

Comparison of concentration of 17 β -estradiol (E2) and total testosterone (TT) in serum in young males of European bison with or without spermiogenesis

Elżbieta Czykier

Department of Histology and Embryology, Medical University in Białystok, Poland

Abstract:

– The aim of our study was to determine, how often spermiogenesis in young males of European bison up to 3 years old occurs and what is the concentration of 17 β -estradiol (E2) and total testosterone (TT) in serum of those animals in comparison to the ones without spermiogenesis in the same age group. The animals were divided into two 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 study was performed on sections of testes and epididymis collected from 51 males in the age between 2–3 years old. The animals were culled during the autumn-winter seasons (after rutting period) in years 1994–2008 in the Białowieża Primeval Forest. Moreover the concentration levels of E2 were determined in serum of 51 individuals and TT in serum of 46 animals. There was no significant difference between mean values of E2 and TT concentration in serum of young male European bison up to 2 and 3 years old.

Key words: *Bison bonasus*, juveniles, sex hormone, spermiogenesis

Introduction

The research performed on postnatal development of spermiogenesis in male European bison *Bison bonasus* (L.) led in 1969–1993 has shown that in the group of youngest males in the age of up to 2 years old (37 individuals) the spermiogenesis does not occur (Czykier *et al.* 1999). In the same test it was revealed that within males up to 3 years old (13 individuals) only 3 specimens had spermiogenesis. To verify the results next research was performed in 1998–2003 on 45 males European bison in the age ranges from 3 months to 3 years old. The obtained data showed that in calves up to one year old spermiogenesis does not occur, however in animals up to 2 years old it is present within the youngest case 15 months old (Czykier, Krasińska 2004). The occurrence of spermiogenesis in young male European bison up to 3 years old was also confirmed in that investigation (Czykier, Krasińska 2004).

The main hormones responsible for spermiogenesis are total testosterone (TT) and 17 β -estradiol (E2). In Gill's monography (1999) the author compares levels of TT and E2 in serum of young and adult European bison. Other authors

describe the level of free testosterone (FT) in serum of European bison in different age groups (Czykier, Krasińska 2006) and compare the concentration of FT in serum of young male bison with spermiogenesis to those without (Czykier 2008).

In many other mammals the level of TT in serum was determined, also in the wild ungulates: red deer, wapiti, roe deer, sika deer, white-tailed deer (*Cervus elaphus*, *Axis axis*, *Capreolus capreolus*, *Cervus nippon*, *Odocoileus virginianus*) (Lincoln, Kay 1979; Haigh *et al.* 1984; Loudon, Curlewis 1988; Suzuki *et al.* 1992; Bubenik *et al.* 2005) and livestock: cattle (Wearthesbee, Lodge 1976; Earl Gray *et al.* 2006), horses (Roser, Hughes 1992; Hoffman, Landeck 1999). Parallel research about concentration of estrogens in serum was conducted for many years, mainly in horses (Ganjam, Kenney 1975; Roser, Hughes 1992; Inoue *et al.* 1993; Raeside, Christie 1997; Stewart, Roser 1998; Hoffman, Landeck 1999; Lemazieur *et al.* 2002) and other species subsumed to ungulates: white-tailed deer (*Odocoileus virginianus*) (Bubenik *et al.* 2005), cattle (Wearthesbee, Lodge 1976; Eiler, Graves 1977).

The aim of our study was to compare concentration levels of E2 and TT in serum in young male European bison in the age range from 18 to 36 months old, both with and without spermiogenesis in the same age groups.

Material and Methods

The histological material originated from testes and epididymis of 51 male European bison in the age ranged from 18 to 36 months old. The material was collected from the individuals culled during autumn-winter season in 1994–2008 (after rutting period) in the Białowieża Primeval Forest (Krasiński, Raczyński 1967). 43 specimens in the age from 18 months to 36 months old were from free-ranging population, and 8 animals in the age from 18 to 27 months were from captivity.

The animals were eliminated due to routine reduction in the population number. The choice was made with a variety of reasons i.e.: diseases, with the special stress on posthitis/balanoposthitis, poor condition, exterior defects, injuries of various origin, aggression towards people or senile age (Krasińska, Krasiński 2007).

