

Cases of spermiogenesis in young European bison

Elżbieta CZYKIER and Małgorzata KRASIŃSKA

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Development of spermiogenesis and sizes of seminiferous tubules of the testes and epididymal duct were studied in 45 young European bison *Bison bonasus* (Linnaeus, 1758) males from an enclosed breeding centre and a free-ranging population in the Białowieża Forest. Of 13 males in age class II (up to two years), four showed the presence of elongated late spermatids: one male aged 15 months from the enclosed breeding centre and three from the free-ranging population (2 males aged 18 months and one about 24 months old). Of 7 males from age class III (up to 3 years), four showed spermiogenesis, of which the youngest, 26 and 32 months old, were from enclosed breeding. Spermiogenesis was observed in males with high body weight in a given age class. In males of age class I (up to one year old), the mean diameter of the seminiferous tubules was 49.8 μm , in class II – 110.7 μm , and in class III – 162 μm , the mean diameter of the epididymal duct being 110.8 μm , 187.2 μm and 273.4 μm , respectively. Measurements of seminiferous tubules and epididymal duct were significantly correlated with age and body weight of males and differed significantly between the three age classes.

Department of Histology and Embryology, Medical University, 15-089 Białystok, Kilińskiego 1, Poland, e-mail: czykier@amb.edu.pl (EC); Mammal Research Institute, Polish Academy of Sciences, 17-230 Białowieża, Poland, e-mail: mkrasin@bison.zbs.bialowieza.pl (MK)

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Introduction

In the study on the postnatal development of spermatogenesis in the European bison *Bison bonasus* (Linnaeus, 1758) conducted in the years 1969–1993 spermiogenesis was not observed in the youngest males aged up to two years (37 individuals) and only single specimens developed spermiogenesis at the age of 3 years. Four-year-old males, however, were characterized by fully developed spermatogenesis (Czykier *et al.* 1999). Studies performed by other authors based on direct observations of males and analysis of pedigree data from the European Bison Pedigree Book revealed successful matings by males aged 15–20 months in captivity (Zablocki 1949, Mohr 1952, Jaczewski 1958). Also Bomba (1995) demonstrated the presence of spermatozoa in the electron microscopic picture in one 1.5-year-old male European bison.

In European bison males, living in the Białowieża Forest, the disease of prepuce and penis defined as posthitis has been reported since 1980. It has been observed in

males of all age classes (Pusiński *et al.* 1997, Krasińska and Krasiński 2004). Since 1995 another pathological change, namely epididymal cysts, has been observed in the European bison (Matuszewska and Sysa 2000, 2002).

The aim of the study was to examine the development of spermiogenesis in young male European bison aged up to 36 months, in relation to breeding conditions (enclosed breeding, free-ranging population) and to body weight, as well as to estimate the changes in the diameters of the seminiferous tubules and epididymal duct in relation to age and body weight of young males. The likely effect of such pathologies as the posthitis disease and epididymal cysts on the histological structure of the testes of young males was also taken into consideration.

Material and methods

The material used for analysis was collected from the European bison living in a free-ranging population in the Białowieża Forest (33 individuals aged from 3 months to 3 years). The other samples were obtained from bison kept in captivity (12 individuals aged 6–32 months). The animals were divided into three age groups: class I – calves up to one year old, class II – juveniles up to two years old, and class III – juveniles up to three years old (Table 1). The age of bison from the free-ranging population was established by Z. A. Krasiński (BNP) according to a sequence of tooth eruption, degree of tooth wear (Węgrzyn and Serwatka 1984) and growth of horns (Krasiński *et al.* 1982). The age of bison from enclosed breeding was known from pedigree data.

The animals were culled between October and April in the years 1998–2003 (most males were culled in the period of December–March) for several reasons, including a reduction in number, injuries, poaching or a male disease defined as necrotic posthitis. The relatively numerous material was obtained from diseased males with posthitis ($n = 23$) and epididymal cysts ($n = 18$).

