



DAGENE 2025

*International Association for the Conservation of
Animal Breeds in the Danubian Region*

BOOK OF ABSTRACTS

*34th Annual Meeting of the
International Association for the Conservation of Animal Breeds
in the Danube Region (DAGENE),*

*Novi Sad, at University of Novi Sad, Faculty of Agriculture,
Department of Animal Science, Republic Serbia,
17–19 September 2025*

BOOK OF ABSTRACTS

34th Annual Meeting of the International Association for the Conservation of Animal Breeds in the Danubian Region (DAGENE)

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**Novi Sad, at University of Novi Sad, Faculty of Agriculture,
Department of Animal Science, Republic Serbia, 17–19 September 2025**

Editors: Prof. dr Saša Dragin and MSc Jovana Grba Ilić-Faculty of Agriculture, University of Novi Sad

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Dear Participants and Colleagues,

We are very pleased to welcome you to the 34th Annual Meeting of the International Association for the Conservation of Animal Breeds in the Danube Region, "DAGENE 2025," from September 17. to 19. 2025., in Novi Sad, Republic of Serbia. The conference is organized by the Department of Animal Science at the Faculty of Agriculture, University of Novi Sad, with the co-operation of the "DAGENE" Association, Republic of Hungary.

The aim of the conference is the presentation of current research results and legislative information regarding animal biodiversity with an actual point on modern scientific methods in service to preserve the genetics and variety of autochthonous and rare traditional animal breeds in modern production environment. This conference will provide an opportunity to gather scientists and academics engaged in this field of research to exchange their skill and experience as well as to establish potential collaboration in the given points at issue. Organizers appreciating the participation of colleagues from various research institutions and universities at this conference.

We would like to wish you a nice atmosphere for the presentation and creative discussion and stimulating ideas during your stay in Novi Sad.

Special thanks go to “Salon u Radničkoj” for providing its facilities without financial demands so that the conference can be held in its beautiful atmosphere.

Prof. dr Saša Dragin

President of the Scientific Committee
and

Asst. Prof. Dejan Beuković

President of the Organizing Committee

Novi Sad, September 17th, 2025.

DAGENE 2025 – Scientific Program
International Association for the Conservation of Animal Breeds in the Danube
Region 17–19 September 2025 Novi Sad, Serbia

Time	Details
17 September	Location: „Salon u Radničkoj“
18:00–20:00	Welcome Cocktail (Registration & Networking)
18 September	Location: „Salon u Radničkoj“
08:00–08:45	Registration & Coffee/Tea
08:45–09:00	Opening of Symposium: Chairperson: Habil. Prof. dr András Gáspárdy
09:00–12:30	Plenary Session Chairpersons: Asst. Prof. Dejan Beuković, Prof. dr Peter Chrenek
12:30–13:00	Coffee Break
13:00–15:00	Plenary Session Chairperson: Prof. dr Saša Dragin
15:00–16:30	Lunch Break
16:30–17:30	Poster Session Chairperson: Habil. Prof. János Posta
18:30–21:00	Dinner at Restaurant „Macchiato Fax“
19 September	Organized trip to Special Nature Reserve "Zasavica"
08:00–17:00	(guided tour and lecture)

CONTENTS

Chrenek Peter, Olexikova Lucia, Makarevich Alexander, Curlej Jozef, Vasicek Jaromir, Balazi Andrej, Kuzelova Lenka, Vozaf Jakub: Conserving Cattle Biodiversity: „Population Analysis, Cryopreservation and Genetic Resource Banks for Pinzgauer and Slovak Spotted Breeds“	1-2
Gáspárdy András, Kaltenekker Endre, Borics Imre : Generation Interval Determined in the Case of Hungarian Grey Cattle Progenies Born from Revitalized “Archive Sires”	3
Jovana Grba Ilic, Sasa Dragin, Peter Chrenek, Alexander Makarevich: Investigation of Cryopreservation-Induced Damage in Ram Spermatozoa Using Fluorescent Markers	4
Kovačević Sara, Dimitrijević Vladimir, Gáspárdy András, Savić Mila, Trailović Ružica, Tarić Elmin, Čobanović Nikola, Dominiković Nina, Paskaš Snežana, Rašeta Mladen, Cekić Bogdan, Becskei Zsolt: Morphometric Analysis and Sex Dimorphism of the Autochthonous Lipe Sheep	5
Elmin Taric, Zsolt Becskei, Nina Dominikovic, Branislav Vejnovic, Sara Kovacevic, Jelena Janjic, Bogdan Cekić, Mladen Raseta, Snežana Paskas, Mila Savic, Vladimir Dimitrijevic: Strengthening the Sustainability of Sjenica Sheep	6
Žan Metka, Bojkovski Danijela, Flisar Tina: Financial Support Measures fof Endangered Local (Autochthonous) Breeds in Slovenia	7
Kovacs Máté, Gyúró Daniella, Posta János: The Lipizzan Horse and its Fertility Characteristics in Hungary	8
Vašíček Jaromír, Svoradová Andrea, Baláži Andrej, Vozaf Jakub, Olexiková Lucia, Chrenek Peter: Magnetic Nanopurification of Ram Spermatozoa Using Annexin V and Lectin-coated Nanoparticles to Improve Sperm Viability and Motility	9
Ivan Vlahek, Jasna Kusanović, Velimir Sušić, Anamaria Ekert Kabalin, Sven Menčik, Aneta Piplica, Niko Popović, Maja Maurić Maljković: Exterior and Production Characteristics of Brač Sheep	10

