.43 kg; however, the difference was statistically insignificant (Table 1). The mean weight of the right testis with spermiogenesis was 92.08 g and was statistically significantly higher ($p<0.03$) than the mean weight of the right testis without spermiogenesis – 38.5 g (Table 2). However, the mean weight of the left testis with spermiogenesis was 55.13 g, being lower compared to that without spermiogenesis – 68.04 g, although the difference was statistically insignificant ($p=0.78$) (Table 2). In this age class, 8 (53.3%) European bison males had epididymal cysts.

In the two study groups, all European bison males

showed normal histological picture of the testes (Figs. 1, 2, 3, 4) and epididymes, except for the presence of epididymal cysts, which were also revealed histologically.

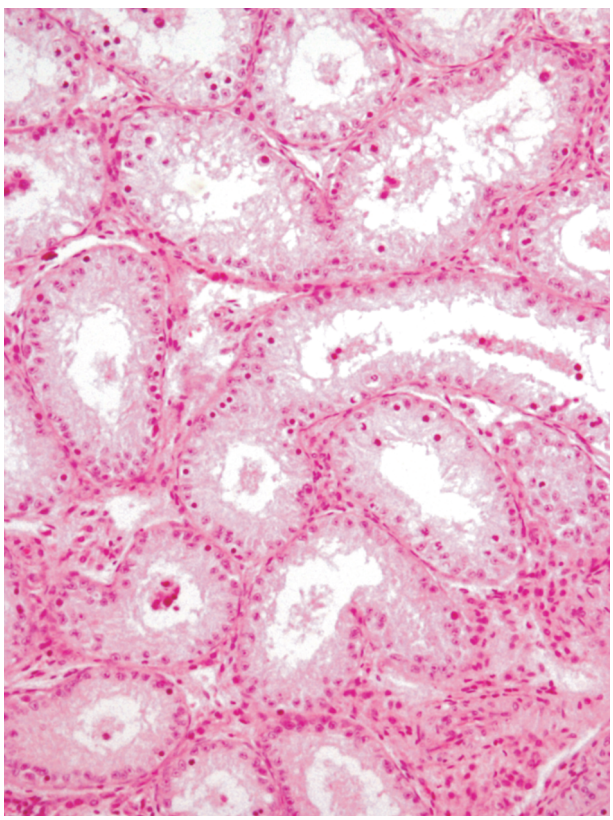

Fig. 3. Fragment of the testis in a 24-month-old male European bison. The seminiferous tubules with a visible lumen, lined with the seminiferous epithelium showing predominance of Sertoli cells and clusters of Leydig cells between tubules. H+E. Magnification x 200.

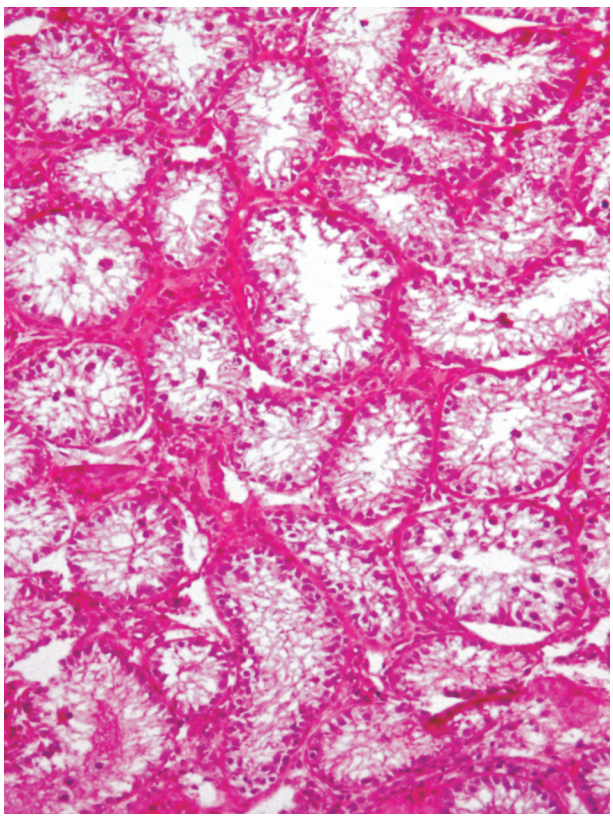

Fig. 4. Fragment of the testis in a 36-month-old male European bison. Sertoli cells still dominate in the tubules. H+E. Magnification x 200.