Culled animals were always provided in the same way. The material for histological investigations was collected by workers of the Białowieża National Park (BNP) and Mammal Research Institute, Polish Academy of Sciences in Białowieża, and preserved. Sections from the upper pole of the testis and, separately, fragments of the epididymal corpus, were obtained for examination. The material was fixed in Bouin's fluid; 6 μ m paraffin sections were stained with hematoxylin and eosin (H+E). Microscopic preparations of the testes and epididymes were subjected to histological examination using a light microscope (maximum magnification – 400 \times). The diameters of seminiferous tubules and epididymal duct were measured in histological preparations. The measurements were taken on 50 transverse cross-sections of the seminiferous tubules and on 50 transverse cross-sections of epididymal ducts from each animal (5 vision fields from each preparation, 10 measurements of diameters of seminiferous tubules on the transverse cross-section in each vision field and 10 measurements of diameters of the epididymal duct on the transverse cross-section in each vision field), using the morphometric program analySIS (Olympus). The diameter of the epididymal duct was measured in age class I only in 20 individuals, because of the lack of material from all the males.

Statistical analysis of the results of the three groups of European bison was based on the statistical program SPSS 8.0 PL using the Mann-Whitney *U*-Wilcoxon Rank Sum *W* Test. A linear Pearson correlation analysis was performed to evaluate the relationship between the diameter of seminiferous tubules of the European bison testes and body weight and age of these animals, between the diameter of epididymal duct and body weight and age and between the diameters of seminiferous tubules and epididymal duct of the animals. We also compared the percentage of animals with posthitis or epididymal cysts among the groups using the Chi-square test. *P* values < 0.05 were considered significant.

The presence of spermatids in seminiferous epithelium or the presence of spermatozoa in the lumen of the seminiferous tubules of the testes and/or presence of spermatozoa in the epididymal duct were considered the criteria of spermiogenesis. Data referring to body weight and to records of posthitis were obtained from the Białowieża National Park.

Results

Calves up to one year old

The mean diameter of seminiferous tubules of the testes on the cross-section was $49.8 \pm 11.25 \mu\text{m}$ (Table 1). The mean epididymal duct diameter was $110.84 \pm 42.14 \mu\text{m}$ ($n = 20$) (Table 1).

In the study group of 25 animals, in 23 calves the histological picture of the testes was relatively homogenous. The seminiferous tubules had no lumen and were filled with primary Sertoli cells lying on the basement membrane and few primary spermatogonia situated proximally to the central part of the tubule (Fig. 1). The mean diameter of the seminiferous tubules in these 23 animals was $47.5 \pm 8.3 \mu\text{m}$. Epididymal duct epithelium was not fully developed, resembling cylindrical monolayer rather than pseudostratified epithelium; stereocilia were not visible (Fig. 2). In these animals, the mean diameter of the epididymal duct on the cross-section was $103.4 \pm 36.5 \mu\text{m}$ ($n = 18$).

In two specimens from the enclosed breeding centre the testes were histologically more mature than in the other males of the same age class. In these two

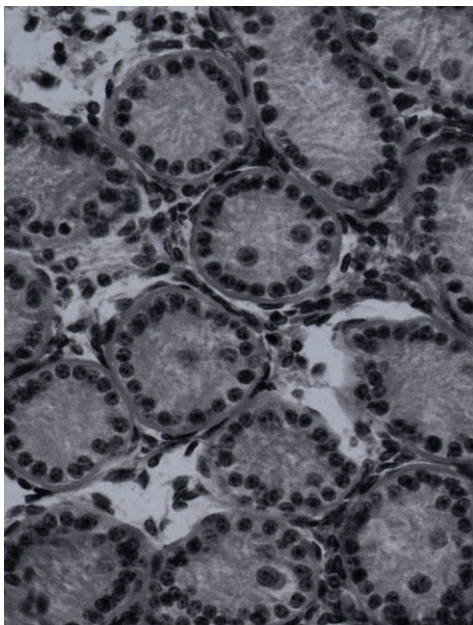


Fig. 1. Fragment of the testis of a 5-month-old male. Seminiferous tubules are devoid of lumen, have a small cross-section diameter. Seminiferous epithelium shows a predominance of Sertoli cells; young spermatogonia (1–3 in number) are most frequently situated above the nuclei of these cells. H+E. Magnification 200.