Aneta Piplica, Maja Maurić Maljković, Anamaria Ekert Kabalin, Velimir Sušić, Ivan Vlahek, Niko Popović, Sven Menčik: Characterization of Reproductive Traits in the Buša Cattle Breed: Implications for Conservation and Productivity	11
Niko Popović, Maja Maurić Maljković, Ivan Vlahek, Anamaria Ekert Kabalin, Sven Menčik, Velimir Sušić, Aneta Piplica: Buša-an Indigenous Cattle Breed in the Cow-calf Production System	12
Maja Maurić Maljković, Aneta Piplica, Niko Popović, Anamaria Ekert Kabalin, Sven Menčik, Velimir Sušić, Ivan Vlahek: Indigenous Donkey Population in Croatia – Current Situation and Trends	13
Anamaria Ekert Kabalin, Maja Maurić Maljković, Ivan Vlahek, Maja Črnjević Diklić, Bojan Matković, Velimir Sušić, Aneta Piplica, Niko Popović, Sven Menčik: Morphological Measurements of Dogs: Practical Approach to Standardization of Autochthonous Breeds	14
Dominiković Nina, Becskei Zsolt, Tarić Elmin, Kovačević Sara, Savić, Mila – Dimitrijević, Vladimir: The Water Buffalo (<i>Bubalus bubalis</i>) as an Autochthonous Genetic and Dairy Resource in Republic of Serbia	15
Anna Mária Vásárhelyi, András Lévai, András Gáspárdy: Breed History and Current State of Milking Tsigai Sheep in Hungary	16
Alexander Atanasoff, Galin Nikolov, Dimitar Spasov: The black gold of the Danube River. Sturgeons in Bulgaria: life history, present status and future challenges	17
Jelena Ramljak, Branko Kovačić, Sanja Jakrlin, Josip Trogrlić, Nikica Prvanović-Babić: Current Trends and Future Perspectives in Gidran Horse Breeding in Croatia	18
Renáta Klein, János Oláh, János Posta: Partial Correlations Between Various Inbreeding Coefficients of Horse Breeds Developed in Mezőhegyes	19
Bácsi Eszter Ilona, Klein Renáta, Egerszegi István, Bodnár Ákos, Oláh János: Changes in Production Parameters of Indigenous Tsigai	20
Éva Váradi Kissné, Árpád Drobnyák, Zsuzsa Szabó, Éva Török, Barbara Végi: Cryopreservation Studies to Establish a Sperm Bank of Hungarian Giant Rabbit	21

Barbara Végi, Eszter Várkonyi, Éva Váradi, Árpád Drobnyák, Kitti Buda, Bence Lázár, Nóra, Pálincás-Bodzsár, Csilla Eiben, Krisztina Liptói: Slovak - Hungarian Interreg Project from the Perspective of the Hungarian Partner	22
Terpai Vasyli: Indigenous Carpathian Cattle Breeds – the Basis for the Scientific and Trans-boundary Aspects of the Implementation of the “Carpathian Convention” and Its Protocols	23
Árpád Drobnyák, Éva Kissné Váradi, Zsuzsa Szabó, Éva Török, Tímea Rácz, Tamás Sipos, Edit Zajách, Barbara Végi: Sperm Freezing Studies in Honey Bee (<i>Apis mellifera carnica</i>)	24
András Gáspárdy, András Lévai: Pedigree Based Analysis of Population Structure in Hungarian Sheep (Racka Sheep)	25

Conference facts:

Number of participants: 28

Number of countries represented: 8

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Number of countries represented in List of Reviewers: 8

