

## **AGE CHANGES OF FRUCTOSE AND CITRIC ACID LEVELS AS WELL AS HYALURONIDASE ACTIVITY IN THE EPIDIDYMAL SECRETION AND VESICULAR GLANDS OF THE PODOLSKY PLANT TYPE BULLS OF THE UKRAINIAN BLACK-DAPPLED DAIRY BREED**

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**Abstract.** This study presents data on the age changes of hyaluronidase activity in epididymal secretion as well as the presence of fructose and citric acid in vesicular glands (*glandulae vesiculares*) secretion. The results of age changes in the amount of epididymal secretion and its spermatozoa concentration in the Podolsky plant type bulls of the Ukrainian black-dappled dairy breed are also given. The research was conducted on 54 individuals, aged 1–13 months. It was found that fructose and citric acid in vesicular glands secretion as well as hyaluronidase activity and mature spermatozoa in epididymal secretion emerge for the first time in 5-month-old bulls. The highest concentrations of fructose ( $742 \pm 63$  mg/per cent) and citric acid ( $914 \pm 81$  mg/per cent) in vesicular glands secretion and the highest hyaluronidase activity (1.6 thousand units) in epididymal secretion were found in 12-month-old bulls. A strong positive correlation between hyaluronidase activity and spermatozoa concentration in epididymal secretion was also observed.

**Key words:** bulls, citric acid, epididymis, fructose, hyaluronidase activity, vesicular glands

### **INTRODUCTION**

It is known that spermatogenesis processes in the testicles, epididymis and accessory genital glands (*glandulae genitales accessoriae*) of sires are connected with a number of morphological, physiological and biochemical changes. One indicator of the beginning of spermatogenesis in the males of farm animal species is the

emergence of hyaluronidase activity in the testes, which, according to Males and Turkington [1970], acts as a biochemical marker of spermatozoa progress, and also the presence of fructose and citric acid in the vesicular glands (*glandulae vesiculares*) secretion of sires [Pavlyuk and Vovk 2010 a].

Fructose and citric acid production by vesicular glands of sires is regulated to a large extent by testosterone, which determines the progress of their sexual features and has an influence on the regulation of physiological functions, their progress and development [Bell et al. 1980, Pavlyuk and Vovk 2010 b]. An increase in fructose and citric acid concentration in vesicular glands secretion is an evidence of proper spermatogenesis [Fedorovych and Siratsky 2007].

It is also established that testosterone affects spermatogenesis processes and the vitality of spermatozoa. It is also necessary for the ultimate maturation of spermatozoa in the epididymis [Blaquer et al. 1972, Zirkin et al. 1989].

Taking into consideration the above-mentioned facts, the aim of our work was an analysis of age changes of fructose and citric acid contents as well as hyaluronidase activity in the vesicular glands and epididymal secretion during the progress of spermatogenesis in the Podolsky plant type bulls of the Ukrainian black-dappled dairy breed.

## MATERIAL AND METHODS

The research was carried out between 1999 and 2013 at the Teophiopol private joint-stock company “Zernoproduct MXP” 3711337, Teophiopol district, Khmelnytsky region.

Epididymides and vesicular glands, used as research material, were taken during the slaughter of the Podolsky plant type bulls of the Ukrainian black-dappled dairy breed being at the age of 1 to 13 months.

Fructose content in the vesicular glands secretion was determined by the Kulka-Selivanova method [Petruhin 1992] and the citric acid content by the Bazarnova method [Zubkov et al. 2001].

The content of epididymal secretion of bulls and spermatozoa concentration were determined by us [Vlizlo et al. 2004], whereas hyaluronidase activity in epididymal secretion was assayed in accordance with Mclean’s method modified by Mohilevsky and Kogan [Zubkov et al. 2001].

In order to determine significant differences between the groups, the F-test and Duncan’s multiple range test were used ( $P \leq 0.01$ ,  $P \leq 0.05$ ). All calculations were performed using Statistica® software [StatSoft 2009].

## RESULTS AND DISCUSSION

Fructose and citric acid in seminal vesicles secretion began to emerge in 4-month-old bulls. After that their contents in seminal vesicles secretion increased rapidly in accordance with bulls' age.

In particular, a sharp rise in the fructose level in vesicular glands secretion was observed in 6- and 10-month-old bulls. Afterwards, its level was practically the same, up to the age of 13 months. Fructose contents in the vesicular glands obtained from 4- to 6-month-old bulls increased 8.8 times, that is, from  $56 \pm 8$  mg per cent to  $496 \pm 35$  mg per cent, and in the glands from 6- to 9-month-old bulls, it increased 1.4 times (to  $686 \pm 69$  mg/per cent). Until the age of 12 months, fructose level in the vesicular glands increased only 1.08 times and its content in secretion was  $742 \pm 63$  mg per cent. The accession of fructose content was not observed in 13-month-old bulls.