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

Moreover full blood was collected from femoral artery (*post mortem*), and then the serum was centrifuged in the Mammal Research Institute, Polish Academy of Sciences in Białowieża. Then the samples of serum were frozen and stored in the temperature of -20°C . The animals 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. As the criteria of presence of spermiogenesis was existence of late spermatidis in seminiferous epithelium of testes or presence of spermatozoa in the lumen of the seminiferous tubules of the testes and/or frequency of spermatozoa in duct of epididymis (Czykier, Krasinska 2004; Czykier 2008). Calves up to 1 year old were discarded from the analysis, because such young animals do not shown any sign of spermiogenesis. As the limiting age 3 years old was established, because of previous histological research showed that all males in the age of 4 years old all had spermiogenesis in seminiferous tubules of the testis (Czykier *et al.* 1999).

In serum of 51 males bison the concentration levels of 17β -estradiol were determined with immunoenzymatic assay (ELISA) with sensitivity 15 pg/ml from IMMUNO-BIOLOGICAL LABORATORIES company (No RE 52041, Hamburg, Germany). Total testosterone concentration was also determined with immunoenzymatic assay (ELISA) in serum of 46 male bison with sensitivity 0.04 ng/ml with test from Diagnostic Systems Laboratories (No DSL-10-4000, Webster, USA). The measurements of concentration levels of E2 and TT in male bison serum were performed in Laboratory of the Department of Endocrinology, Diabetology and Internal Diseases, Medical University in Bialystok.

The quantity variables were described by the mean value and standard deviation (SD). The comparison of groups was performed with t-Student test. 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

In this age class ($n=36$) the values of E2 concentration in serum of animals' with spermiogenesis were very high or medium in comparison to the serum of bison without spermiogenesis (Fig. 1). The mean value of E2 concentration

Table 1. Mean value of 17β -estradiol (pg/ml) concentration in serum of young males European bison from two age groups (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-non significant difference, n -number of individuals

Age class		n	17 β -estradiol pg/ml				p
			Mean	SD	Min	Max	
I	A	6	193.20	141.86	62.1	450.0	ns
	B	30	183.63	326.56	4.6	1800.0	
II	A	8	111.35	167.15	32.0	510.0	ns
	B	7	90.23	54.60	3.3	180.0	

in serum of animals with spermiogenesis ($n=6$) was higher than mean value of E2 concentration in animals without spermiogenesis in the same age class, however this difference was not statistically significant (Tabl. 1). Nevertheless the value of TT concentration in serum of animals with spermiogenesis was low, medium and high in comparison to the TT concentration value of ones without spermiogenesis in the same age class (Fig. 2). The mean value of concentration of TT in serum of animals with spermiogenesis ($n=4$) was lower than mean value in serum of animals without spermiogenesis in the same age class, however it was not statistically significant difference (Tabl. 2).

Young males up to three years

In this age class ($n=15$) concentration values of E2 in serum of young males with spermiogenesis reached high and medium level in comparison to concentration values of E2 in serum of animals without spermiogenesis (Fig. 1). However, concentration values of TT in serum of animals with spermiogenesis reached high, medium and low levels in comparison to concentration values of TT in serum of animals without spermiogenesis which often were at the low level (Fig. 2). The mean value of E2 concentration in animals serum with spermiogenesis was higher, than mean value of E2 concentration in serum of animals without spermiogenesis in the same age group, however the difference is not statistically significant (Tabl. 1). Although the mean value of TT concentration in serum of animals with spermiogenesis was two times higher than mean value of TT concentration in serum of animals without spermiogenesis in the same age group, the difference is not statistically significant (Tabl. 2).

Table 1. Mean value of total testosterone (ng/ml) concentration in serum from two age groups of male 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-non significant difference, n -number of individuals

Age class		n	Total testosterone ng/ml				p
			Mean	SD	Min	Max	
I	A	4	5.55	5.04	1.57	12.51	ns
	B	28	6.29	7.32	0.45	32.81	
II	A	7	10.75	11.79	0.22	31.77	ns
	B	7	5.09	7.98	0.42	22.30	

Discussion

Young males with spermiogenesis (both up two and three years old) demonstrated high E2 concentration values in serum in comparison to animals without spermiogenesis. Whereas TT concentration values in serum of