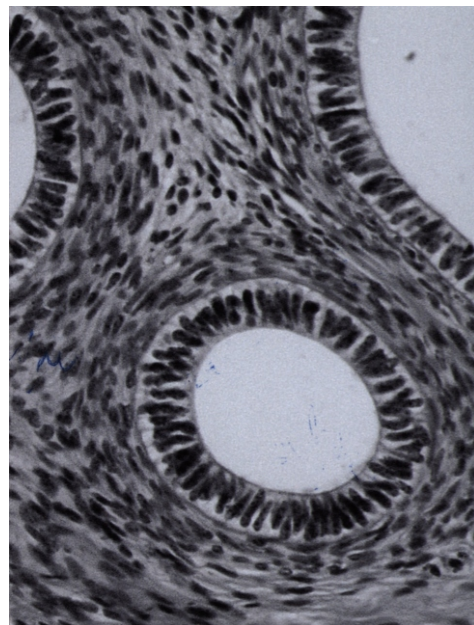


Fig. 2. Fragment of the epididymis of a 9-month-old male. Epididymal duct epithelium is not fully developed, resembling cylindrical monolayer rather than pseudostratified epithelium; stereocilia are not visible. H+E. Magnification 400.

Table 1. Comparison of the presence of spermatozoa, epididymal cysts, symptoms of posthitis and ranges of body weight, diameter of seminiferous tubules, diameter of epididymal duct in males of the European bison of three classes of age.

Age class	Age (months)	n	Body weight (kg)	Number of animals with:			Diameter of seminiferous tubules (μm)			Diameter of epididymal duct (μm)		
				Spermatozoa	Epididymal cysts	Symptoms of posthitis	Avg	SD	Range	Avg	SD	Range
I	3-10	25	38-220	-	10	10	49.8	11.2	34.0-77.5	110.8	42.1	50.4-207.9
II	15-24	13	160-266	4	5	9	110.7	29.1	50.7-166.2	187.2	50.3	105.9-267.9
III	26-38	7	240-410	4	3	4	162.1	41.5	86.8-198.6	273.4	46.3	104.0-319.3

Table 2. Average values of body weight and diameters of seminiferous tubules and epididymal duct in European bison males with or without the presence of spermatozoa in the testes in two age classes. Mann-Whitney *U*-test was used for comparison. ns - not significant.

Parameter	Individuals with spermatozoa			Individuals without spermatozoa			Significance
	n	Avg	SD	n	Avg	SD	
Body weight				Males up to two years old			
Diameter of seminiferous tubules	4	241.2	17.5	9	207.8	25.6	$p < 0.05$
Diameter of epididymal duct	4	130.6	32.8	9	101.9	24.1	ns
	4	193.8	60.4	9	184.3	49.0	ns
Body weight				Males up to three years old			
Diameter of seminiferous tubules	4	335.0	54.5	3	293.3	46.2	ns
Diameter of epididymal duct	4	181.4	14.9	3	136.2	55.4	ns
	4	280.0	52.8	3	264.7	45.3	ns

calves, lumen started to appear in the seminiferous tubules (Fig. 3). Their mean diameter was $76.1 \pm 2.0 \mu\text{m}$, being significantly higher than in the remaining 23 calves in this age class ($U = 0.0$, $W = 49.0$, $p < 0.021$).

Epididymal duct epithelium in these two European bison males was fully developed. It was pseudostratified, with distinct fine stereocilia. The mean diameter of the epididymal duct in these animals was $177.7 \pm 33.7 \mu\text{m}$ and was larger than in the remaining 18 calves of this age class, the difference being on the border of significance ($U = 3.0$, $W = 36.0$, $p < 0.059$) (Table 2). In one of these two calves, inflammatory changes of posthitis type were observed, and both of them had epididymal cysts. These two males were the heaviest in their age class.

Of 25 males of the age class I, 10 had symptoms of posthitis and 10 suffered from epididymal cysts. Only four specimens had both cysts and posthitis.

