The frequency of morphological defects of epididymal sperm in exotic animal species of the family *Bovidae* kept in the Czech Republic

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Abstract

Epididymal sperm morphology was evaluated by strict criteria using the DeSMA software. A total of 134 animals from the Dvůr Králové ZOO were included in the study. The aim of this study was to determine the prevalence of morphological defects of epididymal sperm in animals of the family *Bovidae* in species that are not native to the Czech Republic and are bred here only in zoos. Our results showed that the frequency of abnormalities was higher in exotic species of the *Bovidae* family compared to domestic species. Furthermore, the acquired defects prevailed over developmental abnormalities. Cytoplasmic droplets are a characteristic feature of epididymal sperm. Their average prevalence in the whole *Bovidae* family is almost 30%. However, distal droplets are not considered to be sperm pathology and, therefore, they are not the most frequent defect. The most common abnormalities are acquired head defects, followed by acquired tail defects, and then by acquired neck defects, mainly represented by proximal cytoplasmic droplets. The lowest prevalence was found for developmental abnormalities, with heads being more often affected than tails. The remaining defects were sporadic.

Antelopes, spermatology, DeSMA

Even though the need for establishing genetic reserves for endangered animal species is increasing today, very few authors deal with the characterization of sperm of these species (Cassinello et al. 1998; Sontakke et al. 2009). In addition, the possibilities of obtaining sperm from wildlife species are very limited.

A number of methods for obtaining sperm from various animal species have been described. One of the most common semen collection methods in wildlife species is electroejaculation described by many authors (Wildt et al. 1986; Roth et al. 1998; Suwanpugdee et al. 2009). A disadvantage of this technique is that it can cause considerable stress. However, it can be used repeatedly in the animals. Nowadays, the technique of *post mortem* sperm retrieval allowing collection of sperm from different parts of the epididymis is often used, especially for research purposes. This method can be effectively used in animals after sudden death or after emergency slaughter, and the extracted sperm can then be used for preservation of genetic material or used in further studies. It is well-known that sperm from the tail of the epididymis have an adequate degree of maturity and the ability to fertilize the oocytes (Turner 1995; Foote 2000). A comparison of two different methods of sperm recovery from the epididymis of red deer was made by Martinez-Pastor et al. (2006). Based on the total number of obtained sperm, degree of contamination, and sperm quality indicators, these authors preferred the method of flushing sperm from the epididymis to the method of epididymis slicing. On the other hand, the method of slicing (Songsasen et al. 1998; An et al. 1999; Yu and Leibo 2002; Hishinuma et al. 2003) or cutting (Kishikawa et al. 1999) of epididymal tissue while allowing the sperm to swim up to a buffered medium for a few minutes is preferred in small animals. This technique can also be used in large animals (Hishinuma et al. 2003). Another possibility is the retrograde flushing of sperm from the tail of the epididymis using a needle connected to a syringe,

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containing either a flushing medium (Garde et al. 1998; Herold et al. 2004) or the air (Kikuchi et al. 1998; Ikeda et al. 2002).

Morphology evaluation has been one of the major parts of semen analysis for several decades. One of the first scientists to characterize and classify abnormal forms of sperm was Lagerlöf (1936), later followed by Bretschneider and Vaniterson (1948), Bonadona (1956) and many others. The development of a strict sperm morphology assessment (Kruger et al. 1986; Menkveld and Kruger 1995) was another step leading to more accurate sperm classification. Sperm morphology in some species, such as sheep and mice, is almost invariable, whilst morphology of bull (Gravance et al. 1996) and dog sperm (Dahlbom et al. 1997) differs between individual animals. Likewise, wild animal species exhibit significant differences in sperm morphology.

