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of Animal Breeds in the Danube Region
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Research articles

Announcements

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Magnetic nanopurification of ram semen using Mase V particles for depletion of morbid and DNA fragmented spermatozoa

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Abstract

The aim of this study was to enhance the quality of fresh ram semen by purification of spermatozoa using combination of annexin V and lectin (PNA and LCA) coated nanoparticles. Semen samples from two rams were collected regularly during the breeding season using electro-ejaculation. Samples (n=5) with poor quality (decreased sperm motility and viability) were chosen for the nanopurification procedure. Fresh (control) samples and purified (depleted from damaged spermatozoa) samples were analysed by CASA for total and progressive motility. Flow cytometry was used to check presence of apoptotic-like changes (annexin V-FITC) and integrity of acrosome (PNA-AF488 and rhodamine LCA) as well as proportion of dead cells (propidium iodide) in semen samples before and after nanopurification. Transmission electron microscopy (TEM) was performed to assess changes in sperm ultrastructure and interaction with nanoparticles. Purified ram spermatozoa exhibited increased viability ($p<0.01$) and both motility parameters ($p<0.05$) and decreased proportion of spermatozoa with damaged acrosome ($p<0.05$). On the other hand, no significant differences were observed for annexin V and LCA positive spermatozoa between fresh and purified samples. TEM analysis revealed occurrence of spermatozoa with swollen acrosome and damaged plasma membrane as well as abundance of nanoparticles surrounding the cells or bound to the membrane in positively stained spermatozoa. In conclusion, nanopurification process can improve the quality of ram semen, mainly in terms of sperm viability, motility and presence of damaged spermatozoa, which might result in better fertilization ability of insemination doses or even higher cryosurvival rates of stored spermatozoa.

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Keywords: ram, spermatozoa, nanopurification, CASA, flow cytometry

Introduction

In general, animal semen may contain different proportions of intact viable, damaged or even dead spermatozoa, which can greatly affect the overall fertility. It has been reported that high number of dead spermatozoa in semen sample is associated with insufficient reproductive outcomes of *in vitro* fertilization as well as with poor cryopreservation (ROCA et al., 2013). The elimination of non-viable spermatozoa, which cause the main damage, from the semen using current technology has been previously studied with different results. The most recent, a nanotechnology-based technique (nanopurification) has been developed to non-invasive target and remove the moribund spermatozoa. Due to the interactions with specific magnetic nanoparticles, the process of nanopurification may have a potential to enrich insemination doses with viable spermatozoa resulted in the improvement of animal breeding efficiency (DURFEY et al., 2017).

In human assisted reproduction, magnetic-activated cell sorting (MACS) has been used to eliminate spermatozoa with deteriorated membranes and apoptotic-like features (AGARWAL et al., 2009; BUCAR et al., 2015; GLANDER et al., 2002; VENDRELL et al., 2014). In addition, the same technique using manual MACS instruments were used in rabbit (VASICEK et al., 2014) or boar (MRKUN et al., 2014). Recently we used for the first time a fully automated magnetic cell sorter to remove deteriorated spermatozoa from ram semen samples (VAŠÍČEK et al., 2020; VAŠÍČEK et al., 2021). Unfortunately, the used technique was not sensitive enough either to efficiently deplete the dead spermatozoa, or to improve the sperm motility itself. In fact, all above mentioned studies applied nanoparticles conjugated with annexin V, which bind to phosphatidylserine exposed on the surface of cells undergoing apoptosis. However, other ligands such as those of lectins, PNA (peanut agglutinin from *Arachis hypogaea*), PSA (*Pisum sativum* agglutinin), and LCA (*Lens culinaris* agglutinin) are present on the surface of defective spermatozoa with revealed acrosome (CROSS & WATSON, 1994; ODHIAMBO et al., 2011). Nanopurification of animal spermatozoa using these lectins in combination with or without of annexin V has already been reported in bull (ODHIAMBO et al., 2014), boar (DURFEY et al., 2017; DURFEY et al., 2019; FEUGANG et al., 2015) or camel (RATEB, 2021). On the other hand, such combination of glycoproteins has never been used to improve the quality of ram spermatozoa.

The main objective of this study was to magnetically eliminate the ram defective spermatozoa using the combination of nanoparticles conjugated with annexin V, PNA and LCA proteins.

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Material and methods

Sexually mature and clinically healthy rams (n=2) of the Native Wallachian and Slovak dairy sheep breed at the age of 2.5–5 years were used in this study. Animals were kept at a breeding facility of NPPC – RIAP Nitra (Lužianky, Slovak Republic) and fed as reported previously. Semen samples were collected twice a week by electro-ejaculation and immediately transported to the laboratory throughout the whole study as reported previously (VOZAF et al., 2021). The basic semen quality assessment consists of sperm motility and viability analysis. Based on this assessment, five semen samples with impaired quality were chosen for further nanopurification process to improve the ram sperm attributes.
The animals and sample collection were carefully handled in accordance with ethical guidelines as stated in the Slovak Animal Protection Regulation RD 377/12, which conforms to European Union Regulation 2010/63..

CASA analysis

The motility and sperm movement were analysed by CASA (SpermVision™ software, Minitube, Tiefenbach, Germany) with light microscope (at the 200× magnification; AxioScope A1, Carl Zeiss Slovakia, Bratislava, Slovakia) and Makler counting chamber (Microptic, Barcelona, Spain) as described previously (VOZAF et al., 2021). Briefly, samples were diluted by saline (0.9% NaCl; Braun, Nuaille, Germany) at ratio 1:20 (v/v). A drop of diluted semen (10 µL) was transferred to a counting chamber and analysed with manufacturer's pre-set parameters for rams. Mainly total (TM) and progressive motility (PM) were observed in this study.

Flow-cytometric analyses

Viability and apoptosis
The viability of spermatozoa in ram semen samples were evaluated as the proportion of dead spermatozoa stained by propidium iodide (PI) dye at the final concentration of 50 µg/mL. The apoptotic-like changes in ram spermatozoa were observed by staining with Annexin V-FITC (AnV) in combination with dead cell dye DRAQ7 as described previously (VASÍCEK et al., 2022).

Acrosomal status

The integrity of acrosome was determined using two different fluorescent probes: Alexa Fluor 488 conjugated PNA in combination with DRAQ7 dye and rhodamine conjugated LCA in combination with SYTOX Green dye as reported in previous study (VASÍCEK et al., 2022).

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Stained semen samples were immediately analyzed using flow cytometer FACS Calibur equipped with a Cell Quest Pro software (BD Biosciences, San Jose, CA, USA). A least 10,000 events (spermatozoa) were acquired and analysed in each sample.

Nanopurification of ram spermatozoa

To remove dead and morbid spermatozoa from the ram semen, Mase V iron nanoparticles (Clemente Associates Inc., USA) were used. The particles were coated with three glycoproteins, Annexin V, PNA and LCA, which bind either to the apoptotic-like spermatozoa, or spermatozoa with compromised acrosome. Briefly, an aliquot of ram semen (10^8 spermatozoa) diluted in 1.3 mL of HEPES buffer (VAŠÍČEK et al., 2020; VAŠÍČEK et al., 2021) was incubated at orbital shaker for 15 min. at room temperature with a proper volume of Mase V nanoparticles (at final concentration of 4 mg/mL) calculated according to the producer's manual and following formula:

Y (mL) = ax + b, a = - 0.22 b= 22.23 and x is the sperm motility

Then magnet was placed against the tube for 10 min. Afterwards, supernatant containing intact viable spermatozoa (negative fraction) was gently decanted to new tube, while the DNA fragmented and damaged spermatozoa (positive fraction) were still attached to the particles against the wall of the tube and magnet. The sorted (negative) spermatozoa were analysed by CASA system to check the sperm motility parameters and by flow cytometry to determine sperm viability (PI), apoptosis-like changes (AnV) and acrosomal status (PNA, LCA) as mentioned above.

Transmission electron microscopy of sorted ram spermatozoa

To analyse the interaction of nanoparticles with damaged ram spermatozoa, the sample of positive fraction after nanopurification was processed for transmission electron microscopy (TEM) as described previously (VOZAF et al., 2022). Briefly, positively sorted ram spermatozoa were fixed in a fixative solution (2% paraformaldehyde and 2.5% glutaraldehyde in 0.15 mol/L sodium cacodylate buffer, pH 7.1–7.3). Then, sample was washed three times in cacodylate buffer for 15min. and postfixed in 1% osmium tetroxide in cacodylate buffer during 1h and embedded into 2% agar. Subsequently, sample was dehydrated by passing them through an acetone series and embedded into PolyBed resin (Polysciences Inc., USA). Ultrathin sections (70 nm) were placed on nickel grids, contrasted and examined under the transmission electron microscope (JEM-2100, JEOL, Japan) operating at 200 kV.

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Statistical Analysis

Experiments with nanopurification of ram spermatozoa were performed five times. Due to the high variability of obtained data, an unpaired nonparametric Mann-Whitney t test was used for statistical analysis using GraphPad Prism version 9.5.1 for Windows (GraphPad Software, San Diego, CA, USA). The data are represented as means ± SD. Differences at $p<0.05$ were considered statistically significant.