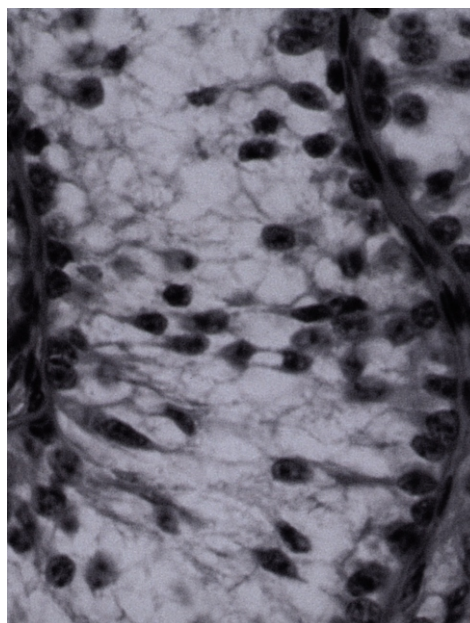


Fig. 3. Fragment of the testis of a 10-month-old male. The testes are histologically more mature. A lumen starts to appear in the seminiferous tubules. H+E. Magnification 400.

Juveniles up to two years old

The mean diameter of the seminiferous tubules was $110.7 \pm 29.1 \mu\text{m}$ (Table 1), being significantly larger compared to the age class I ($U = 12.0$, $W = 404.0$, $p < 0.001$). The mean diameter of the epididymal duct was $187.2 \pm 50.4 \mu\text{m}$ (Table 1) and was also statistically significantly larger than in class I ($U = 28.0$, $W = 323.0$, $p < 0.001$).

Among 13 males in the age class II, four had spermiogenesis, including a 15-month-old male from enclosed breeding and three bison from free-ranging population (two 18- and one about 24-month-old). Two males with spermiogenesis had the highest body weight in their age class and no pathological changes in the epididymis or prepuce. The other two were also characterized by high body weight reaching the maximum values of this age class and had posthitis but not cysts (Table 1). These four males with spermiogenesis had a significantly greater body weight ($U = 4.5$, $W = 41.5$, $p < 0.037$) compared to the remaining nine bulls in this age class (mean 241.2 ± 17.5 and 207.8 ± 25.6 kg respectively) (Table 2), despite

younger age. Among 13 males in this age class, nine had symptoms of posthitis, 5 had epididymal cysts, and 3 males had both pathological changes.

In these four bison with spermiogenesis the seminiferous tubules of the testes (mean diameter $130.6 \pm 32.8 \mu\text{m}$) (Table 2) contained elongated late spermatids within the seminiferous epithelium (Fig. 4). However, there were no spermatozoa in the epididymal duct (mean diameter $193.8 \pm 60.5 \mu\text{m}$) (Table 2).

The remaining 9 individuals with no features of spermiogenesis had seminiferous tubules (mean diameter $101.94 \pm 24.08 \mu\text{m}$) (Table 2) with a visible lumen, lined with seminiferous epithelium which showed predominance of Sertoli cells and clusters of Leydig cells between the tubules (Fig. 5). In the epididymal duct of these animals (mean diameter $184.3 \pm 49.0 \mu\text{m}$) (Table 2), pseudostratified epithelium with low stereocilia was observed. These males had distinctly smaller diameters of seminiferous tubules of the testes and smaller diameters of the epididymal ducts, compared to those in two-year-old males with spermiogenesis, the difference being statistically insignificant (Table 2).

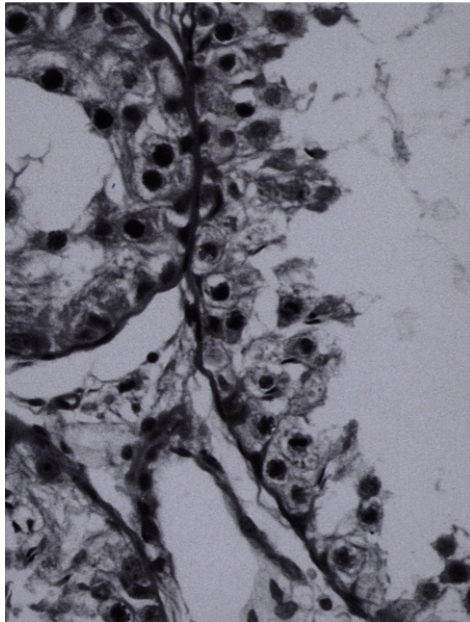


Fig. 4. Fragment of the testis of a 24-month-old male. The seminiferous tubules show increased tortuosity, enlarged diameter and there is a considerably decreased amount of loose connective tissue between them. The seminiferous tubules contain elongated late spermatids within seminiferous epithelium. H+E. Magnification 400.