Taking into account the content of citric acid in vesicular glands, its level increased 4.6 times from 4 to 6 months, 1.4 times from 6 to 9 months and 1.1 times up to the age of 12 months.

The dynamics of fructose and citric acid contents in the vesicular glands of bulls is shown in Figure 1.

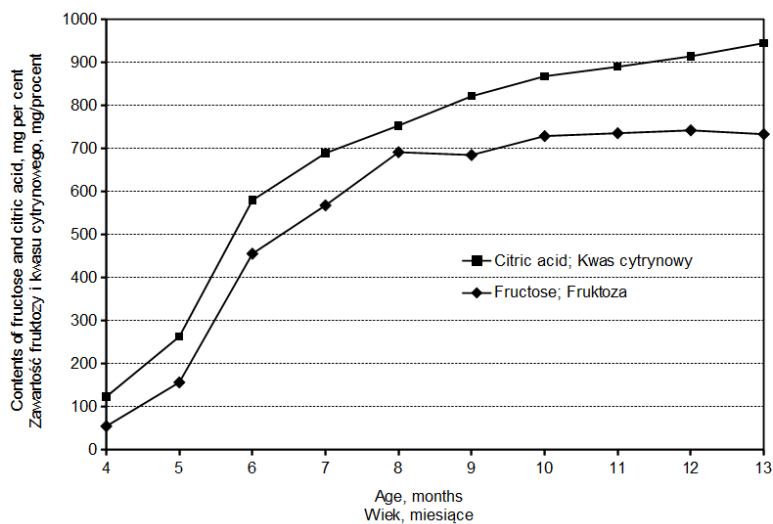


Fig. 1. The dynamics of fructose and citric acid contents in the vesicular glands of bulls

Rys. 1. Dynamika poziomu fruktozy i zawartości kwasu cytrynowego w gruczołach pęcherzykowych byków

The data presented in the figure show that the secretion of vesicular glands of bulls contains a higher level of citric acid in comparison with that of fructose at all ages in the investigated bulls.

It must be noted that the most observable accession of fructose and citric acid levels in the vesicular glands of bulls takes place in 5- and 6-month-old animals.

The determination of the amount of secretion in the epididymis, spermatozoa concentration in the secretion and hyaluronidase activity were the separate aspects of our study of the Podolsky plant type bulls of the Ukrainian black-dappled dairy breed depending on their age (Table 1).

Table 1. Age changes in the content of epididymal secretion, spermatozoa concentration and hyaluronidase activity in bulls

Tabela 1. Zależność między wiekiem a zmianami intensywności wydzielania przez najądrza, koncentracją plemników i aktywnością hialuronidazy u byków

Age, months Wiek, miesiące	n	Amount of epididymal secretion, ml Intensywność wydzielania przez najądrza, ml	Spermatozoa concentration, billion · ml <sup>-1</sup> Koncentracja plemników, mld · ml <sup>-1</sup>	Hyaluronidase activity, thousand units Aktywność hialuronidazy, tys. jedn.
3	7	0.11 ±0.06	–	–
4	5	0.21 ±0.05	–	–
5	4	0.54 ±0.08	0.14 ±0.11	0.2
6	6	2.76 ±0.15	0.21 ±0.15	0.4
7	5	2.89 ±0.18	0.27 ±0.14	0.5
8	4	3.03 ±0.16	0.34 ±0.20	0.6
9	4	3.19 ±0.24	0.38 ±0.24	0.8
10	3	3.28 ±0.23	0.64 ±0.22	1.1
11	6	3.36 ±0.24	0.95 ±0.22	1.3
12	5	3.44 ±0.17	1.32 ±0.28	1.6
13	5	3.46 ±0.19	1.39 ±0.24	1.6

The data given in Table 1 show that the content of epididymal secretion in 3-month-old bulls is very low, amounting to 0.11 ±0.06 ml, and it is at a rather low level in 6-month-old bulls. The amount of epididymal secretion in 6-month-old bulls was 2.76 ±0.15 ml. The highest content of secretion (3.46 ±0.19) was obtained in the epididymis of 13-month-old bulls.

The hyaluronidase activity in the epididymal secretion emerged in 5-month-old bulls. It should be mentioned that the emergence of hyaluronidase activity in epididymal secretion coincides with that of mature spermatozoa in this secretion.

The graph given below (Figure 2) shows the relationship between hyaluronidase activity in the epididymal secretion and spermatozoa concentration.