Results and discussion

The removal of defective spermatozoa is one of the big challenges how to improve the assisted reproductive outcomes. Here, we used the magnetic nanoparticles to purified ram spermatozoa by depleting spermatozoa with apoptotic-like changes bound to annexin V and/or spermatozoa with compromised acrosome integrity bound to PNA or LCA. The flow cytometry revealed that apoptosis-like process was not significantly present either in fresh, or purified spermatozoa (Figure 1A). On the other hand, significant ($p<0.05$) enrichment of acrosome intact spermatozoa was achieved in purified spermatozoa compared to fresh samples (8.8±4.8% vs. 21.7±12.9% of PNA-positive cells, respectively; Figure 1B). The decrease of LCA-positive spermatozoa in purified samples was also observed, although not significant (Figure 1C). However, a significant ($p<0.01$) removal of dead spermatozoa was noticed after nanopurification of ram spermatozoa compared to fresh sperm samples (5.9±3.6% vs. 24.8±11.7%, respectively; Figure 1D). The nanopurification even significantly ($p<0.05$) improved the motility attributes of ram spermatozoa, both total and progressive motility in comparison to fresh sperm samples (82.9±9.4% vs. 71.0±6.2 and 80.0±8.6% vs. 65.2±8.7%, respectively; Figure 1E and 1F).

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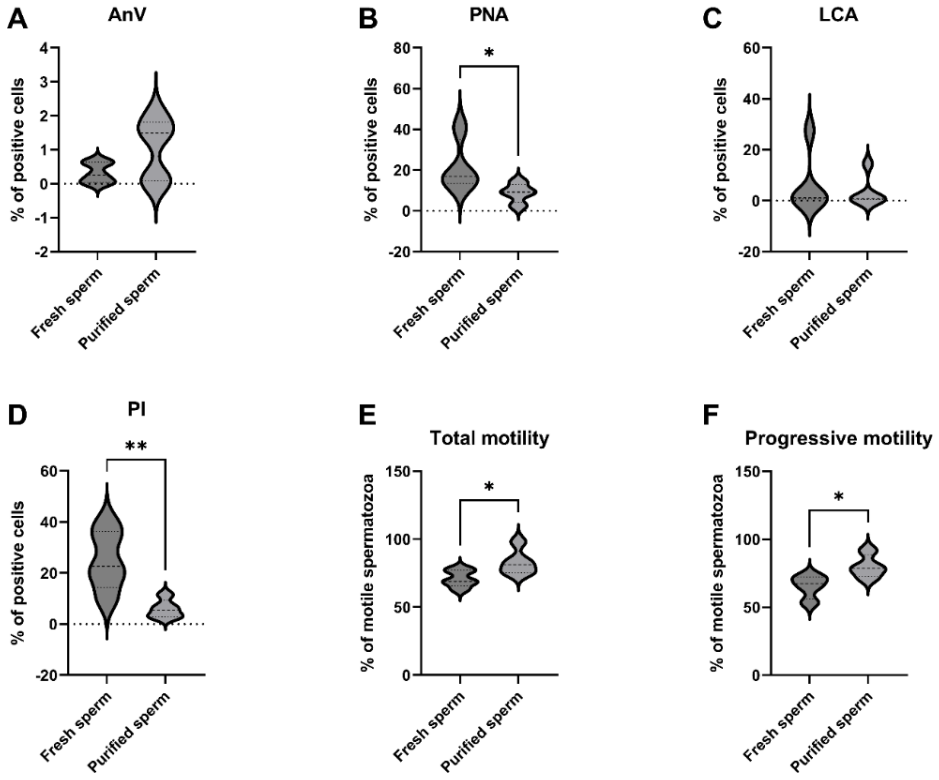

Figure 1: Sperm quality attributes in fresh and purified ram spermatozoa analysed by flow cytometry and CASA system. Fresh and magnetically purified ram spermatozoa were stained with annexin V to detect apoptotic-like changes (A), with PNA and LCA to detect disturbed acrosome (B and C, respectively) and with PI to target dead spermatozoa (D). CASA analysis was used to assess total and progressive motility (E and F, respectively) of fresh and purified ram spermatozoa. The data are expressed as the means ± SD; difference is statistically significant at * $p<0.05$ and ** $p<0.01$.

Similarly, increase in the viability of cryopreserved bull spermatozoa or in the total and progressive motility of fresh boar spermatozoa, both nanopurified with PNA conjugated nanoparticles, was observed in previous studies (FEUGANG et al., 2015; ODHIAMBO et al., 2014). DURFEY et al. (2017; 2019) also found significantly higher sperm progressive motility in fresh boar semen samples, in which defected spermatozoa were depleted with nanoparticles coated with lectins and annexin V. On the other hand, plasma integrity or acrosome reaction of nanopurified spermatozoa determined by PI or PNA staining respectively, was not significantly different compared to nonpurified spermatozoa (DURFEY et al., 2019). Contrarily, a dramatic improvement in post-thaw sperm motility and viability as well as in the

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proportion of spermatozoa with intact acrosome was reported in the camel cryopreserved semen after purification with lectins and annexin V conjugated nanoparticles (RATEB, 2021).

To confirm the effective interaction of nanoparticles with ram spermatozoa as well as to check the quality of sperm plasma membranes, we performed an ultrastructural analysis of purified sperm sample using TEM. Majority of the positively selected spermatozoa exhibited swollen acrosome and damaged plasma membrane with nanoparticles surrounding the cells or bound to the membrane (Figure 2). Similar observation was made in nanopurified boar spermatozoa (DURFEY et al., 2019).

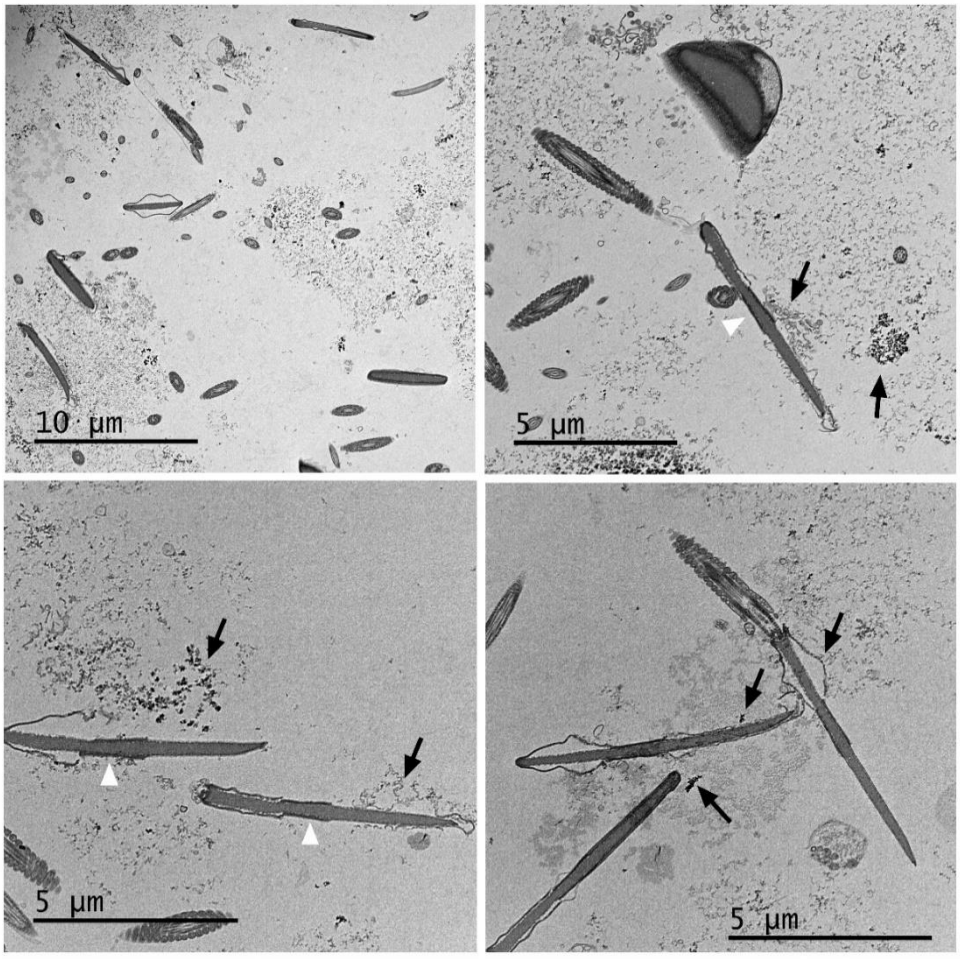

Figure 2: TEM analysis of ram spermatozoa retained using magnetic nanoparticles and magnetic field within the sorting tube. Nanoparticles are indicated with black arrows, while spermatozoa with damaged plasma membrane are marked by white arrowheads.

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Conclusion and recommendation

In conclusion, gentle manual magnetic nanopurification of ram semen samples may be a better alternative to automated MACS sorting regarding great sensitivity of ram spermatozoa. Moreover, purification of spermatozoa with the combination of annexin V and lectins such as PNA evidently much more improve the ram semen quality in terms of the sperm viability, motility and acrosome integrity than sperm sorting with nanoparticles coated only with annexin V itself. The presented method might be eventually used to enhance the semen quality of valuable ram intended for cryopreservation or artificial insemination. Anyway, further study is required to investigate if this method could also improve the post-thaw quality of already cryopreserved semen samples.

Acknowledgement

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Hungarian Grey Cattle calves created by use of cryopreserved semen and the length of the generation interval thus formed

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Abstract

The Hungarian Grey Cattle, an important historical breed of livestock, is now one of Hungary's national treasures. The breed's population reached a critically low level by the early 1960s, which is one of the determinants of its current diversity and a warning for its careful maintenance. To strengthen the rare bull lines, an initiation was accomplished by the Hortobágy Nature- and Gene Preservation Nonprofit Ltd. and Association of Hungarian Grey Cattle Breeders. During the breeding program, which lasted for three years (2020-2022), cryopreserved semen of the breeding bulls was used that born in the 1970s. Seventy-eight animals became pregnant from 150 synchronized heifers and delivered calves (2021-2023). Twelve young bulls completed own performance test and became qualified and licensed. The dam-offspring relationship resulted in significantly ($p<0.001$) fewer years (4.2) than the sire-offspring relationship (43.9). Next to the revitalization of rare sire lines, the genetic pool of the herd becomes renewed by this investigation. By comparing the individuals born from the applied procedure with those born from contemporary sires, any phenotypic changes occurring in the breed can be detected. Last but not least, the use of the reproductive material from time to time also serves to control the reliability of the sperm freezing technique.