Discussion

The present findings concerning the frequency of spermiogenesis in young European bison males up to 2 years old are similar to results obtained previously in this field (Czykier et al. 1999, Czykier and Krasińska

Table 2. Weight of right and left testis of young males of European bison (I – young males up to 2 years old, II – young males up to 3 years old). RT – right testis, LT – left testis, A – young males with spermiogenesis, B – young males without spermiogenesis, ns – insignificant difference, *n* – number of individuals.

Age class			*n*	Weight of testis (g)				
				Mean	SD	Min	Max	*p*
I	RT	A	4	65.92	18.33	40	83.1	$p<0.03$
		B	18	42.37	21.09	14.7	93	
	LT	A	4	69.23	36.12	31.5	103.1	$p<0.05$
		B	21	34.12	12.3	14.7	52	
II	RT	A	6	92.08	27.82	70	137.7	$p<0.03$
		B	6	38.5	36.4	19.6	112.5	
	LT	A	6	55.13	32.18	18.2	92.3	ns, $p<0.78$
		B	5	68.04	27.8	19.8	82	

2004, Czykier 2008). In this study, in young males up to 2 years old spermiogenesis was rare and could be observed in a marginal number of animals (16.7%). The present findings prove that spermiogenesis in European bison aged up to 2 years is definitely an occasional phenomenon.

However, the present study has revealed that over half of the young 3-year-old European bison males (53.3%) examined demonstrate the presence of spermatozoa in the epididymal duct, which indicates that the definite occurrence of spermiogenesis in 3-year-old animals is more frequent than in the group of the 2-year-olds. This finding becomes clear when we take into consideration that at the age of 4 years all the male European bison examined had spermiogenesis and entitles us to verify the existing ideas concerning the frequency of spermiogenesis in 3-year-old European bison. In comparison to other ungulates, spermiogenesis in European bison appears quite late (Czykier et al. 1999). For example in male wapiti (*Cervus elaphus*), the sperm was found to be present in ejaculate in 2-year-old specimens (Haigh et al. 1984), in male fallow deer (*Dama dama*) at the age of 16 months (Chapman and Chapman 1970, Chaplin and White 1972), in male red deer (*Axis axis*) between 12 and 15 months (Webster et al. 1992) and in male North American bison *(Bison bison)* from 18 to 33 months of age (Helbig et al. 2007).

The present study revealed that both 2- and 3-year-old European bison that had spermiogenesis also demonstrated higher body weight than animals in their age groups without spermiogenesis. This regularity was also observed in other investigations (Czykier and Krasińska 2004). However, in the present study, the differences in body mass between young males up to 2 years old with and without spermiogenesis were statistically significant. Although young males up to 3 years old with spermiogenesis showed a clear tendency to reach higher body weight in comparison to the animals without spermiogenesis in this age group, the difference was statistically insignificant. We presume that the considerably lower number of the animals aged 3 years might have influenced the lack of statistical significance in the analysis of differences between the mean values of this parameter. In the present study, higher body weight in European bison with spermiogenesis was observed both in free-ranging and captive-bred animals. It seems that the origin of European bison (free-range, captive breeding) and the related feeding factor do not influence significantly the frequency and onset of spermiogenesis in 2- and 3-year-old European bison.

In the present study, the young 2-year-old European bison males with spermiogenesis were found to have higher weight of the testes than their peers without spermiogenesis. The same regularity was observed in the young 3-year-old European bison for the right testis, being heavier in the animals with as compared to those without spermiogenesis. However, the finding concerning the left testis in the 3-year-old animals was totally the opposite. The mean weight of the left testis with was lower than that without spermiogenesis. The European bison show great differences in the weight of the testes, reaching even 30% in the same specimen (Krasińska et al. 2009), however with no regularity as both the right and the left testis can be larger (Gill 1999). In our study, the finding concerning the weight of the left testis in the 3-year-old European bison may be due to the asymmetry of the testicular weight observed in these animals.

The present analysis indicates that in 3-year-old European bison males spermiogenesis is more common than in the 2-year-old animals. Moreover, at the age of 3 years, more than half of the European bison examined reach sexual maturation, which is manifested by the presence of spermiogenesis.

Acknowledgment

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