Number of presented scientific papers: 24 (4 poster presentation)

Additional Conference information contacts: sasa.dragin@stocarstvo.edu.rs
jovana.grba@stocarstvo.edu.rs
gaspardy.andras@univet.hu

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Conserving Cattle Biodiversity: „Population Analysis, Cryopreservation and Genetic Resource Banks for Pinzgauer and Slovak Spotted Breeds “

Chrenek Peter^{1,2}, Olexikova Lucia², Makarevich Alexander², Curlej Jozef¹, Vasicek Jaromir^{1,2},
Balazi Andrej², Kuzelova Lenka¹, Vozaf Jakub.^{1,2} (*invited paper*)

¹ *Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Science, Slovakia;*

²*NPCC - Research Institute for Animal Production Nitra, Slovakia;*

Abstract

Ratification of the Convention on Biological Diversity in 1992 obliges the Slovak Republic to protect biodiversity, guarantee the sustainable use of its components, and ensure fair and equitable access to the benefits arising from genetic resources. The availability of animal genetic resources has an impact on present and future quality of life, as well as an important effect on food security. Data for the regular updating of the European Farm Animal Biodiversity Information System (EFABIS) revealed different trends across species. In cattle, a decrease in dairy breeds was observed, while an increasing trend was recorded in Slovak Spotted cattle and beef breeds. In sheep, a rapid decrease in the population size of traditional breeds was noted. Goat populations of traditional breeds increased. Numbers of pigs, horses, poultry, and rabbit breeds were more or less stabilized. In many cases, the preference of farmers for a single breed and their personal dedication are the only reasons for maintaining certain populations. In this regard, raising awareness is a very important part of animal genetic resources conservation. The number of cattle, particularly the Pinzgau breed, has decreased significantly over the past 30 years. In 1990, more than 1.5 million cattle were bred in Slovakia, of which 550,000 were cows; in 2020, the total dropped to 442,000, including 191,000 cows. During the same period, the number of Slovak Spotted cattle decreased from 385,000 to 162,000, and Slovak Pinzgau cattle from 70,000 to 10,000. The Slovak Pinzgau cattle originated from crossing the original red and Carpathian cattle with Pinzgau cattle imported from Austria. It is a modest, healthy, and hardy breed with good walking ability, suitable for sub-mountain and mountain areas. A characteristic trait of the breed is its coloring: chestnut to cherry-red across the entire body, including the head. Breeding of the Pinzgau breed dates from 1870 to 1880, as evidenced by written records of its expansion in the northernmost counties of old Hungary. The breed became more clearly established in the late 19th and early 20th centuries. In 1994, the Pinzgau breed was registered by the UN FAO as endangered. Milk production ranges from 5,500 to 6,500 kg, with 3.40% protein and 4.00% fat content. Regarding meat efficiency, the average daily gain of Slovak Pinzgau bulls in fattening reaches 1,200–1,300 g, with a total slaughter yield of 58%. The Slovak Spotted breed is a combined meat-dairy type with a medium-to-large body frame, harmonious body structure, and very good musculature. Its basic coloring ranges from yellow-spotted to red-spotted. A typical feature of the Slovak Spotted breed is the white coloring of the head, cheeks, and legs, a legacy of crossing with the Simmental breed. The skin is pink, while horns and hooves are wax-colored. Milk yield ranges from 5,500 to 6,000 kg per lactation for first-calf cows, and 6,500–7,500 kg for older cows. Protein production averages 280 kg, with 3.5% protein content and 4.0% fat content over 4–5 lactations.