The knowledge of the maximum acceptable percentage of sperm with morphological anomalies is used in the production of insemination doses. In practice, certain cut-off values for sperm morphology have been set up over time for both farm and domestic animals. For example, in bulls, due to the association between the number of pathological forms of bovine sperm in the ejaculate and their fertilizing ability, the discriminating threshold value of 20% sperm with abnormal morphology was established. When exceeded, the ejaculate is not suitable for processing into insemination doses. Such thresholds have not yet been established for wild species of *Bovidae*, although some authors have already dealt with antelope semen characteristics (Cassinello et al. 1998; Sontakke et al. 2009).

Today, the *Bovidae* family comprises two subfamilies, *Bovinae* and *Antilopinae* (Robinson and Ropiquet 2011). According to the current taxonomy, all species of the *Bovidae* family are divided into 12 taxonomic units (tribus, tribes) (Groves and Grubb 2011). The *Bovinae* include *Bovini*, *Tragelaphini*, and *Boselaphini*; *Antilopinae* include *Hippotragini*, *Alcelaphini*, *Antilopini*, *Aepycerotini*, *Caprini*, *Reduncini*, *Cephalophini*, *Neotragini* and *Oreotragini*. The present study follows this classification of the species.

The aim of this study was to determine the prevalence of morphological defects of epididymal sperm in animals of the family *Bovidae* in species that are not native to the Czech Republic and are bred here only in zoos.

Materials and Methods

In the present study, testes and epididymides of wild species of the *Bovidae* family were used. The majority of organs were provided by the Dvůr Králové nad Labem ZOO and several *Taurotragus oryx* originated from the University Farm Estate Lány of the Czech University of Life Sciences Prague. The sampling was conducted for 11 years from 2007 to 2018, regardless of the season. The samples were taken after animals' death and were then transported to the laboratory on ice in a cooling box within 24 h. The animals were either found dead or were slaughtered. The tail of the epididymis was gently dissected free from the reproductive organs. Great emphasis was put on avoiding contamination of the samples with blood. Latex-free gloves were used during the work. Samples were processed immediately upon receipt in the laboratory. The samples were moistened with phosphate-buffered saline at 4–8 °C to prevent the tissues from drying out. After processing, the tail of the epididymis was cut into small pieces. Where the veins could not be removed completely, the tail of the epididymis was cut in a vessel-free area. An appropriate amount of phosphate-buffered saline (pH 6.8) was added to immerse the prepared tissue. The supernatant was collected after 10 min using a Pasteur pipette. A smear was made, fixed with 3.5% formalin and stained with 5% aniline blue after 15 s and with 0.5% crystal violet after further 15 s (Aniline blue CI 427805; Crystal violet CI 42555; Sigma-Aldrich, St. Louis, USA). Morphological sperm defects were evaluated under a Nikon ECLIPSE 50i light microscope (Nikon Corporation Instruments Company, Yokohama, J) with × 1000 magnification using immersion oil (Sigma-Aldrich, St. Louis, USA).

Multiparametric analysis was performed using the DeSMA software (Detailed Sperm Morphology Analysis, https://www.vri.cz/en/research/applying_results/software). All abnormalities were recorded for 200 spermatozoa in every smear. A total of 134 samples of sperm from animals classified in 32 species and 8 tribes were evaluated in this study. Ten species were represented by only one animal each. These were not included in the between-species comparison of parameters, but they were included in the statistical comparison of tribes. The method of analysis of variance (ANOVA) using the SPSS software package, v.18 for Windows (SPSS, Inc., Chicago, USA) was used for testing the agreement of the means. A significance level of 0.05 was chosen for the testing of differences.

Results

The average number of abnormal sperm in our group of animals of the *Bovidae* family was 51.2% (SD 20.3). Acquired defects overnumbered the developmental defects. The percentage of sperm showing at least one acquired defect was 38.9% (SD 17.5), while the percentage of sperm showing developmental defects was 17.8% (SD 13.9). The teratogenic index reflected the number of defects per abnormal sperm at a value of 1.2 (SD 0.1). The following sperm defects were the most common: acquired head defects in 19.9% (SD 17.6), mainly represented by acrosome defects in 10.1% (SD 10.4), and free heads in 6.8% (SD 11.8). The acquired abnormalities included tail defects in 14.9% (SD 7.9) and defects of the sperm neck in 12.6% (SD 15.1), with the vast majority formed by proximal cytoplasmic droplets in 12.3% (SD 15.1).