Keywords: cryopreservation, artificial insemination, rare breed conservancy

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Introduction

The centuries-old history of the Hungarian Grey Cattle is intertwined with the people living in the Carpathian Basin: for a long time it played an indispensable role in the steppe animal husbandry and was an excellent, leg-driven meat animal for the slaughterhouses of Western countries (BODÓ, 2000). Today, the Hungarian Grey Cattle is our national treasure, protected as a Hungarikum. The breed almost became extinct in the 1950s when it was ordered that all Hungarian Grey bulls be sent to the slaughterhouse and the cows were ordered to be crossed with the Soviet Kostroma breed. Since 1960, the pure breeding and mating of Hungarian Grey cows with Hungarian Grey bulls has been officially banned (BODÓ et al., 2004). In the most critical year in the country, 1961, the breed was survived by eight bulls and about 250 cows, which form the basis of the current population. Due to the persistent initiative to preserve the breed, the authorities in 1961 permitted the further pure breeding in two state farms (Hortobágy State Farm and Középtisza State Farm), which were soon joined by a third (Városföld State Farm). This marked the beginning of so-called *reserve-like breed preservation* in 1963. This meant that the farms had to generate the resources necessary to preserve the breed themselves. It can be dated from 1973 when the Ministry of Agriculture adopted a resolution for general central support for the maintenance of autochthonous breeds. This marked the beginning of a *de jure breed preservation*, which continues to this day.

The generation interval (GI) is the average age of the parents at the birth of the offspring (LUSH, 1945). Taking gene transfer into account, this is more precisely the average age of the parents at the birth of the offspring that are involved as breeding animals in the creation of the next generation. MCMANUS et al. (2019) have proposed the “mean age of parents” as the name for the first interpretation, and the “generation interval” for the second. If the aim of breeding is to maintain the population, a long generation interval is considered to be suitable (GROENEVELD, 2009).

Today, the living population of the bull lines has become uneven. In order to strengthen the rare bull lines (B, M, T, and V), an initiation was accomplished by the Hortobágy Nature- and Gene Preservation Nonprofit Ltd. and Association of Hungarian Grey Cattle Breeders. During the first part of the breeding program, which lasted for few years, cryopreserved semen of the breeding bulls was used (2020-2022) that born in the 1970s. This breeding program also provides insight into the applicability of biotechnical procedures and the comparison of traits over time in a heritage breed. This study reports on the experiences of the breeding program so far.

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Material and methods

The part of the special breeding program that we report on here lasted from artificial insemination (AI) (2020-2022), through the birth (2021-2023) and raising of calves, to the evaluation of bull candidates (2023-2024).
On the course of the program 150 heifers (born 2017 and 2018) were synchronized (between January 1 and March 30 each year), then inseminated. For the insemination, semen from eight bulls (MÓZES - 1976, MISKA I - 1977, TÚR - 1977, VÁNDOR - 1978, BOTOND III - 1978, TÜSKE - 1979, TŐZSÉR - 1979, MÉLYKÚT - 1979) was selected and used. The year of birth is shown after the bulls' names. These bulls represented four sire lines. The success of breeding program is reported through the birth and relocation of the calves. Bull calves completed the own performance test on-farm (Hortobágy Ltd.) or central (Bos-Genetik Ltd.) in 2023-24 (Figure 1).

Figure 1: A three-year-old young candidate bull in own performance test at centralized test station of Bos-Genetik Ltd. (left). Young bulls, including those born from archive sires at the age of two and three in Hortobágy (right).

The Association's database was used for the processing. The pedigree of the 78 calves born includes 1438 individuals, of which 308 are sires and 1130 are dams. The ancestry of calves born from cryopreservation was known from this.
The length of the generation interval was determined from the birth dates of the calves and their parents. The length of the generation interval was expressed as the age of the parent at the birth of offspring, taking into account all of them, so the GI corresponds to its first interpretation. The generation interval was calculated in the following different paths: parent-offspring, dam-offspring, sire-offspring, as well as dam-daughter (DD), dam-son (DS), sire-daughter (SD), and sire-son (SS).
Statistica software (TIBCO Software Inc., 2020) was used to calculate GI and to test the differences between paths (single trait ANOVA with Tukey Honestly Significant Difference, HSD).

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Results and discussion

A total of 78 viable individual calves were born from the inseminated heifers, 2021-2023. The calving rate was thus 52%. This rate is particularly good, for example, when compared to the 42.9% value obtained by DICKINSON et al. (2019) under similar conditions (replacement heifers underwent oestrous synchronization for fixed-time AI).
Of the calves, 40 were bull calves and 38 were heifer calves. Accordingly, the sex ratio was 51.3♂:48.7♀. This ratio is consistent with previous experience in cattle, e.g. KASIMANICKAM et al. (2021) reported 52:48 after artificial insemination.
All heifer calves born became breeding cows without exception.
Of the bull calves, twelve became qualified bulls (sired by BOTOND (2x), TÜSKE (6x), MISKA I (2x), and VÁNDOR (2x)) in 2023-24 after completing the own performance test on-farm (Hortobágy Ltd.) and central (Bos-Genetik Ltd.) (Figure 1). In parentheses, the number of licenced progeny-bulls per archive sires is shown after the bulls' names.
The bull calves born to the sires MÓZES, TÚR, TÖZSÉR, and MÉLYKÚT did not meet the breed requirements, so they were slaughtered.

As shown in Table 1, the average generation interval was 24.2 years. The dam-offspring relationship resulted in significantly ($p<0.001$) fewer years (4.2) than the sire-offspring relationship (43.9). In the case of the dam-offspring relationship, this value is low because heifers calved. This value corresponds to the age at first calving, which is the starting limit of the generation interval.

Table 1: The generation interval using cryopreserved semen of archive sires and live heifer partners

Pathways	Number of connections	Years, Mean ±SD	F-value, P-value	$LSD_{5\%}$, $LSD_{1\%}$
Parent-offspring	156	24.2 19.9		
Dam-offspring	78	4.2[a] 1.4	41971	0.380
Sire-offspring	78	43.9[b] 1.0	<0.001	0.499
Dam-daughter	38	4.5[a] 1.9	14111	0.535
Dam-son	40	4.0[a] 0.1	<0.001	0.703
Sire-daughter	38	43.9[b] 1.1		
Sire-son	40	43.9[b] 1.0		

[a,b] different superscript letters differ significantly

In the previous processing of the Hungarian Grey Cattle herd book, the age at first calving was 3.8 years on average over four decades (GUNVOR, 2002). Previous calculations in the breed indicate that the length of the dam-offspring and sire-offspring relationships (7.9 and 7.3 years, respectively) seems to be more balanced

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during everyday breeding, but the difference between them is confirmed ($p<0.001$) (GYRI, 2002).
In the horse, the Hungarian Hutzul and Haflinger and small horse breeds, POSTA et al. (2020) and GAÁL and POSTA (2024) found an average of 10.3 and 8.8 years for the generation interval, respectively. In sheep, the endangered Gyimes racka, SCHÜTZ et al. (2023) estimated an average generation interval of 4 years. In the rabbit, the Pannon White breed, POSTA et al. (2024) calculated an average generation interval of 1.1 years. According to the parental paths in the cited breeds, in practice the generation interval is relatively balanced.

Conclusion and recommendation

The special breeding program aimed at using cryopreserved semen can be considered successful. Several beneficial conclusions can be drawn from this. One of them is that the chain of biotechnical procedures applied in Hungarian Grey Cattle (semen collection, deep freezing, cryopreservation, cycle synchronisation, artificial insemination) has proven to be reliable.
The revitalization of four narrowed sire lines has been successful. The Association enriches the breed diversity and cow pairings with twelve breeding bulls born from archive bulls.
The breeding program also had unintended consequences, resulting in the exclusion of some bull calves from breeding.
The strict requirements were not met by brownish hair coat (red hairs on the shoulder, rump, and on the poll) remained after first shedding. These were often found among the offspring of TŐZSÉR. Not fully pigmented, pinkish tongue and palate came to light mainly after the archive sire TÚR. Reasons for exclusion included constitutional weakness and low growth potential. Some progenies had weaker back and a weaning weight under 170 kg. Furthermore, the bull calves born to the sires MÓZES and MÉLYKÚT did also not meet the breed standard requirements, so they were slaughtered.
Semen has been collected from Hungarian grey cattle bulls on several occasions. The insemination material is stored in containers with liquid nitrogen at the Bos-Genetik station of the University of Veterinary Medicine Budapest. It is recommended to use them purposefully from time to time. It is also recommended to continuously expand the frozen storage of reproductive material with newly collected semen.
As a new goal, we plan to calculate the inbreeding coefficient of the offspring born in the program, as well as the relatedness coefficient between them.