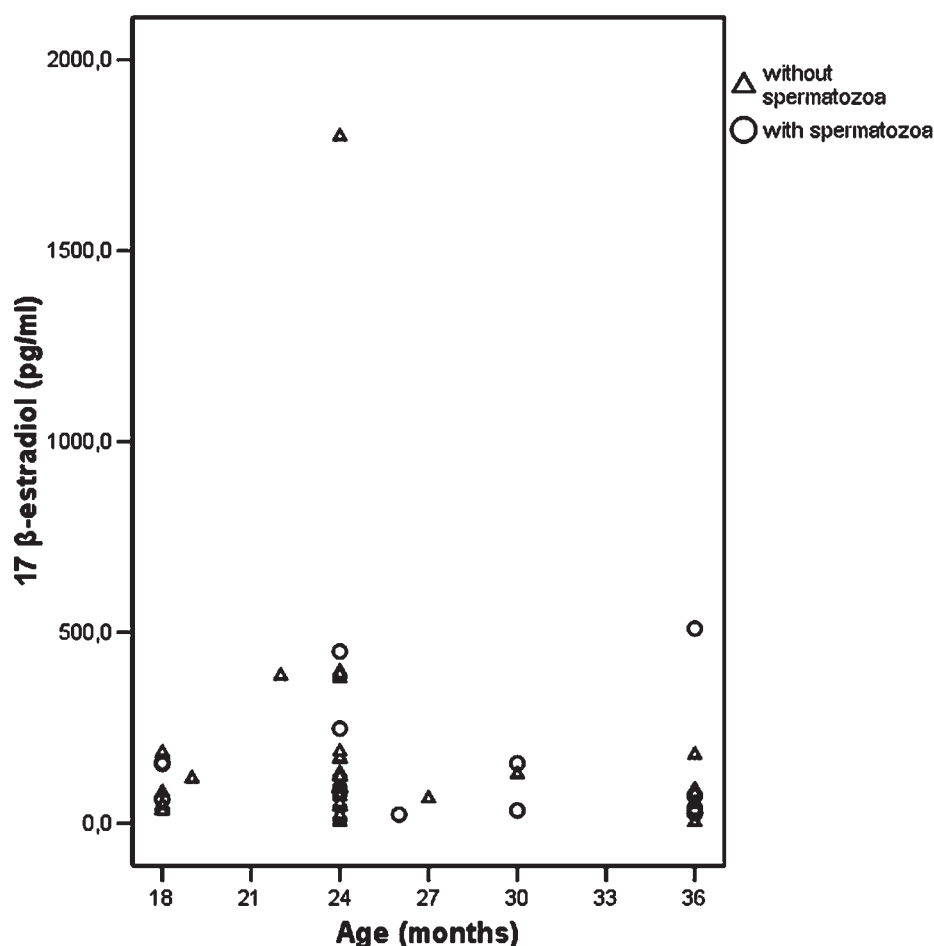


Figure 1. Concentration of 17β-estradiol (pg/ml) in serum of all European bison (up to two and three years old).

animals with spermiogenesis from both age groups varied from low, through medium to high level, similarly to the results observed in other males without spermiogenesis. The obtained results were much diversified because in all examined animals individual differences in E2 and TT concentration in serum were considerable in single specimens. This regularity was also described by other authors (Gill 1999; Czykier 2009a; 2009b). Moreover considerable difference between sex hormones in European bison are observed not only in TT and E2 but also in remaining hormones like FT (Czykier, Krasińska 2006; Czykier 2008).

In our research the mean value of E2 concentration in serum of young males up to two and three years old in both groups: with and without spermiogenesis are lower than average E2 concentration mean value in serum of young