Developmental defects were most often found on the sperm heads in 11.0% (SD 11.1), followed by the tail defects in 4.9% (SD 11.1), neck defects in 3.4% (SD 4.1) and, finally, a very small percentage of defects of the midpiece in 0.4% (SD 0.6). The most common abnormality was the distal cytoplasmic droplet found in 17.7% (SD 18.1) which, however, is not considered as a defect. The average percentages of sperm defects in different species are shown in Table 1. The average percentages of defects in different tribes are shown in Table 2. Only defects with significant between-tribe differences are presented.

Statistical comparison of morphological defects in different species

The percentage of developmental defects of sperm heads in the *Hippotragus equinus* was significantly higher compared to *Taurotragus oryx*, *Nanger dama ruficolis*, *Connochaetes taurinus taurinus*, *Kobus megaceros* and *Kobus leche kafuensis.* The percentage of acquired defects of sperm heads in the *Aepyceros melampus* was significantly higher compared to *Addax nasomaculatus*, *Hippotragus equinus*, *Taurotragus oryx*, *Hippotragus niger*, *Connochaetes taurinus taurinus*, *Kobus ellipsiprymnus defassa* and *Kobus leche kafuensis*. This difference was even bigger in the percentage of defective acrosomes, in which the *Aepyceros melampus* again differed from *Addax nasomaculatus*, *Hippotragus equinus*, *Taurotragus oryx*, *Hippotragus niger*, *Connochaetes taurinus taurinus*, *Kobus ellipsiprymnus defassa* and *Kobus leche kafuensis,* but also from *Damaliscus pygargus phillipsi*, *Tragelaphus imberbis*, *Tragelaphus angasii*, *Tragelaphus spekii*, *Kobus megaceros* and *Kobus ellipsiprymnus ellipsiprymnus*. The highest percentages of sperm with proximal cytoplasmic droplets were found in *Oryx dammah* and *Addax nasomaculatus* compared to *Taurotragus oryx*, *Hippotragus niger,* and *Connochaetes taurinus taurinus*. In addition, this percentage in *Oryx dammah* was higher than in *Nanger dama ruficolis*, *Kobus megaceros*, and *Aepyceros melampus*. The highest percentage of acquired defects of the sperm tail was observed in *Hippotragus niger* and differed significantly from *Taurotragus oryx*. Furthermore, the difference in the teratogenic index was significantly higher in *Aepyceros melampus* and *Hippotragus equinus* compared to *Connochaetes taurinus taurinus*. *Aepyceros melampus* also exceeded *Ammotragus lervia*. No significant differences were observed between different species in the percentages of normal sperm, distal droplets, free heads, developmental defects of the tail, defects of the midpiece, total number of developmental defects and total number of acquired defects.

Statistical evaluation of morphological defects in individual tribes

The highest but non-significant average percentage of normal sperm was detected in the *Caprini*. However, the tribe *Alcelaphini* significantly exceeded the values in the *Hippotragini*. The highest percentage of developmental defects was found in the *Hippotragini* which significantly differed from the *Antilopini*, *Caprini*, *Alcelaphini* and *Tragelaphini*. Acquired head defects were the most common finding in the *Aepycerotini* and differed significantly from the *Bovini*, *Hipotragini*, *Reducini*, *Caprini*, *Alcelaphini* and

Table 1. Morphological defects of epididymal sperm of individual species of the family *Bovidae*.