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Strengthening the Sustainability of Sjenica Sheep

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Abstract

The Sjenica–Pešter Plateau (43.27306 N, 19.99944 E), due to its natural characteristics, holds significant potential for sheep production. Breeding the Sjenica sheep in this region represents a key mechanism for securing livelihood for the local population, reducing unemployment, and preventing depopulation. However, uncontrolled crossbreeding with highly productive breeds (such as Württemberg and Île-de-France) has led to a loss of authenticity and reduced genetic diversity of this autochthonous strain, thereby threatening biodiversity, agroecosystems, and the cultural heritage of Serbia and the surrounding region. As a response to these challenges, the most reliable conservation strategy involves promoting sustainable low-input breeding systems within traditional habitats. This study focuses on the characterization of lamb stelja - a cured meat product - as a value-added traditional resource obtained through extensive production relying exclusively on local plant-based forage. Fatty acid analysis of the stelja revealed a highly balanced nutritional profile. The proportion of monounsaturated fatty acids (MUFA) was 48.82 ± 2.00, saturated fatty acids (SFA) accounted for 46.05 ± 1.30, while polyunsaturated fatty acids (PUFA) comprised 3.08 ± 0.52. The dominant fatty acid was oleic acid (C18:1 cis-9) at 46.90 ± 1.75, followed by palmitic acid (C16:0) at 23.12 ± 0.48 and stearic acid (C18:0) at 19.30 ± 1.33. Omega-3 fatty acids contributed 0.95 ± 0.07 of the total content, with an omega-3 to omega-6 ratio of 0.46 indicating a favourable lipid

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composition in terms of human nutrition. These findings confirm that stelja derived from the Sjenica sheep represents a valuable regional product that offers not only nutritional benefits but also ecological and cultural significance. As an authentic, well-adapted, and traditionally bred animal, the Sjenica sheep deserves systematic protection and affirmation through the integration of its genetic traits, local production practices, and sustainability - serving the purpose of rural development and preserving the cultural identity of the Pešter Plateau and the region.

Keywords: Sjenica sheep, lamb stelja, fatty acids, autochthonous genetic resource, rural development

Introduction

The Pešter Plateau represents a unique area where, across approximately 1000 km², there are no industrial facilities that could harm the environment—namely, pollute water, soil, or air in any way (DANGIĆ, 1998; DRAGOVIĆ, 2004). The climate is significantly harsher and, in some aspects, more extreme than in other regions of Serbia with similar altitudes. Winters are typically long and severe, with deep snow cover, although occurrences of black frost with extremely low temperatures can also negatively affect cultivated plants, including grasses—especially young sown pastures—in terms of overwintering and productivity. The Sjenica–Pešter Plateau is well recognized in the domestic market for its traditional products. All products originating from this region bear specific characteristics shaped by local climate, soil, vegetation, and traditional production methods (e.g., Sjenica cheese, Sjenica lamb, kaymak, prosciutto, sudzuk, dried sheep meat—*stelja*, pastrma, *jardum*, etc.). Livestock production in the Pešter region is based on the traditional *katun*-style pastoralism involving cattle, sheep, and goats, characterized by extensive and semi-extensive farming systems. The Pešter Plateau is also marked by rich plant biodiversity (LAZAREVIĆ, 2014). The long-standing tradition of dried sheep meat (*stelja*) production in this area is a result of skills, knowledge, and experience passed down through generations. This product is traditionally made during the winter months, taking full advantage of the local climatic conditions. Dried sheep meat (*stelja*) is a salted and smoked meat product obtained from the deboned carcass of sheep, including associated fat and connective tissue, but excluding the shoulder and most bones (except for the shank). The traditional production of *stelja* involves the use of the autochthonous Sjenica Pramenka breed, which is raised in the Sjenica–Pešter Plateau at an altitude of 1150–1250 meters, in southwestern Serbia (43.27306 N, 19.99944 E). The animals are mainly fed on vast open pastures (Savić et al., 2014). Toward the end of summer and the beginning of winter, mostly female animals are selectively culled from the flock for various reasons—age, infertility, or

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udder damage due to mastitis—and removed from the production herd. Animals intended for slaughter must be between 1.5 and 5 years of age. During this period, selected animals are additionally fed with grains (barley, wheat, corn) to improve body condition. Only sheep meat and salt are used in the production of *stelja*. The product is obtained from the whole deboned carcass of mature sheep, with kitchen salt added as a preservative. The salting, drying, and smoking process is carried out in traditional smokehouses, where conditions (temperature, humidity, and ventilation) are influenced by the local climate. The traditional production of ovčija stelja (dried sheep meat) is closely linked to climatic conditions, as the entire process is carried out during the winter period when low temperatures play a crucial role in achieving the desired product quality. The aim of this study was to determine the fatty acid composition as one of the quality parameters of ovčija stelja produced in traditional rural households.

Material and methods

Production of Dried Sheep Meat (*Ovčija Stelja*)

Traditional **dried sheep meat** was produced from deboned meat with the addition of coarse salt. The raw material was obtained from Sjenica Pramenka sheep, with an average age of approximately 4 years. All animals were born and grazed on the Pešter Plateau. The smoking and drying process was carried out in a small traditional smokehouse, where conditions (temperature, humidity, and ventilation) were influenced by the local climate.

Sampling

After the smoking and drying process was completed, six finished products from six different producers were selected. From each product, three subsamples were taken from three different anatomical locations (shoulder, *m. longissimus dorsi*, and hind leg), which were then pooled into a single composite sample to obtain average values for better assessment of the composition and quality of **dried sheep meat**. The dimensions of the individual subsamples were 10 × 10 cm. The samples were wrapped in aluminum foil, stored at +4°C, and transported to the laboratory. Fatty acid composition was determined according to ISO standards (ISO 5509, 2000).

Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 6 (GRAPHPAD, SAN DIEGO, CA, USA). Descriptive statistics included arithmetic mean, standard deviation, and coefficient of variation.

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Results and discussion

The fatty acid composition of the traditional product dried sheep meat (Table 1) shows a notable presence of monounsaturated fatty acids (MUFA), which on average account for 48.82 ± 2.00% of total fatty acids.

Table 1: Fatty acid composition of traditional dried sheep meat (ovčija stelja)

Fatty acids	Dried sheep meat
C14:0	1.44±0.06
C15:0	0.52±0.08
C16:0	23.12±0.48
C16:1	0.86±0.13
C17:0	1.54±0.30
C18:0	19.30±1.33
C18:1trans-11	1,96±0.60
C18:1cis	46.90±1.75
C18:2cis	2.09±0.44
C20:0	0.13±0.05
C18:3n-6	-
C18:3n-3	0.69±0.34
C20:1	0.72±0.07
C20:2	0.14±0.27
C20:3n-6	0.01±0.02
C22:1+C20:4	0.10±0.03
C20:5n-3	0.02±0.02
C22:5n-3	0.10±0.03
c9,t11CLA	0.01±0.02
t10c12CLA	0.01±0.02
SFA	46.05±1.30
MUFA	48.82±2.00
PUFA	3.08±0.52
n-6	2.13±0.46
n-3	0.95±0.07
n-3/n-6	0.46±0.06
n-6/n-3	2.23±0.31

The most abundant fatty acid among them is oleic acid (C18:1cis, 46.90 ± 1.75%), which aligns with previous findings for sheep meat products (ENSER et al., 1998). A high content of MUFA is desirable as it contributes to a more favourable lipoprotein profile in consumers and plays a role in the prevention of cardiovascular diseases (WOOD et al., 2008). Saturated fatty acids (SFA) are present at 46.05 ± 1.30%, dominated by palmitic acid (C16:0, 23.12 ± 0.48%) and stearic acid (C18:0, 19.30 ± 1.33%). Stearic acid is considered metabolically neutral as it does not significantly affect serum cholesterol levels (HUNTER et al., 2010).

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Polyunsaturated fatty acids (PUFA) constitute a smaller portion of the total fatty acid profile (3.08 ± 0.52%), which is typical for ruminant adipose tissue due to the active biohydrogenation process in the rumen (JENKINS et al., 2008). The most abundant PUFA is linoleic acid (C18:2cis, 2.09 ± 0.44%). Although present in low concentrations (0.01 ± 0.02%), conjugated linoleic acids (CLA) indicate a certain functional potential of the product, given their anti-inflammatory and anticancer properties (BENJAMIN & SPENER, 2009). The n-6/n-3 fatty acid ratio (2.23 ± 0.31) is particularly important, as it falls within the recommended limits (4:1), thus achieving a nutritional balance crucial for reducing the risk of inflammatory processes and cardiovascular diseases (SIMOPOULOS, 2002). The favourable value of this ratio may result from the traditional grazing system of sheep, which contributes to a higher proportion of n-3 fatty acids (SCOLLAN et al., 2006). Overall, the results indicate that dried sheep meat has a balanced and functionally valuable fatty acid profile. The high MUFA content and favourable n-6/n-3 ratio represent important nutritional qualities that contribute to the quality and potential health benefits of this traditional product.

Conclusion and recommendation

Based on the conducted research aimed at characterizing *dried sheep meat*, it can be concluded that this product belongs to the category of traditional dry-cured meat products originating from the autochthonous Sjenica Pramenka breed from the Pešter Plateau. The preparation technique is specific to this region, which distinguishes the product from other dry-cured meats. Additionally, the production is characteristic of the artisanal sector and is primarily carried out in rural households for personal use.

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The role of the Muraközi horse and conservation options today

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Abstract

The Muraközi (Murinsulaner) horse is a traditional Hungarian breed connected to the area called Muraköz. There was bustling trade in this area in the 18th century. This required horses with good trotting ability and high load-bearing capacity. The local smallholders owned 2-4 mares. These mares could be paired with a state or licensed private stallions. According to the description of the 1883 economic newspaper, Muraközi horses could be purchased in large amount at the fairs of Szentkereszt, Szentanna, Csáktornya, Perlak and Varsad during the autumn season. In the Muraköz region, horse breeding was the only noteworthy livestock breeding activity, which was so significant that it was considered the basis of the entire economic system. The farmers here insisted on the Nori-type horses, as they were excellently suited for both agricultural and yoke work. During the chaotic period after the World War I, the Muraköz was divided with the division of the country. The statistics of the Ministry of Agriculture in September 1945 states that 58 percent of the Hungarian horse population had been lost. The changes that came in the second half of the 20th century endangered our traditional breeds, including the Muraközi horse. At the same time, there was a necessity to save traditional breeds. Recognizing the traditional genetic values, the Directorate of the Őrség National Park finally took on the task. In 2003, the regeneration of the Muraközi began. The Muraközi horse has almost disappeared by now in Slovenia, Croatia and Hungary, and is already completely extinct in Austria. Today, a significant number of horses

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representing the breed can be found in Őriszentpéter, but fortunately, private breeders are once again starting to recognize the values of this breed.