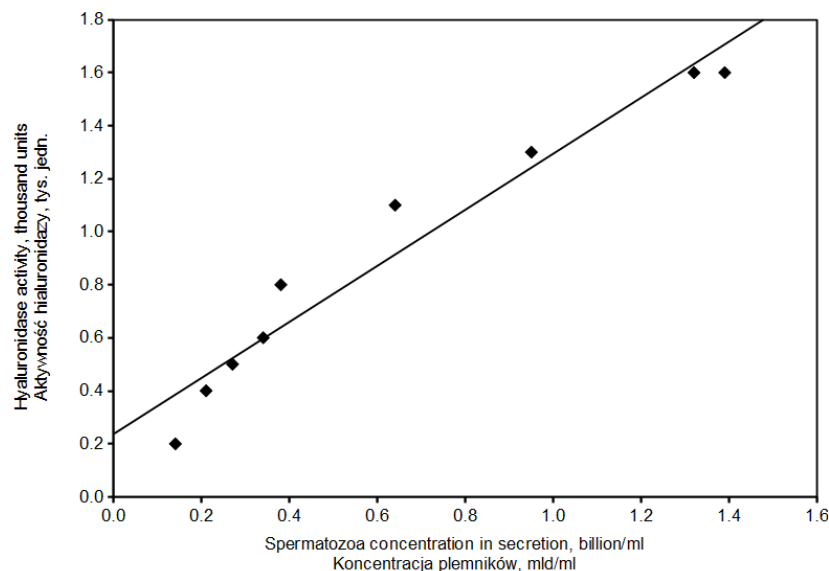


Fig. 2. Relationship between hyaluronidase activity in the epididymal secretion and spermatozoa concentration

Rys. 2. Zależność między aktywnością hialuronidazy w wydzielinie najądrzy a koncentracją plemników

Figure 2 shows the linear relationship between hyaluronidase activity in the epididymal secretion of bulls and spermatozoa concentration. To summarize the results of our study, it should be mentioned that a positive correlation exists between hyaluronidase activity in the epididymal secretion of bulls and spermatozoa concentration ( $r = 0.96$ ).

## CONCLUSIONS

1. Fructose and citric acid in the vesicular glands secretion as well as hyaluronidase activity and mature spermatozoa in the epididymal secretion emerge for the first time in 5-month-old bulls of the Podolsky plant type of the Ukrainian black-dappled dairy breed.
2. The highest concentrations of fructose ( $742 \pm 63$  mg/per cent) and citric acid ( $914 \pm 81$  mg/per cent) in the vesicular glands secretion and the highest hyaluronidase activity (1.6 thousand units) in the epididymal secretion of the Podolsky plant type bulls of the Ukrainian black-dappled dairy breed were found in 12-month-old animals.

3. It can be stated that there is a strong positive correlation between hyaluronidase activity and spermatozoa concentration in the epididymal secretion of the Podolsky plant type bulls of the Ukrainian black-dappled dairy breed ( $r = 0.96$ ).

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**ZALEŻNOŚĆ MIĘDZY WIEKIEM A ZMIANAMI W STĘŻENIU FRUKTOZY, KWASU CYTRYNOWEGO I AKTYWNOŚCIĄ HIALURONIDAZY W WYDZIELINIE NAJĄDRZY I DODATKOWYCH GRUCZOŁÓW PŁCIOWYCH U BUHAJÓW RASY PODOLSKIEJ PO KROWACH UKRAIŃSKICH CZARNO-BIAŁYCH**

**Streszczenie.** W niniejszej pracy przedstawiono dane dotyczące aktywności hialuronidazy w wydzielinie najądrzy (*glandulae vesiculares*) w zależności od wieku, obecności fruktozy i kwasu cytrynowego w kanalikach nasiennych oraz wpływu wieku i aktywności wydzielniczej najądrzy na liczebność i koncentrację plemników u buhajów rasy podolskiej po krowach ukraińskich czarno-białych. Badania przeprowadzono na 54 osobnikach, w wieku od 1 do 13 miesięcy. Wykazano, że fruktoza i kwas cytrynowy pojawiają się w wydzielinie najądrzy i gruczołów płciowych dodatkowych u pięciomiesięcznych buhajów. Ponadto obserwuje się wzrost aktywności hialuronidazy i pojawianie się dorosłych plemników. Największe stężenie fruktozy ( $742 \pm 63$  mg/procent) i kwasu cytrynowego ( $914 \pm 81$  mg/procent) wykazano w gruczołach płciowych dodatkowych, a najwyższą aktywność hialuronidazy (1,6 tysiąca jednostek) wykazano w wydzielinach najądrzy u 12-miesięcznych buhajów. Wykazano wysoką dodatnią korelację pomiędzy aktywnością hialuronidazy a koncentracją plemników w wydzielinach najądrzy.

**Słowa kluczowe:** aktywność hialuronidazy, buhaje, fruktoza, kanaliki nasienne, kwas cytrynowy, najądrza

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