Tribe + species	N	Normal spermatozoa (%)	Head abnormalities (%)			Neck abnormalities (%)		
			Primary	Acrosome	Loose heads	Primary	Secondary*	Proximal droplet
Aepycerotini								
Aepyceros melampus	6	39.3 (21.4)	8.7 (4.1)	32.6 (27.5)	15.6 (25.5)	2.8 (2.1)	0.3 (0.6)	6.6 (6.9)
Alcelaphini								
Damaliscus p. phillipsi	4	62.0 (19.2)	6.4 (4.5)	5.9 (4.5)	2.6 (1.1)	0.5 (0.4)	0.1 (0.3)	13.9 (20.4)
Connochaetes gnou	5	50.5 (20.4)	11.5 (3.2)	12.2 (9.4)	3.4 (1.7)	4.3 (2.1)	0.6 (0.7)	9.1 (13.6)
Connochaetes t. albojubatus	1	52.5	10.5	6.0	1.5	2.0	0.0	1.5
Connochaetes t. taurinus	6	65.2 (9.9)	5.7 (5.0)	6.3 (3.4)	1.9 (2.6)	4.2 (2.9)	0.2 (0.4)	4.8 (8.8)
Antilopini								
Antidorcas marsupialis	2	50.8 (17.3)	1.3 (1.8)	10.8 (6.0)	11.3 (13.1)	0.8 (0.4)	0.3 (0.4)	7.8 (11.0)
Gazella leptoceros	1	49.5	3.0	2.0	14.0	3.0	0.5	2.0
Madoqua kirkii	1	54.0	21.5	5.5	2.0	3.5	0.5	6.5
Nanger dama ruficolis	5	46.8 (12.7)	4.9 (5.5)	12.7 (11.1)	14.4 (12.0)	3.3 (2.1)	0.6 (0.8)	7.7 (11.0)
Bovini								
Bos indicus	1	39.0	8.5	17.0	5.5	2.5	0.0	16.0
Bos taurus (Dahome)	1	64.0	2.5	6.5	1.0	0.5	0.0	2.0
Syncerus c. caffer	2	33.8 (13.1)	10.8 (5.3)	6.8 (1.1)	4.8 (3.2)	2.3 (1.1)	0.0 (0.0)	23.8 (33.6)
Taurotragus oryx	18	62.4 (17.5)	4.6 (3.6)	8.6 (5.3)	6.8 (6.5)	3.0 (2.0)	0.3 (0.5)	7.1 (10.8)
Caprini								
Ammotragus lervia	2	65.0 (9.2)	3.3 (1.1)	7.8 (4.6)	1.5 (0.0)	2.0 (1.4)	0.0 (0.0)	1.0 (0.0)
Capra hircus	1	81.5	1.5	9.5	1.5	2.5	0.0	0.0
Ovibos moschatus	1	46.0	16.0	5.5	1.5	7.5	0.5	10.0
Ovis aries	2	68.5 (12.0)	3.3 (2.5)	19.0 (10.6)	2.5 (0.7)	2.5 (1.4)	0.3 (0.4)	0.8 (0.4)
Hippotragini								
Addax nasomaculatus	6	36.6 (14.0)	16.6 (5.8)	6.8 (5.4)	4.8 (2.3)	1.9 (2.6)	0.6 (1.2)	35.0 (15.4)
Hippotragus equinus	6	35.3 (22.3)	29.8 (18.9)	6.0 (4.0)	2.7 (3.0)	3.6 (3.3)	0.1 (0.2)	22.1 (23.9)
Hippotragus niger	10	43.8 (16.4)	17.4 (17.3)	9.8 (8.8)	5.5 (4.3)	2.6 (2.0)	0.4 (0.4)	5.1 (4.9)
Oryx dammah	3	27.3 (7.6)	19.2 (12.6)	8.8 (3.7)	3.3 (0.6)	2.8 (1.3)	0.7 (0.8)	46.8 (5.3)
Oryx gazella	1	63.5	6.5	22.0	0.5	1.0	0.0	1.5
Oryx leucoryx	1	43.0	12.0	21.0	3.5	6.0	0.0	5.5
Reduncini								
Kobus e. defassa	8	37.7 (15.6)	19.6 (7.7)	3.9 (1.5)	4.3 (3.6)	5.7 (5.2)	0.3 (0.7)	19.7 (10.2)
Kobus e. ellipsiprymnus	5	37.0 (20.9)	22.2 (19.6)	6.8 (2.8)	4.3 (1.4)	2.2 (0.9)	0,2 (0.4)	14.7 (2.5)
Kobus leche kafuensis	7	57.3 (22,5)	7.4 (5,6)	7.8 (5,8)	2.0 (1.5)	2.5 (2.6)	0.2 (0.4)	12.4 (20.5)
Kobus megaceros	10	48.4 (30.0)	10.4 (14.0)	9.2 (4.6)	12.3 (26.9)	6.1 (12.2)	0.2 (0.2)	9.0 (13.3)
Redunca fulvorufula	1	55.5	17.5	6.5	0.5	10.5	0.0	5.0
Tragelaphini								
Tragelaphus angasii	3	55.3 (23.0)	5.8 (3.2)	5.7 (4.9)	5.5 (3.3)	1.2 (0.8)	0.0 (0.0)	15.2 (16.5)
Tragelaphus imberbis	3	57.7 (9.6)	5.8 (2.5)	6.8 (5.3)	5.2 (5.5)	2.7 (1.0)	0.3 (0.3)	8.2 (11.6)
Tragelaphus spekii	7	40.2 (21.7)	9.4 (5.6)	12.0 (6.1)	16.9 (22.8)	4.3 (3.1)	0.9 (1.5)	17.9 (16.2)
Tragelaphus strepsiceros	4	34.3 (18.7)	6.3 (3.5)	23.8 (25.3)	15.8 (10.4)	5.1 (2.0)	0.8 (0.9)	14.4 (17.8)