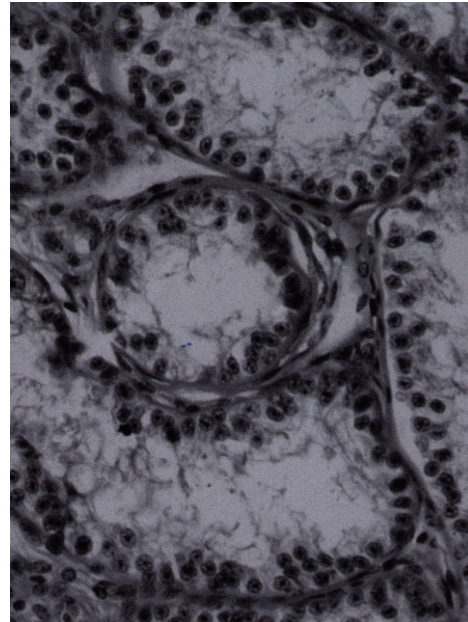


Fig. 5. Fragment of the testis of a 24-month-old male. The seminiferous tubules with a visible lumen, lined with seminiferous epithelium showing predominance of Sertoli cells and clusters of Leydig cells between the tubules. H+E. Magnification 200.

Juveniles up to three years old

The mean diameter of the seminiferous tubules of the testes in males of this age class was $162.1 \pm 41.5 \mu\text{m}$ and of the epididymal duct $273.4 \pm 46.3 \mu\text{m}$ (Table 1). These measurements were significantly larger compared to the lower age classes (diameter of seminiferous tubules for class I:II $U = 12.0$, $W = 404.0$, $p < 0.001$, class I:III $U = 0.0$, $W = 203.0$, $p < 0.001$, class II:III $U = 15.0$, $W = 104.0$, $p < 0.016$, diameter of the epididymal duct for class I:II $U = 28.0$, $W = 323.0$, $p < 0.001$, class I:III $U = 1.0$, $W = 167.0$, $p < 0.001$, class II:III $U = 12.0$, $W = 107.0$, $p < 0.008$).

Among 7 males aged up to 3 years, four had spermiogenesis, ie the presence of elongated late spermatids in the seminiferous tubules of the testes (Fig. 6) and spermatozoa in the epididymal duct (Fig. 7). The process of spermiogenesis varied in intensity between the respective animals. Some individuals had very numerous spermatozoa in the epididymal duct, in others spermatozoa were rare. In the other 3 males (with no spermiogenesis), the histological picture of the seminiferous tubules (Fig. 8) and epididymal duct was typical of this age class. Males with spermiogenesis were 26, 32 and 36 months old and their mean body weight was $335 \pm 54.5 \text{ kg}$, ie higher than of the remaining males ($293 \pm 46.2 \text{ kg}$), although the difference was statistically insignificant (Table 2). The mean diameter of the

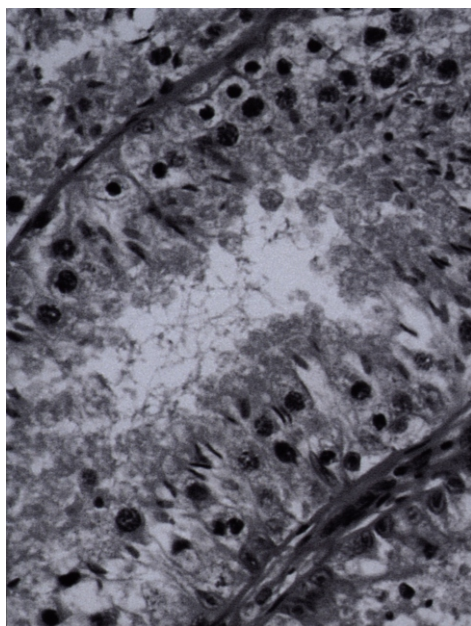


Fig. 6. Fragment of the testis of a 36-month-old male. The seminiferous tubules contain elongated late spermatids. H+E. Magnification 400.