Keywords: Muraközi horse, Murinsulaner horse, breed reconstruction

The history of the Muraközi horse

The Muraközi (Murinsulaner) horse was considered an extremely sought after breed, according to contemporary records. The Muraközi horse can be associated with the region called Muraköz, located between the Drava and Mura rivers. There was bustling trade in this area in the 18th century. This required horses with good trotting ability and high load-bearing capacity. This is how a type could develop, which was bred specifically for this kind of use (MIHÓK, 2017). According to the records, both breeders and wagoner paid large sums of money for a good Muraközi and specifically sought out this type of horse. The local smallholders owned 2-4 mares. These mares could be mated with a state or licensed private stallions. The farmers could not maintain many animals on their small farmlands, this is why they were willing to sell everything, except for their good mares of course, with which they could work with and sell their foals (MONOSTORI, 1894). According to the description of the 1883 economic newspaper, Muraközi horses could be purchased in large amount at the fairs of Szentkereszt, Szentanna, Csáktornya, Perlak and Varsad during the autumn season (ÓCSAG, 1995).

Keeping and breeding the Muraközi horse

Farmers used older mares for breeding (MONOSTORI, 1894). The horse was able to perform full-fledged work at the age of 3, and was considered mature at the age of 4 (MIHÓK, 2017). Hand mating was used at the age of 2-3. The mares spent most of their time with her foal in the stable and working around the farm. The farmer did not starve his horse if he could. The stables are said to have been simple, but not crappy. They were shared with other animals, like cattle or even sheep, but were airy, roomy and clean (MONOSTORI, 1894). When it comes to appearance, the Muraközi horse never showed a unified figure. We can get a closer picture if we look at the contemporary records. There were smaller ones, with a height of around 142 centimetres at the withers, measuring with a stick, more agile horses weighing approximately 400 kilograms, and larger, slower-moving horses weighing approximately 500 kilograms, with the heights between 150 and 162 centimetres at the withers (MONOSTORI, 1894).
The Muraköz region was the centre of the breeding of this traditional Hungarian horse breed, with nearly 9,000 horses in the late 1800s. The Muraközi horses found

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here clearly descends from the Nori-type horses from Salzburg and Styria. In this region of the country, horse breeding was the only noteworthy livestock breeding activity, which was so outstanding that it was considered the basis of the entire economic system. The farmers here kept Nori-type horses, as they were excellently suited for both agricultural and yoke work (KOVÁCS-MESTERHÁZY and NÉMETH, 2023).

In these regions there was a shortage of stud stallions. Due to the high steady demand, the foals were sold, and the low-grade quality foals were used for breeding at the age of two. The county committee was forced to issue a certificate of suitability for these two-year-old stallions, otherwise there would not have had enough stallions to pair the mares with. There was a widespread belief in the region that the quality of the foal was largely determined by the mare, so a good mare would produce a good foal even with a bad stallion. This belief was a problem in breeding, despite having a positive effect on the farmers' efforts to keep good mares. During discussions with breeders it became clear that good quality sires of the Nori type were needed to boost breeding in the region. Accordingly, in 1881, the Ministry of Agriculture established 10 breeding stations in the Muraköz where 20 state-owned stallions of exclusively Nori type were accessible to breeders (KOVÁCS-MESTERHÁZY and NÉMETH, 2023).

The decline of the Muraközi horse and efforts to save it

During the chaotic period after the World War I, the Muraköz was divided with the division of the country. As a result, the future of the Muraközi horses was placed in the hands of four countries. One part was enclosed to Austria, one part to Yugoslavia (today's Slovenia and Croatia) and one part to Hungary (MIHÓK, 2017). According to the 1942 animal draft, there were 900,434 horses in Trianon Hungary. The statistics of the Ministry of Agriculture in September 1945 states that 58 percent of the Hungarian horse population had been lost, which was considered average compared to other species (Table 1). However, the descriptions paint a notably bad picture of the remaining horses. The figures also only approximately cover the grim reality. The data could primarily be determined by estimation, since the majority of farmers did not own up to their assets accurately, mainly because of the fear of confiscation (DUNAI, 2024).

Table 1: War casualties in Hungarian horse population (after DUNAI, 2024)

in 1942	in 1945
900,434 horses	58 % loss
740,000 horse tacks	148,000 horse tacks

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The continuance of every genetically important breed has a justification, since extinct breeds can no longer be resurrected and we cannot know what characteristics will be needed in the future (BODÓ, 2011). The changes that came in the second half of the 20th century endangered our traditional breeds, including the Muraközi horse. At the same time, there was a necessity to save traditional breeds from both a professional and cultural perspective. The Horse Breeding Department of the Animal Breeding Research Institute took on the task of saving the Muraközi horse. The program started in 1957 and lasted until 1972 (MIHÓK, 2017). By 1972, the results of the professional work seemed to be fruitful, as the Muraközi was recognized as an independent breed. The breed's right to exist was clearly confirmed. However, the success did not last long, the last decade of the 20th century almost completely wiped out the Muraközi, so much so that at the beginning of the 21st century it was questionable whether it could still be saved. The breeding took place within the confines of the Hungarian Cold-blooded Horse Breeding Association, but saving the breed was not among the goals. Recognizing the traditional genetic values, the Directorate of the Őrség National Park finally took on the task. In 2003, the regeneration of the Muraközi began. However, finding horses with the appropriate ancestry and appearance criteria was not an easy task. Eventually, 12 mares were selected that met the requirements of ancestry and appearance. The pairing was carried out based on a mating plan which was prepared taking into account the above criteria. The ancestry of the selected mares was traced back and then they were divided into families (KOVÁCS-MESTERHÁZY and NÉMETH, 2023). As part of the efforts to maintain the breed, the breeders contacted the Croatian breeders' association, and they also visited some Croatian breeders in 2004. A suitable mare was found, but not a suitable stallion, since the Croatian breeding goal was meat production. In 2005, a visit was also made to the Dobsina stud farm in Slovakia, where the breed called the Murányi horse has been bred for forestry work since the 1960s. The breed was developed by pairing local mares with Noriker stallions, but it would not have improved the Hungarian Muraközi stock in terms of appearance, so breeding stock were not imported (KOVÁCS, 2008).

Can the Muraközi breed be saved?

With the loss of our traditional breeds, our self-esteem would also be lost, and our almost unprecedented register in Europe, which we have been keeping for almost 200 years, would also come to nothing (MIHÓK, 2008). Recently, the efficiency of selection programs for different breeds and their increase have received more attention. Thanks to this, the use of molecular genetic methods has also become more frequent (CSIZMÁR et al., 2017). The preservation of the gene pool, which aims to preserve an endangered (small) population in an unchanged form, is a special area of animal breeding. Saving gene reserves is not economical in the short term, but

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there are cultural and technical arguments in favour of it. One such argument is that our domestic animal breeds are the results of human work, similar to our buildings. From a professional perspective, we must take into account that we cannot know what humanity's needs will be in the near or even distant future. It is possible that methods of exertion that are now considered long lost or obsolete will come forth again. Good adaptability to extreme conditions may be needed or specific traits may be necessary in crossbreeding (BODÓ, 2011). In phylogenetic studies, the examination of mitochondrial DNA has a great importance in mammals, with which it was established, for example, that domesticated horses can be traced back to several maternal founders. Genetic markers can be used not only for pedigree verification but also enable the comparison of populations. Such a mitochondrial DNA examination was conducted, for example, on mares of the Hungarian cold-blooded breed (CSIZMÁR et al., 2017) or reconstructed stock of the Szekler horse breed (GÁSPÁRDY et al., 2023).

Pedigree analysis or genetic marker studies make it possible to learn about the genetic composition and background of populations based on pedigree data. By using indicators calculated with pedigree analysis, inbreeding can be kept at a low level in livestock (KLEIN et al., 2022). It is of great importance to know the genetic structure of populations as accurately as possible. This is also vital in the case of breeds with small populations in a situation similar to the Muraközi (KLEIN et al., 2022). A small population can be associated with the risk of losing genetic diversity. The main task of gene conservation is to save genetic diversity among livestock. The FAO (Food and Agriculture Organization) classifies breeds into categories according to the degree of endangerment, depending on whether the population size is increasing, decreasing or remaining unchanged (SCHERF, 2000). The decrease in genetic diversity is closely related to the effective population size, which is used to design gene conservation strategies in gene conservation (VOSTRÁ-VYDROVÁ et al., 2016). The effective population size expresses the population size that must be taken into account during inbreeding. The effective population size corrects the number of males and females in breeding to a 1:1 sex ratio, random mating (BODÓ, 2011).

Genetic diversity is crucial for adaptation and response to selection. Its reduction is associated with reduced individual fitness and, in the long term, has a negative impact on the longevity of the population. Pedigree analysis is an effective way to determine genetic diversity, especially in breeds with small populations where more expensive technology is not available. The estimates can be used to optimize breeding methods (ABLONDI et al., 2018). However, the challenges of modern times have brought changes that have resulted in a decrease in the number of horses and may lead to genetic erosion. To avoid this, the genetic diversity of horses must be preserved (LIU et al., 2024).