Tribe + species	N	Distal droplet (%)	Primary midpiece abnormalities (%)	Tail abnormalities (%)		Terato spermia index	Total sperm abnormalities (%)	
				Primary	Secondary		Primary	Secondary
Aepycerotini								
Aepyceros melampus	6	32.3 (22.3)	0.4 (0.4)	3.0 (1.1)	14.2 (6.4)	1.4 (0.2)	13.3 (4.3)	52.8 (24.7)
Alcelapini								
Damaliscus p. phillipsi	4	29.1 (28.6)	0.3 (0.3)	4.3 (2.3)	12.8 (4.2)	1.2 (0.1)	11.0 (6.5)	30.9 (18.0)
Connochaetes gnou	5	12.5 (8.4)	0.0 (0.0)	5.8 (4.4)	17.6 (13.5)	1.3 (0.1)	19.1 (5.1)	36.8 (19.7)
Connochaetes t. albojubatus	1	61.0	0.0	3.0	31.5	1.2	13.5	38.5
Connochaetes t. taurinus	6	16.4 (28.5)	0.1 (0.2)	4.5 (3.7)	12.3 (7.6)	1.1 (0.0)	12.3 (6.4)	24.3 (10.7)
Antilopini								
Antidorcas marsupialis	2	2.8 (1.8)	0.0 (0.0)	5.3 (7.4)	17.5 (7.1)	1.1 (0.0)	7.3 (8.8)	43.0 (9.2)
Gazella leptoceros	1	0.5	0.0	6.0	23.5	1.1	12.0	41.0
Madoqua kirkii	1	9.5	0.0	0.5	16.5	1.2	23.5	28.5
Nanger dama ruficolis	5	16.7 (15.7)	0.4 (0.5)	4.5 (3.1)	16.4 (5.7)	1.2 (0.1)	12.4 (7.0)	44.3 (11.3)
Bovini								
Bos indicus	1	13.0	0.0	7.0	20.5	1.3	17.0	49.0
Bos taurus (Dahome)	1	38.5	0.0	1.0	26.0	1.1	4.0	33.5
Syncerus caffer caffer	2	7.8 (8.1)	1.8 (1.1)	9.8 (2.5)	19.5 (6.4)	1.2 (0.0)	22.3 (6.7)	50.0 (19.8)
Taurotragus oryx	18	26.1 (15.7)	0.2 (0.3)	5.9 (6.2)	8.8 (4.0)	1.2 (0.1)	12.8 (9.1)	27.6 (12.1)
Caprini								
Ammotragus lervia	2	3.3 (4.6)	0.0 (0.0)	0.0 (0.0)	21.5 (7.8)	1.1 (0.0)	5.0 (2.8)	30.8 (11.7)
Capra hircus	1	17.0	0.0	0.0	7.0	1.2	4.0	16.5