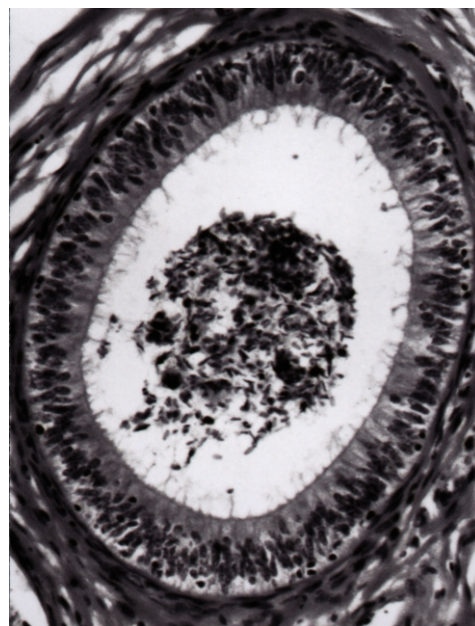


Fig. 7. Fragment of the epididymis of a 32-month-old male. The epididymal lumen shows numerous spermatozoa. H+E. Magnification 200.

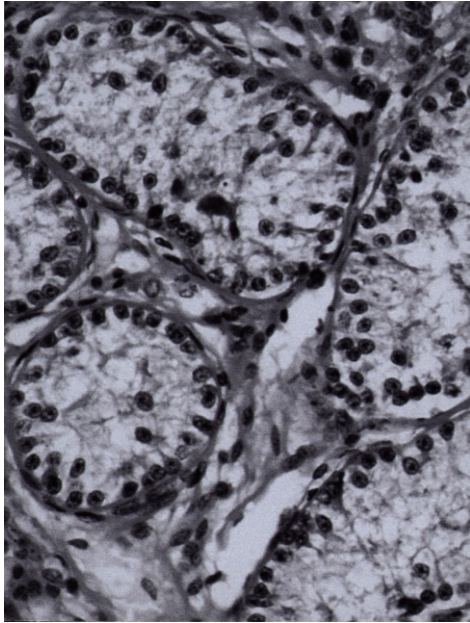


Fig. 8. Fragment of the testis of a 36-month-old male. Sertoli cells still dominate in the tubules. H+E. Magnification 200.

and the diameter of the epididymal duct show correlation with age and mass (Fig. 9a, b, c, d). The changes in both parameters are more body weight- than age-dependent. At the same time these two measurements – the diameter of the seminiferous tubules and the diameter of the epididymal duct correlate with each other (Fig. 9e).

No statistically significant correlation was noted, comparing the percentages of animals with posthitis (age class I–II, $p < 0.2071$, I–III, $p < 0.3507$, II–III, $p < 0.075$) or with epididymal cysts (age class I–II, $p < 0.6042$, I–III, $p < 0.7185$, II–III, $p < 0.744$) among the groups using the Chi-square test.

Discussion

The analysis of the histological structure of the testes and epididymes of 45 European bison culled in the years 1998–2003 has allowed the assumption that spermiogenesis may appear in some males already at the beginning of the second year of life (15 and 18 months). High body weight of the European bison seems to promote spermiogenesis. In all age classes analysed in the study, spermiogenesis occurred in males with the highest body weight or approximating the maximum values in a given age class. In the material obtained from the earlier period (Czykier

seminiferous tubules on the cross-section was $181.4 \pm 14.9 \mu\text{m}$ and of the epididymal duct $280.0 \pm 52.8 \mu\text{m}$, being larger than in the remaining males in this age class ($136.3 \pm 55.4 \mu\text{m}$ and $264.7 \pm 45.3 \mu\text{m}$, respectively), but the difference was not statistically significant (Table 2).

Two of them, the youngest (aged 26 and 32 months), were from enclosed breeding, the other two (around 3 years of age) from free-ranging population. Males with spermiogenesis exhibited high body weight and only one of them had inflammatory changes in the prepuce and two had cysts in the epididymis.