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The current situation of the Muraközi horse

The Muraközi horse has almost disappeared by now in Slovenia, Croatia and Hungary, and is already completely extinct in Austria (POTOČNIK et al., 2011). Efforts to save the Muraközi horse have shown significant results after 14 years. In 2016, the Muraközi research program was launched in Őriszentpéter, with the clear goal of making the Muraközi an independent breed. By then, the initial population in 2003 had greatly increased, which gave room for selection. The success of the program is based on increasing the number of mares owned by private breeders (Table 2), which, despite the difficulties, shows very positive results (KOVÁCS-MESTERHÁZY and NÉMETH, 2023).

Table 2: Development of the number of association members and number of animals between 2018-2023 (after KOVÁCS-VÁRHELYI, 2024; n = number of animals)

Year	Association member	Horse owner's member	Broodmare (n)	Stallion (n)	Foal (n)
2018	17	11	46	7	21
2019	23	13	46	5	22
2020	50	49	76	7	21
2021	76	62	115	15	49
2022	90	75	128	16	74
2023	100	81	133	17	61

Conclusion and recommendation

Based on the literature review, we can say that the Muraközi horse was very popular in its time. The breed associated with the Mura region was ideal for hauliers. Their breeders held their horses in high esteem, putting the needs of their mares above all else. The Muraközi horse was almost completely lost in the storm of history. The realization that our traditional breeds are needed and must be protected has given the Muraközi horse a chance to regain its strength. Unfortunately, at the national level, experts pay very little attention to saving the Muraközi. Today, a significant number of horses representing the breed can be found in Őriszentpéter, but fortunately, private breeders are once again starting to recognize the values of this breed. These private breeders are brought together by the Muraközi Horse Breeding Association, established in 2018, which aims to carry out a breed reconstruction program to reconstruct the Muraközi horse, register the stock, register certificate, administer breed maintenance and performance testing tasks, and also works to popularize the breed even more widely. To this end, the association organizes various events, breeding reviews, professional days, exhibitions, publishes publications and books. Such events include the Muraközi Horse Day for school groups and the Muraközi

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Professional Day. In the Őrség National Park, visiting the Muraközi stud farm, which is unique in our country, or taking a horse-drawn carriage ride with Muraközi horses, has become a tourist destination. Since we see that the number of horses and breeders is continuously increasing, we can rightly conclude that it will continue to hold its own in today's modern world.

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Description of the Hungarian Nonius horse population based on pedigree information

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Abstract

Maintaining genetic diversity is very important during gene preservation. The aim of the present study was to analyse the pedigree information of Hungarian Nonius horses. The base pedigree information was given by the Nonius Horse Breeding Association. The total pedigree file contained the data of 30,826 animals. Various measures of genetic variability were calculated. The dataset was analysed using the POPREP software.
The mare "272 Nonius XL-68 Dorka" and the stallion "3665 Nonius XVII-30 (IV tm.)" appeared with the most offspring in the dataset. There was only one youngster after the 75% of the mares and 54% of the stallions in our dataset. The most offspring selected as breeding animals was found for the Nonius mare "47 Nonius Ürmény VI (Idill)" and stallion "1814 Nonius VI-24 (XVII tm.)", respectively.

Keywords: pedigree analysis, Nonius horse, offspring analysis

Introduction

The human-horse relationship is six thousand years old. From the beginning of human history to the first half of the 20th century the horses were indispensable for the armies. The Nonius breed originated from The Mezőhegyes Stud which was founded in 1784 by Joseph II. Holy Roman Emperor. The breeding aim of the Nonius breed was originally to produce draft and driving horses especially for military purposes (SZ. BOZSIK, 1985). Nowadays the utilization of horses has been changed.

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Horses not important for the military, due to the motorization horses were lose ground in the agriculture, public and personal transport. They became from farm animal to a popular pet animal (BODÓ and HECKER, 2013). The Hungarian indigenous horse breeding was influenced by a lot of things in the past centuries. Their breeding aims were changed from the military purposes to a hobby usage that changed them characterize too. The 32/2004. (IV. 19.) decision of parliament were declared Nonius horse breed – with other Hungarian indigenous breeds as well – to national treasure.

Additionally, the study of the population structure and demography can highlight important circumstances affecting the genetic history of the population. (POSTA – MIHÓK, 2015)

The main goal of breed conservation is to prevent genetic loss and maintain both genetic and phenotypic diversity, essentially aiming to minimize inbreeding. However, for small, closed populations such as the Nonius, complete avoidance of inbreeding is unrealistic due to limited possibilities for immigration. Therefore, breeding strategies should focus on managing and slowing the rate of inbreeding to preserve genetic diversity at an acceptable level (MIHÓK, 2023) The sustainable preservation of small breeds requires population growth to improve selection opportunities for desirable breeding animals in terms of utility, type, and genetic value. Effective selection should align with both the breed's original purpose and the preservation of genetic diversity. In situ gene conservation focuses on maintaining and integrating existing populations of breeds whose traditional functions have declined, adapting them to contemporary value systems (MIHÓK, 2023).

Material and methods

The base pedigree information was given by the Hungarian Nonius Breeding Association. The basis of the current study was the Hungarian breeding population of the Nonius Horse breed in 2019. The active population was 521 horses that were chosen as a reference when needed. There were the pedigree data of 30826 animals in the developed database.

The population was described by the number of progenies and the number of selected progenies. All measurements were calculated using the POPREP software package (GROENEVELD et al., 2009).

Results and discussion

The preservation of genetic diversity is a key objective in conservation breeding programs. To ensure the maintenance of genetic variability across generations, the number of selected offspring per individual is an important parameter. Ideally, every

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animal would have the opportunity to contribute genetically to the next generation. However, in real populations, this balance is disrupted by selection pressure, economic limitations, and other factors, leading to substantial variation in the number of progeny per breeding animal. From a conservation perspective, the optimal approach would involve each broodmare producing four selected female offspring, thereby ensuring the preservation of the maternal genotype. In practice, however, this model is rarely achievable due to various constraints affecting all breeds.

The 30 breeding stallions having the most selected progeny in the analyzed whole database was presented in Figure 1. There were 13 stallions in the whole population having more than 50 selected progenies and 3 having more than 80. Nonius XVII (1814 Nonius VI-24) breeding stallion had 104 selected offspring. The Nonius IV (3665 Nonius XVII-30) breeding stallion in the third position had the most selected foals in the active population. Due to the breeding method of the Nonius breed, 18 English Thoroughbreds were founded among stallions having the most selected progenies. There was some high-impact stallion among these horses like St. Simon xx, Hermit xx, Herod xx, and Galopin xx. The other 12 horses were Nonius. Moreover, eleven of them were breeding stallions.

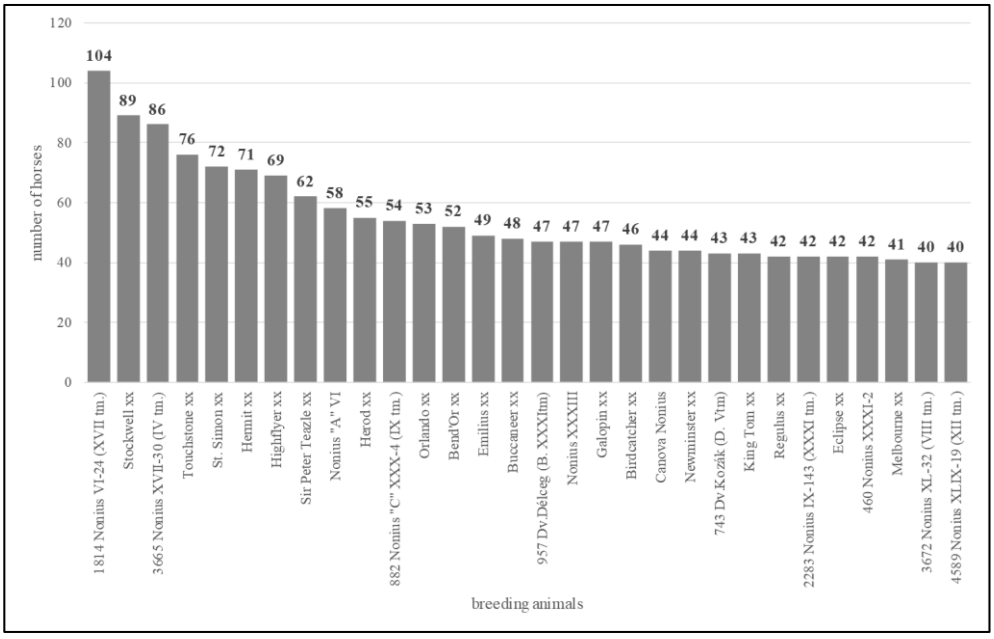

Figure 1: Stallions having the most selected progeny

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The number of selected foals per sire was illustrated on Figure 2. The 54% of stallions so more than 3000 individuals had just 1 selected foal in the herd book, and another 17% had just two foals. Approximately 6% had more than 15 selected progenies. Just a few of these stallions had 20 and more selected offspring. The maximum number of selected progeny was 104, as you could see in the Figure 1.