Ovibos moschatus	1	8.0	0.5	8.0	13.5	1.2	28.0	30.0
Ovis aries	2	0.3 (0.4)	0.0 (0.0)	0.5 (0.7)	6.3 (3.2)	1.1 (0.1)	6.3 (4.6)	26.3 (7.4)
Hippotragini								
Addax nasomaculatus	6	23.2 (12.9)	0.3 (0.3)	5.9 (3.7)	10.9 (6.1)	1.3 (0.1)	22.9 (8.4)	51.4 (16.6)
Hippotragus equinus	6	18.2 (17.9)	1.3 (2.8)	4.3 (3.1)	20.1 (8.1)	1.4 (0.2)	34.3 (17.6)	44.3 (19.8)
Hippotragus niger	10	7.0 (8.8)	0.4 (0.6)	6.6 (9.4)	20.6 (9.5)	1.2 (0.1)	24.2 (22.3)	36.5 (10.9)
Oryx dammah	3	13.8 (14.0)	0.3 (0.3)	5.5 (2.6)	9.7 (3.3)	1.3 (0.2)	24.2 (12.6)	61.0 (7.4)
Oryx gazella	1	0.5	0.0	0.5	6.5	1.1	8.0	29.0
Oryx leucoryx	1	8.5	1.0	6.0	18.0	1.3	24.0	41.5
Reduncini								
Kobus e. defassa	8	8.5 (5.5)	0.6 (1.0)	6.7 (7.8)	18.8 (9.6)	1.3 (0.1)	29.4 (15.8)	42.0 (7.8)
Kobus e. ellipsiprymnus	5	16.6 (20.8)	0.6 (0.7)	8.3 (12.8)	20.0 (8.0)	1.3 (0.0)	30.2 (21.9)	41.9 (6.7)
Kobus leche kafuensis	7	27.4 (22.0)	0.4 (0.6)	3.7 (2.9)	15.9 (3.5)	1.2 (0.1)	13.1 (9.6)	33.5 (20.9)
Kobus megaceros	10	20.0 (26.1)	0.3 (0.4)	4.9 (4.3)	13.2 (9.2)	1.2 (0.2)	18.6 (22.8)	39.5 (25.0)
Redunca fulvorufula	1	0.5	0.0	4.5	19.1	1.1	26.5	19.5
Tragelaphini								
Tragelaphus angasii	3	23.2 (19.0)	0.5 (0.0)	4.5 (4.0)	13.0 (9.0)	1.1 (0.1)	11.5 (7.5)	36.3 (18.3)
Tragelaphus imberbis	3	11.3 (13.6)	0.0 (0.0)	3.7 (1.4)	17.7 (3.7)	1.2 (0.1)	11.5 (1.8)	34.3 (11.8)
Tragelaphus spekii	7	18.2 (17.0)	1.3 (0.7)	2.7 (1.9)	13.7 (5.3)	1.3 (0.1)	15.6 (6.2)	51.7 (20.9)
Tragelaphus strepsiceros	4	7.4 (7.8)	0.1 (0.3)	3.4 (3.8)	12.0 (9.0)	1.2 (0.0)	14.0 (8.6)	56.9 (20.4)