Among 7 males aged up to 3 years, posthitis type changes were noted in 4 individuals, cysts in 3 animals, and only one bison had these two pathological changes.

We also found that the diameter of the seminiferous tubules of the testes

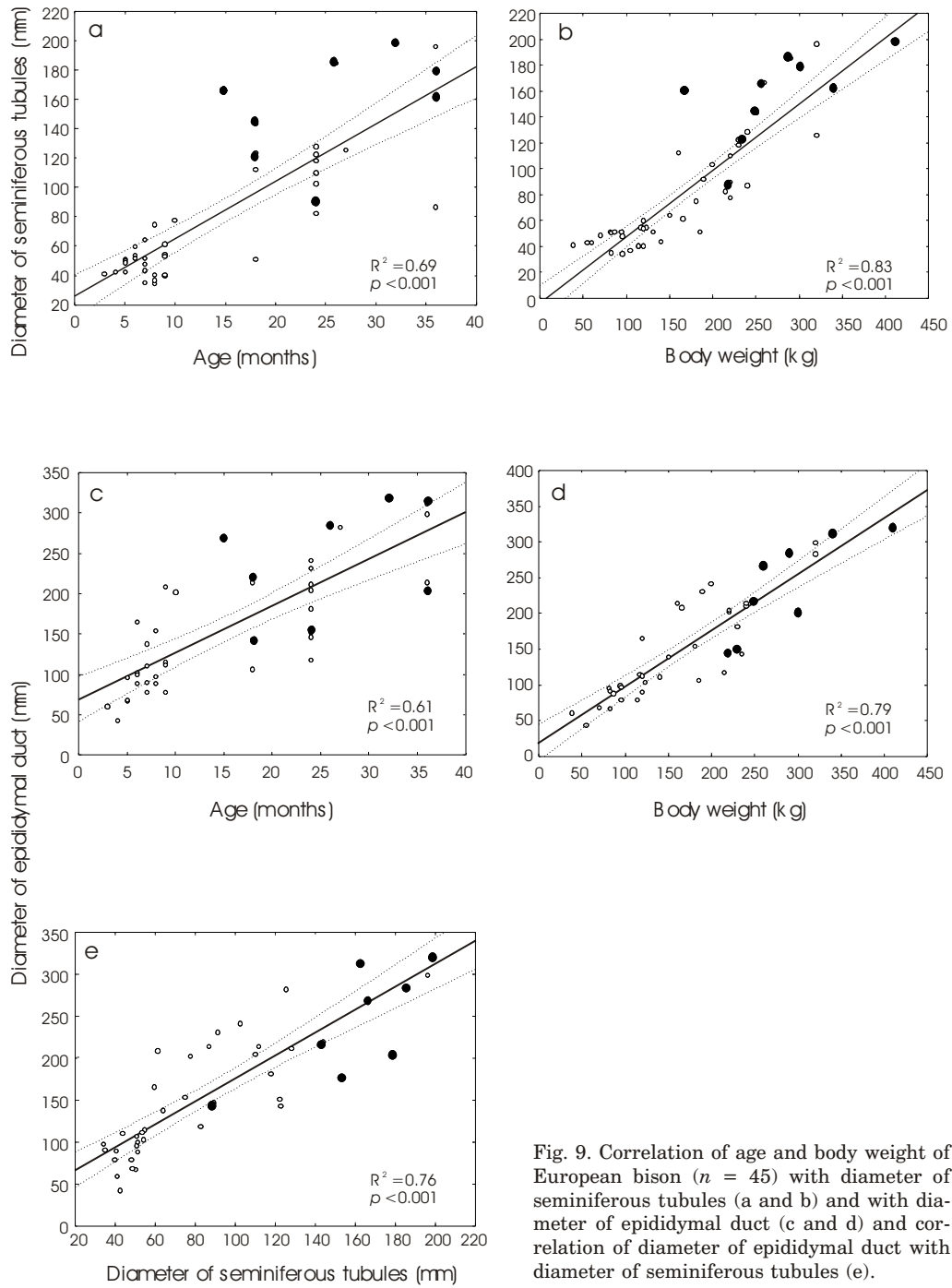


Fig. 9. Correlation of age and body weight of European bison ($n = 45$) with diameter of seminiferous tubules (a and b) and with diameter of epididymal duct (c and d) and correlation of diameter of epididymal duct with diameter of seminiferous tubules (e).