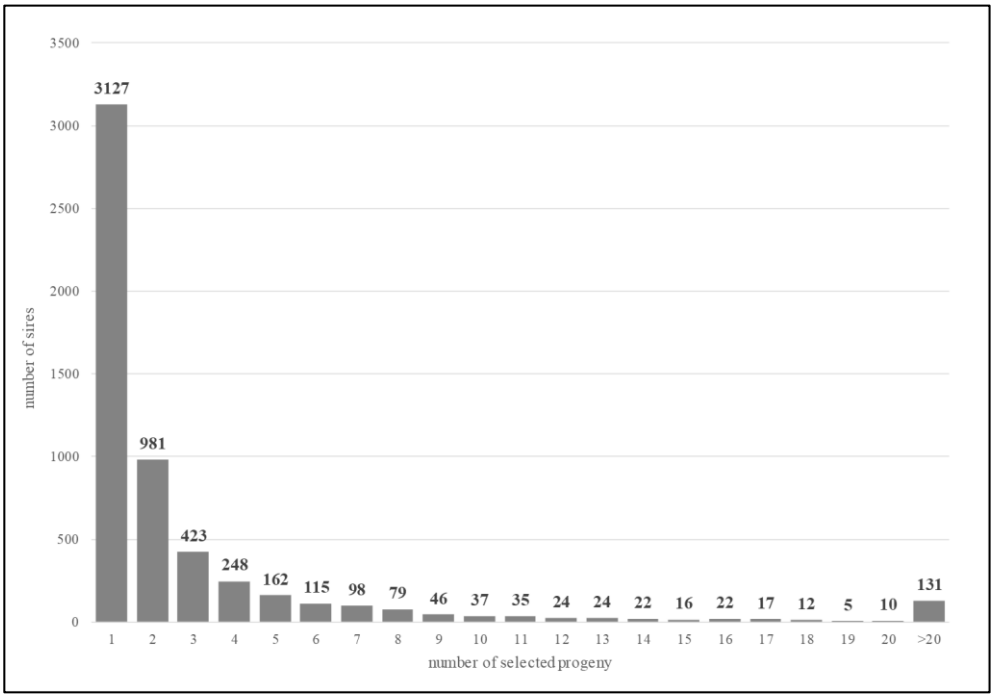

Figure 2: Number of Selected Progeny per Sire

The 30 broodmares having the most selected progeny in the analyzed whole database were presented in Figure 3. In the top 30, every broodmare had at least 6 selected offspring. There were 7 broodmares in the whole population having more than 8 registered progenies. The English Thoroughbred dam Queen Mary xx had the most selected progeny. In the Nonius breed, 47 Nonius Ürmény 6 (Idill) had 8 selected foals. In the active breeding stock 284 Akitos Nonius-55 (Gizike) had the most selected progenies. You can see it easily there were 12 English Thoroughbreds among these horses.

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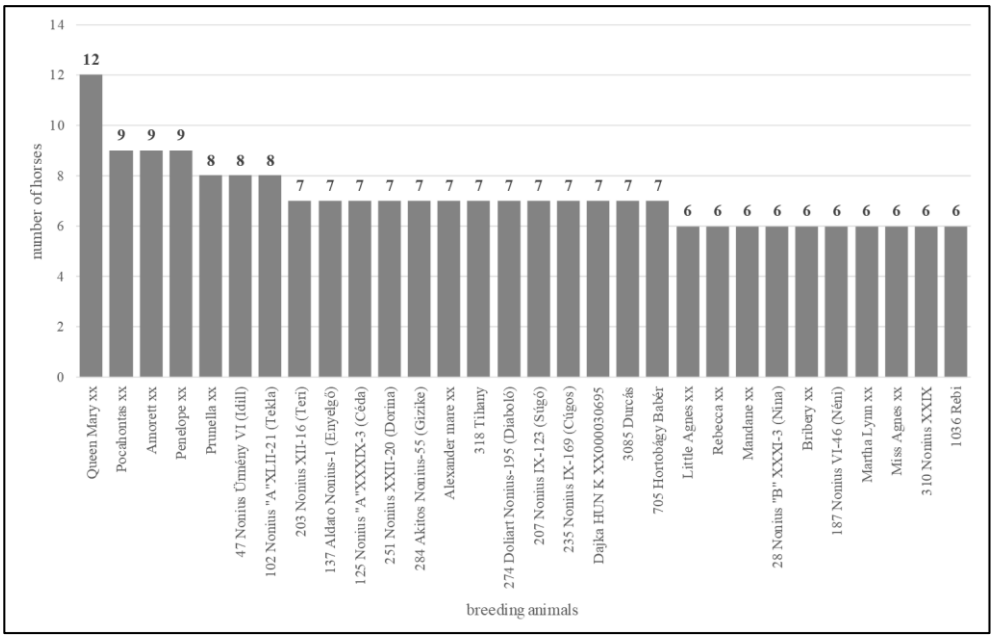

Figure 3: Dams having the most selected progeny

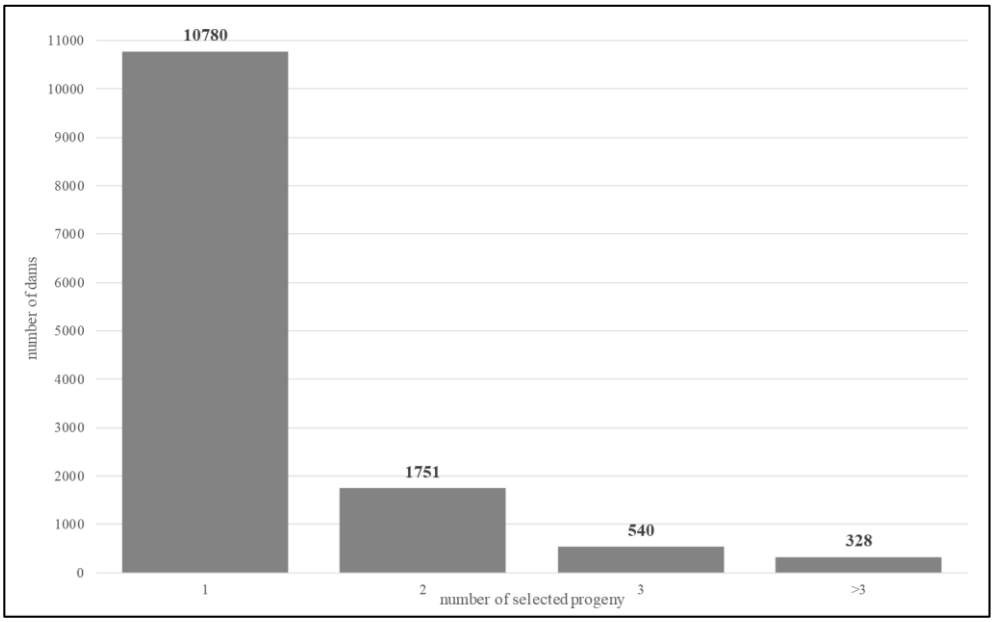

Figure 4: Number of Selected Progeny per Dam

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Figure 4. presents the number of selected foals per dam. More than 80% of broodmares had just 1 selected progeny. Just a few broodmares had 3 and more selected offspring. These 328 horses were nearly 2,5 %. 13% of the broodmares had 2 selected foals.

Conclusion and recommendation

The results of the pedigree analysis demonstrated that the genetic contribution within the Hungarian Nonius horse population is highly unbalanced. A limited number of stallions and mares produced a disproportionately high number of selected offspring, resulting in unequal genetic representation and a potential reduction in the effective population size. Such patterns may accelerate the loss of genetic variability, thereby posing a risk to the long-term sustainability of the breed.
To maintain genetic diversity and ensure the stability of the Nonius population, breeding programs should aim to increase the number of individuals contributing genetically to subsequent generations. Systematic monitoring of pedigree information and the limitation of overrepresentation by highly used stallions are strongly recommended. The inclusion of a broader genetic base in breeding decisions and the application of optimal contribution selection could further support the conservation and sustainable utilization of this indigenous Hungarian horse breed.

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The functioning of the cryobank and the in situ facilities of the indigenous breeds of cattle of the Carpathians is important to science and practice

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Abstract

Climatic conditions and human activity negatively affect the number of native species and mountain meadow ecosystems of the Carpathian region countries due to their scale and unprecedented speed of development. The article describes the main specific features and advantages of local breeds. Their multifunctionality is indicated. The results of the study of the distribution are presented, the current state is revealed, limited numbers, threats of extinction are identified. Attention is drawn to their participation in the restoration of anthropogenically disturbed meadow ecosystems, an important sector for the preservation of genetic biodiversity. Attention is focused on and the feasibility of creating the Carpathian Scientific and Production Centre at the Research Institute of Animal Science, Balice, Poland is scientifically substantiated.

Keywords: meadow high-mountain ecosystems, native breeds, Carpathian Centre, Biodiversity

Introduction

Carpathian Biosphere Reserve
An indicator of the health of natural pasture and meadow ecosystems of the Carpathians is their biodiversity, including indigenous cattle and the processes occurring in them. Energy-saving, ecological technologies, long-term use of these animals ensure the sustainable development of local communities and high-quality food products for the population. For example, 100 g of cheese satisfies the daily human need for proteins by 25-40%, need for fat – by 25-30%.

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The purpose of the work was to highlight the prerequisites for the establishment of the Carpathian Scientific-Production Centre for the development of the potential, research and conservation, management and development of indigenous cattle breeds and natural pasture-meadow ecosystems of the countries of the Carpathian region.

Material and methods

The current state of local cattle breeds was determined by us based on the results of previous research. The analysis was based on the Convention on Biological Diversity and the Report on the State of the World's Animal Genetic Resources for Food Production and Agriculture (FAO, 2015). Mountain meadows are included in the habitats of Resolution 4 of the Bern Convention (E2.3) and Annex 1 of the Habitats Directive of the European Union (6520) (cit. NATIONAL HABITAT CATALOGUE OF UKRAINE, 2018).