Values are presented as means (standard deviation)
* Without proximal cytoplasmic droplet
Primary - developmental; Secondary - acquired

Table 2. Selected morphological defects of tribes of the family *Bovidae* with a significant difference.

Tribe	N	Normal spermatozoa (%)	Head abnormalities (%)			Secondary tail abnormalities (%)	Total sperm abnormalities (%)	
			Primary	Secondary	Acrosome		Primary	Secondary
Aepycerotini	6	39.3 (21.4)	8.7 (4.1)	48.2 (44.7)	32.6 (27.5)	14.2 (6.4)	13.3 (4.3)	52.8 (24.7)
		14.0–64.0	4.0–14.5	15.5–125.0	10.0–75.5	6.5–22.5	7.5–18.5	27.5–83.5
Alcelaphini	16	59.0 (16.2)	8.0 (4.8)	10.5 (7.0)	8.0 (6.3)	15.3 (9.8)	14.2 (6.4)	30.7 (15.5)
		20.5–75.5	0.5–16.0	1.5–32.5	1.5–28.0	5.5–34.0	5.5–24.0	14.0–66.5
Antilopini	9	48.8 (11.2)	5.7 (7.3)	22.6 (11.7)	10.3 (9.0)	17.4 (5.3)	12.4 (7.5)	41.9 (10.1)
		27.0–63.0	0.0–21.5	7.5–43.5	2.0–32.0	8.0–24.0	1.0–23.5	26.5–56.5
Bovini	4	42.6 (16.3)	8.1 (5.0)	13.3 (6.6)	9.3 (5.2)	21.4 (4.8)	16.4 (9.4)	45.6 (14.0)
		24.5–64.0	2.5–14.5	7.5–22.5	6.0–17.0	15.0–26.0	4.0–27.0	33.5–64.0
Caprini	6	65.8 (13.3)	5.1 (5.5)	13.3 (8.1)	11.4 (7.9)	12.7 (8.3)	9.1 (9.6)	26.8 (8.2)
		46.0–81.5	1.5–16.0	6.0–28.5	4.5–26.5	4.0–27.0	3.0–28.0	16.5–39.0
Hippotragini	27	39.2 (16.8)	19.6 (15.1)	13.2 (8.0)	9.0 (7.2)	16.5 (8.8)	25.5 (17.0)	44.2 (15.8)
		4.5–63.5	3.5–61.5	2.5–36.5	1.0–34.0	2.5–33.5	4.5–67.5	11.0–76.0
Reduncini	31	46.0 (23.5)	14.2 (12.8)	13.2 (15.1)	7.0 (4.4)	16.5 (8.1)	22.3 (18.6)	38.5 (17.9)
		1.0–84.0	1.5–55.5	1.5–89.0	0.5–18.5	1.5–34.0	2.5–73.5	12.0–93.0
Tragelaphini	35	53.7 (20.7)	5.9 (4.2)	20.2 (18.9)	10.6 (10.3)	11.2 (5.9)	13.3 (7.7)	37.1 (18.8)
		13.0–82.5	1.0–17.5	3.5–91.5	1.5–61.5	2.0–23.0	3.0–31.0	14.5–81.0

All values are presented as means (standard deviation).
Primary - developmental; Secondary - acquired

Tragelaphini. This phenomenon was mainly observed in acrosome defects, the percentage of which was significantly higher compared to all the other investigated tribes, while the differences in the number of free heads were non-significant. Out of the tail defects, significant differences were only found in the acquired defects, namely between the *Bovini* with the highest and the *Tragelaphini* with the lowest percentage of the defects. The highest overall prevalence of developmental sperm defects was detected in the *Hipotragini* and differed significantly from the *Tragelaphini*. The highest overall prevalence of acquired sperm defects was detected in the *Aepycerotini* and differed significantly from the *Caprini*.