et al. 1999), no male up to 2 years of age exhibited features of spermiogenesis (37 animals). However, most of these young males had lower body weight than bulls in the present study material. The youngest European bison bulls exhibiting spermiogenesis were three males aged 33–39 months, of which only one, the youngest, living in a free-ranging herd, had body weight determined – it was the highest in this age class (Czykier *et al.* 1999). The fact that spermiogenesis was observed at the earliest in males from enclosed breeding in every age class can be associated with their higher body weight, compared to their age matches living in free ranging herds. European bison living in enclosed breeding centres, fed on nutrient-rich fodder throughout the year, reach higher body weight than the European bison living in free-ranging herds in all age-sex classes (Krasińska and Krasiński 2002).

Determination of spermiogenesis in 15-month-old specimens coming from breeding enclosure is consistent with the data reported by other authors who observed successful matings of some males aged 15–20 months in captivity (Mohr 1952, Zablocki 1949, Jaczewski 1958) and who found spermiogenesis in a 1.5-year-old male European bison (Bomba 1995).

The higher body weight are accompanied by greater diameters of the seminiferous tubules and epididymal duct. The dimensions of these two parameters are closely connected with the size of the organ and its ability to initiate spermiogenesis. The fact that the diameters of the seminiferous tubules and epididymal duct correlate more with body weight than with age indicates that high body weight has a great effect on spermiogenesis development.

High body weight exhibited by individual European bison specimens is indicative of rapid metabolic rate, intensive physiological processes and growth, which may be also accompanied by spermiogenesis. However, cases of spermiogenesis in calves are rare, referring only to single animals and reflecting individual physiological state of the animal. High individual changeability of physiological processes in the European bison has been discussed by Gill (1999) based on long-term studies. Spermiogenesis in this animal is not common. Of 95 males (including 50 specimens examined previously, Czykier *et al.* 1999), only 11 males showed features of varied phases of spermiogenesis.

Compared to other ungulates, the European bison shows active spermiogenesis relatively late (Czykier *et al.* 1999). For instance in male wapiti, spermatozoa were found in the ejaculate of specimens up to 2 years old (Haigh *et al.* 1984), in fallow deer at the age of 16 months (Chapman and Chapman 1970, Chaplin and White 1972), while in red deer between 12 and 15 months (Webster *et al.* 1992).

The material examined in the present study was obtained in late autumn, winter and early spring, ie after the rutting period (Krasiński and Raczyński 1967). This fact, however, cannot be associated with lack of active spermiogenesis in young European bison, as the inhibitory effect of the winter period on the spermatogenic activity of the testes refers only to sexually mature individuals. In sexually immature animals, the development of gonads is not affected by the successive seasons of the year (Chaplin and White 1972, Blottner *et al.* 1996).

The disease posthitis observed in the European bison in the Białowieża Forest since 1980 causes no macroscopic changes within the testes (Kraśiński 1994, Kraśiński *et al.* 1994). In our previous study, the histological pictures of the testes of 45 diseased males did not differ from those of the healthy males (Czykier *et al.* 1999). The same result has been observed in the present study in 23 diseased individuals out of 45 studied. The disease probably does not inhibit spermiogenesis, as it was recognized in 3 out of 8 males with spermiogenesis.

Since 1995 another pathological defect, namely epididymal cysts, has been observed in the European bison (in 65 of 107 males examined) (Matuszewska and Sysa 2000, 2002). In this study, epididymal cysts were found in 18 males up to three years old, both in those living in captivity and in free-ranging population. Of 8 males with spermiogenesis, two had epididymal cysts, which however did not restrict spermiogenesis.

Conclusions

1. The sizes of testicular and epididymal structures (seminiferous tubules of the testes and epididymal duct) are significantly correlated with body weight and age of male European bison, the correlation with body weight being higher.

2. Spermiogenesis appears in male European bison with high body weight at the earliest.

3. Spermiogenesis is the earliest in 15-month-old bison male living in enclosed breeding and in 18-months old from free-ranging population.

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