Results and discussion

A study of the status of populations of local cattle breeds in the Carpathians showed the presence of 1 in the Czech Republic, 1 in Hungary, 2 in Poland, 1 in Romania, 1 in Slovakia, 2 in Serbia, 1 in Ukraine, 9 in total. There is instability, a systematic decline in the breeding stock to 451,891 heads, being a genetic reservoir. Environmental conditions, selection, feeding and maintenance have significantly influenced the exterior of these animals. They belong to the mid-season, combined type, are compact, present good phenotypic and productive qualities. They have strong limbs, hooves and well-developed chest, a proportional body structure, adapted to local ecological and feed conditions. They effectively use pasture, green and dry grasses of natural meadows and pastures. They produce milk of very good chemical composition with a special variant of β-casein A2 and the highest cholesterol content compared to Holstein and other factory breeds. On rich green fodder, bulls rapidly increase their live weight, more than 1000 g per day. Milk and meat have excellent taste and technological qualities. At the same time, due to the anomalous reduction in livestock, only 2.5-6.5% of 200,000 hectares of natural meadows and pastures are used in each of the two regions: Podkarpacie, Poland, and Transcarpathia, Ukraine. As studies show in traditional Polonyna farming using rotational grazing, the plant groups of the mentioned agricultural lands, especially high-mountainous ones, are saturated with a variety of forage grasses. After the cessation of livestock grazing, the change of meadow phytocenoses occurs after 4-5 years. Thickets of uncharacteristic species of horse sorrel, ferns, shrubs and trees

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appear. The representative of the Ministry of Agriculture of Austria-Hungary, Ede Egan (EGAN, 2010), studying the natural pasture and meadow ecosystems of the Carpathians back in 1890, pointed out their high value for livestock breeding, which arose on the peaks naturally, on the lower altitude zones – as a result of diverse centuries-old human activity.

The most critical situation is with the Braunvieh (Bruno-Schwyz breed in Romania - 3000 cows), Polish Red breed (Poland - 2399 cows), Pinzgau (Slovakia - 3412 cows), Carpathian Brown breed (Ukraine - 24,051 cows). In some countries there are no sperm cryobanks, lack of system for obtaining, growing and evaluating of bulls-breeders, gene pool herds.
It has been established that only by long-term storage of bull semen, oocytes, DNA samples and embryos the problem of preserving the gene pool of breeds in the Carpathian region is extremely difficult to solve. There is a need to breed populations of female and male individuals, *in situ* conservation. DRAGANESCU (1975) proposed the minimum-optimal size of the reserve native gene pool population for each cattle breed of 10 bulls and 50 cows. All this together becomes a prerequisite for the creation on the basis of the scientific-research Institute of Animal Science in Balice, Poland of a powerful scientific-research and economic structure – the Carpathian Scientific-Production Centre for the development of the potential, research, conservation, use, management and development of aboriginal cattle breeds and natural meadow-pasture ecosystems of the countries of the region, which will serve as a laboratory and will carry out the development and practical implementation of:

- Provisions of the Convention on Biological Diversity, Report on the State of the World's Animal Genetic Resources for Food Production and Agriculture of the FAO, the Framework Convention on the Conservation and Sustainable Development of the Carpathians and its Protocols, On the Conservation and sustainable use of biological and landscape diversity and other documents.
- Regulatory and legal documents, provisions of a systemic and ecological approach to the modernization of production in order to reveal the potential, research, preservation, use, management and development of indigenous cattle breeds and the environment. Conducting a full inventory of indigenous cattle breeds and natural grassland ecosystems of the Carpathians, monitoring the progress of processes and changes that will occur.
- Will generate and implement innovative ideas in production to increase the safety of manufactured products
- Will develop and popularize strategies for the sustainable development of local communities and the region as a whole, using available resources – indigenous breeds and natural grassland ecosystems and unique traditional mountain farming. Target standards for the main exterior features and

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desired types of animals, breeding plans. Will solve the issues of individual selection, centralized reproduction of bulls and their assessment, the formation of the genetic structure of breeds, the creation of necessary reserves, the preservation and targeted use of cryopreserved sperm, the functioning of a virtual gene pool cryo-herd and a transboundary *in situ* facility of indigenous cattle breeds of the Carpathians.

- Will create effective monitoring systems that will make it possible to receive constant information about the ongoing multifaceted processes in populations of indigenous cattle breeds and natural meadow and pasture ecosystems with the prompt identification of threats and, based on the analysis, the adoption of preventive measures to eliminate them.
- Will attract scientists and scientific potential, European investments and grant projects.
- Will create an automated information system on breeding, which will be connected to the global biodiversity information system. Prepare and publish catalogues and studbooks of animals.
- Conduct a systematic assessment of animals according to the main economic characteristics and the degree of realization of their genetic potential in the conditions of interaction "genotype - environment" with current global and regional climate changes. Genetic testing of each breed. Consulting and advisory activities, implementation of scientific developments. International scientific and practical conferences.

Thus, the creation of a centre at the named institution in Poland is advisable due to the presence of complexes of instruments and equipment that allow conducting in-depth research at the molecular, organismal and population levels. It will be a modern material and technical base for creating collection gene pool herds in Krasny (Poland), there is a station for growing, evaluating bulls-breeders, cryopreservation of sperm and artificial insemination.

Conclusion and recommendation

The Carpathian Scientific and Production Centre for the development of the potential, research and conservation, management and development of indigenous cattle breeds and natural pasture-meadow ecosystems of the countries of the Carpathian region will radically change approaches to livestock breeding models, using local resources and environmentally friendly methods that will improve the protective, health-improving, socio-economic attractiveness of the territories. It will conduct fundamental and applied research, generate ideas, projects in the field of biodiversity conservation and natural meadow-pasture ecosystems, and implement

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them for the sustainable development of the Carpathians. It will solve priority problems of humanity.

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In memoriam Prof. Dr. Sc. Pavo Caput (1935 – 2024)

Pavo Caput was born on December 21, 1935, in the village of Dubravca, in the Konavle region near Dubrovnik. He completed primary school in Gruda and graduated from the Teacher Training School in Dubrovnik in 1953. He then worked as a teacher and headmaster at elementary schools in Vitaljina, Pridvorje, and Dubravci, all situated in the Konavle area.

Later, he worked as a research associate at the Institute of Animal and Dairy Science, University of Zagreb Faculty of Agriculture, during which time he completed a specialized course on the application of radioisotopes in animal science organized by the Federal Nuclear Commission in Belgrade. Between 1964 and 1968, he gained hands-on experience in animal production while working at the Agricultural Station in Bjelovar and at the agricultural enterprise PIK "Moslavina" in Kutina. From 1968 to 1982, he was employed at the Livestock Breeding and Selection Centre in Zagreb, where he served as a technical officer, advisor, and later as the head of the institution, focusing on livestock breeding and selection across Croatia. He earned his master's degree with the thesis *"The Influence of Cow Age on Milk Production and the Accuracy of Breeding Value Estimation"* (1979), and his doctoral degree with the dissertation *"The Genetic Composition and Phenotype of the Modern Simmental Cattle in Croatia"* (1982).

In 1982, he joined the University of Zagreb Faculty of Agriculture, where he taught courses in Cattle Breeding, Horse Breeding, and several related subjects until his retirement in 2006. During his academic career, he advanced to the rank of Scientific Advisor and Full Professor with tenure. He supervised numerous undergraduate theses, 12 master's theses, and four doctoral dissertations. He also served as Vice-Dean for Science at the Faculty of Agriculture and as a member of the Governing Board of the University of Zagreb. Between 1996 and 1997, he held the position of Deputy Minister of Agriculture and Forestry of the Republic of Croatia.

His primary scientific interests were animal breeding and selection, as well as the conservation and revitalization of autochthonous breeds. He led several scientific and applied research projects and published, independently and in co-authorship, around 170 scientific and professional papers indexed in international databases and national journals. He was the author of three university textbooks — *Animal Husbandry* (Školska knjiga, 1991), *Cattle Breeding* (Celeber, 1996), and *Conservation of Biological Heritage in Animal Husbandry* (Croatian Dairy

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Association, 2010) — and several professional books, including *The Path of Milk* (HAD, 2000), *Agricultural Advisor* (Znanje, 1985), and *Dubrovnik Cheese* (Celeber, 2003). He served as Editor-in-Chief of the journal *Stočarstvo* from 1990 to 2004 and was a member of the editorial boards of *Agronomski glasnik*, *Poljoprivredna znanstvena smotra*, *Mljekarstvo*, and *Praxis Veterinaria*.

During the 1990s, he chaired the Animal Science Section of the Croatian Academy of Sciences and Arts (HAZ) and served as Vice-President of the regional association *Danubian Countries Alliance for Conservation of Genes in Animal Species (DAGENE,* Budapest) between 1998 and 2010. He chaired the organizing committees of several scientific conferences (DAGENE 1990, Buzet; DAGENE 1994, Zagreb) and co-organized and moderated numerous national and international meetings (EAAP, FAO).

He undertook scientific and professional training and study visits in the United Kingdom, the United States, Canada, Norway, the Netherlands, Denmark, Germany, Switzerland, France, Portugal, Italy, Austria, Hungary, Czech Republic, Slovakia, Russia, Ukraine, Romania, Egypt, and Australia.

For his exceptional contributions to science, he was awarded the *Order of Danica Hrvatska with the image of Ruđer Bošković* by the President of the Republic of Croatia (1996) and received a Certificate of Appreciation from the University of Zagreb Faculty of Agriculture, for his long-standing teaching and research work (1995).

He considered his most significant professional achievements to be the education of numerous generations of animal science students, the development of national breeding programs in cattle and horse breeding, and his dedicated advocacy for the conservation and revitalization of endangered indigenous breeds of domestic animals.

He passed away at the age of 89 in 2024. May he rest in peace.

Dr. András Gáspárdy
President of DAGENE