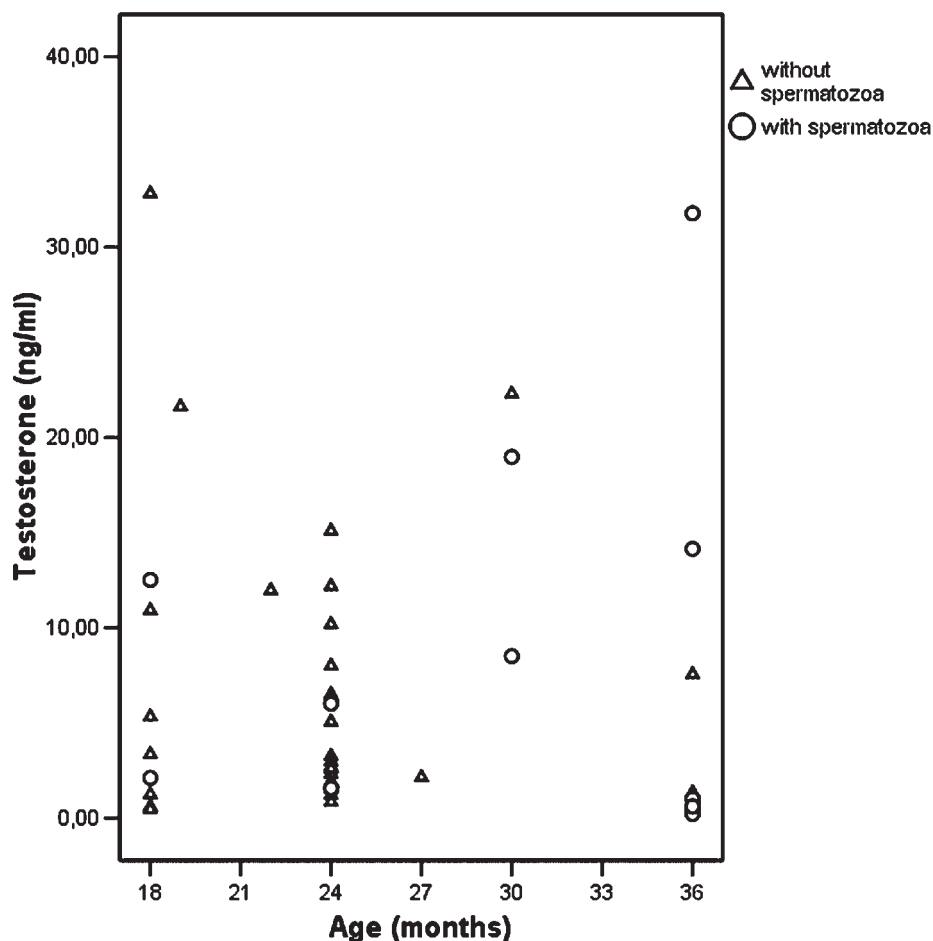


Figure 1. Concentration of total testosterone (ng/ml) in serum of all European bison (up to two and three years old).

European bison described by Gill (1999). Although, they are higher than mean value of E2 serum concentration in adult European bison (Gill 1999).

In our research mean value of TT serum concentration in young males up to two years and up to three years old with and without spermiogenesis are higher than mean value of this hormone observed in young horses (Stewart, Roser 1998) and in bulls (Wearthesbee, Lodge 1976). Elsewhere other authors observed higher mean values of TT serum concentration in prepubertal horses in comparison to our results obtained with European bison (Lemazurier *et al.* 2002).

In our current research we discovered higher mean value of E2 concentration in serum of European bison with spermiogenesis in comparison to the mean value of E2 concentration in animals without, what seem to support the idea

about this hormone participation in the process of spermiogenesis. However in the relation to TT mean value of serum concentration in animals with spermiogenesis we observed quite different tendency. In the age of 3 years old for group with spermiogenesis there is peak of mean concentration of TT in comparison to animals without spermiogenesis. Therefore in the age of 2 years old in animals with spermiogenesis there is drop of mean concentration of TT in serum in comparison to animals without spermiogenesis. This tendency in maintaining the higher mean value of E2 in serum of 2 and 3 years old European bison with spermiogenesis may prove that such levels are needed in the process of spermatogenesis. However gradually approaching the moment of sexual development the TT concentration considerably increases to major amounts in animals with spermiogenesis in age of 3 years old. We believe that lack of statistically significant differences in mean value of E2 and TT in serum of examined animals is strongly influenced by high dispersion of initial values of hormones' concentration in serum.

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Porównanie stężenia 17 β -estradiolu (E2) i testosteronu całkowitego (TT) w surowicy młodych samców żubra z obserwowaną lub nie spermiogenezą

Streszczenie: Celem pracy było sprawdzenie u jakiej części samców żubra w wieku do 3 lat rozpoczyna się proces spermiogenezy oraz ocena stężenia 17 β -estradiolu (E2) i testosteronu całkowitego (TT) w surowicy krwi. Samce były podzielone grupy ze względu na wiek: do 2 lat i w wieku od 2 do 3 lat oraz ze względu na występowanie spermiogenezy. Badania przeprowadzono na wycinkach jąder i najądrzy zebranych od 51 samców eliminowanych w Puszczy Białowieskiej. Żubry były eliminowane w sezonie jesienno-zimowym, po okresie rui, w latach 1994–2008. Nie stwierdzono istotnych różnic między grupami samców, choć stężenie E2 u samców z występującą spermiogenezą było wyższe w porównaniu do grupy rówieśników nie wykazujących spermiogenezy, ale różnica nie była się istotna. Natomiast nie stwierdzono różnic między grupami pod względem stężenia testosteronu.