Discussion

The prevalence of defects in the group of animals investigated in the present study was higher than in the domestic species of the *Bovidae* family. In breeding cattle, the expected prevalence of all sperm defects is up to 20% and the prevalence of developmental defects only up to 10% (Věžník et al. 2004). The number of the evaluated pathologies in our group averaged 51.2%. There are no publications with a similar focus and scope for wildlife.

The values obtained in the animal species investigated in our study differed most considerably from those reported above for *Hippotragus equinus* in the number of developmental abnormalities on sperm heads, *Hippotragus niger* in the number of acquired tail abnormalities, *Aepyceros melampus* in the number of acquired head defects, mainly acrosome defects, and for both *Addax nasomaculatus* and *Oryx dammah* in the number of sperm with proximal cytoplasmic droplets.

In this study, six *Hippotragus equinus* were examined. One of them was 11 years old and showed testicular calcifications, one was 8 years old, two were 2 years old and two were under 2 years of age. All samples were examined within 24 h of sample collection. The majority of developmental pathologies of the sperm head were detected in the oldest animal

with altered testicular structure and in the youngest animal, in which a high percentage of proximal droplets was found. These findings suggested that age and health condition might play an important role in the development of pathologies.

Ten *Hippotragus niger* were examined. Two of them were 6 years old, one was 3 years old and was found dead, two were 2 years old and the remaining animals were under 2 years of age. All samples were processed within 24 h of collection. Even though this group consisted of relatively young animals, an increased number of acquired tail defects was found in one older breeding male as well as in most of the young animals. This indicates that all of these animals could be biological relatives.

Six *Aepyceros melampus* were examined. One of them was a 10-year-old animal suffering from a phlegmon that had fathered the other animals; one was a 6-year-old animal affected by a disease, two animals were under 3 years of age and the remaining ones were under 2 years of age. Sperm from one animal was not examined within 24 h. The worst results were detected in the diseased animal and also in the young animal, the sample of which was not processed soon enough. Due to the fact that this group was relatively small and *Aepyceros melampus* is the only species placed in the *Aepycerotini* tribe, the characteristics of the species were reflected in the characteristics of the entire tribe. The important factors here were the health condition, strong influence of biological relationship, and the time of sample processing.

Six *Addax nasomaculatus* were examined. Samples from all animals were processed within 24 h. One animal was 9 years old, the rest were young animals under 2 years of age; two of them had epididymal cysts.

Three *Oryx dammah* were examined: one was 9 years old and two were under 2 years of age. We assumed that in these animals, especially in *Addax nasomaculatus*, the finding of the increased prevalence of proximal droplets was caused by the young age of the majority of animals in the examined group. This was confirmed by the obtained results in individual animals.

The results showed that the main factors distinguishing one species from another in the group of animals in the present study were the age of the animals, their health condition, and the biological relationship between the animals. Furthermore, time was a very important factor in the sample processing, however, in this group it manifested itself rather at the level of individual animals. Another factor was that epididymal sperm collection did not take place in a natural way as the samples were obtained after the death of the animals. In addition, samples were processed within 24 h, whereas bull semen is processed immediately after collection. It is also important that the animals did not live under natural conditions and the samples were taken throughout the year, regardless of reproductive activity.

A characteristic feature of epididymal sperm in exotic species of the *Bovidae* family is the presence of cytoplasmic droplets. Both distal and proximal cytoplasmic droplets occurred at the same time in almost 30% of sperm. However, distal droplets are not considered as pathologies and thus, acquired head defects were the most common pathology.

The unique collection of animals of the *Bovidae* family used in this study was assembled over almost 10 years. In addition, a sperm bank was created when sample processing and subsequent examination were performed. Finally, it was possible to prepare insemination doses from 70 animals and, by virtue of this, 27 species have been preserved. In the future, the frozen epididymal sperm can be used for fertilization, thus contributing to the reproduction of these animals.

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