Lifetime productivity can exceed 30,000 kg of milk. In terms of meat efficiency, Slovak Spotted breeding bulls in fattening reach an average daily gain of 1,300 g, with a total slaughter yield of 58–60%. Meat quality is comparable to that of Simmental and Pinzgau cattle breeds. As a contribution to the *ex situ* strategies for conserving the Pinzgau cattle breed, our goal was to produce blastocysts of the Pinzgau cattle in vitro and preserve them in the Gene Bank of Animal Genetic Resources. Ovaries from slaughtered Pinzgau cows were transported to the laboratory. Oocytes, isolated by aspiration of ovarian follicles, were matured in vitro and subsequently fertilized using frozen sperm from a Pinzgau bull. Presumed fertilized zygotes were cultured for 6–8 days until the blastocyst stage. In total, 20 blastocysts have been produced so far. Of these, 17 good-quality blastocysts were vitrified using an ultra-rapid cooling method. Cryopreserved blastocysts were stored in the Gene Bank of Animal Genetic Resources and entered into the CryoWeb database. More than 300 insemination doses from breeding bulls are cryopreserved in the Gene Bank of Animal Genetic Resources at NPPC RIAP Nitra. The situation with animal genetic resources in the Slovak Republic is somewhat better than 10 years ago, largely due to the storage of DNA samples, semen doses, oocytes, embryos, and stem cells in the gene bank. We cryopreserve and store sperm from cattle breeds (Pinzgau and Slovak Spotted), horse breeds (Slovak Sport Pony, Slovak Warmblood), sheep breeds (Native Wallachian, Improved Wallachian, Tsigai, and Slovak Dairy Sheep), rabbit breeds (Nitra Rabbit, Zobor Rabbit, Blue of Holič Rabbit, Zeppelin Rabbit, Lipton Bold-Spotted Rabbit, Charbray Rabbit), chicken (Oravka), and goose breeds (Slovak White and Suchovská Goose). In addition, we cryopreserve oocytes and embryos of cattle, as well as stem cells from rabbit, chicken, and sheep breeds. From the last update of animal breed statuses at the end of 2011, it is clear that two pig breeds in the Slovak Republic are at risk of extinction.

Keywords: animal genetic resources, Slovak breeds, cryopreservation, gene bank, Pinzgau cattle, Slovak Spotted cattle

Acknowledgments

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Generation Interval Determined in the Case of Hungarian Grey Cattle Progenies Born from Revitalized “Archive Sires”

Gáspárdy András¹, Kaltenekker Endre², Borics Imre² (*invited paper*)

¹*Institute of Animal Breeding, Nutrition and Laboratory Animal Science, University of Veterinary Medicine Budapest, István utca 2, 1078 Budapest, Hungary;*

²*Association of the Hungarian Grey Cattle Breeders, Lőportár utca 16, 1134 Budapest, Hungary*

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, rare breed conservancy, generation interval

Investigation of Cryopreservation-Induced Damage in Ram Spermatozoa Using Fluorescent Markers

Jovana Grba Ilic¹, Sasa Dragin¹, Peter Chrenek^{2,3}, Alexander Makarevich³ (*invited paper*)

¹*University of Novi Sad, Faculty of Agriculture, Department of Animal Science, Serbia*

²*Faculty of Biotechnology and Food Science, Slovak University of Agriculture, Nitra, Slovak Republic*

³*Research Institute for Animal Production Nitra, National Agricultural and Food Centre (NPPC), Lužianky, Slovak Republic*

Abstract

During the cryopreservation process, the spermatozoa of domestic animals can be damaged, which negatively affects motility, plasma membrane function and the reproductive value itself. Sperm from Improved Wallachian, Native Wallachian and Slovak Dairy rams were used in our experiment. Only ejaculates with initial progressive motility > 60% and < 10% abnormal sperm morphology were investigated. The quality of thawed sperm samples was assessed *in vitro* using computer-assisted sperm analysis (CASA). This research was carried out to improve methods for assessing the quality of ram reproductive material. The results came from the authors' original research at the National Agricultural and Food Centre, Nitra, Slovak Republic. Fluorescent marker SYBR-14/PI was used to assess the integrity of the sperm membrane, which directly affects sperm quality and fertility after cryopreservation. Sperm viability was assessed using a LIVE/DEAD sperm viability kit. Sperm samples were analyzed using a Leica fluorescence microscope. After staining, live sperm showed green fluorescence, dead sperm showed red, while dying or apoptotic sperm showed orange-yellow fluorescence. The results of this research were processed using SPSS version 23 software packages and STATISTICA version 11. A paired-samples t-test was used. Results show a significant increase ($P < 0.05$) in the percentage of dead sperm after cryopreservation. In accordance with the obtained results, two breeds showed statistical significance for propidium iodide (PI, dead cells), while the autochthonous Wallachian sheep did not show statistical significance in dead spermatozoa after cryopreservation. Results will help to optimize the selection of rams based on the quality of frozen ejaculates, making it easier to select sperm samples suitable for cryopreservation in animal banks of genetic resources.

Keywords: Spermatozoa, cryopreservation, fluorescent markers, ram

Morphometric Analysis and Sex Dimorphism of the Autochthonous Lipe Sheep

Kovačević Sara¹, Dimitrijević Vladimir¹, Gáspár András², Savić Mila¹, Trailović Ružica¹, Tarić Elmin¹, Čobanović Nikola³, Dominiković Nina⁴, Paskaš Snežana⁵, Rašeta Mladen⁶, Cekić Bogdan⁷, Becskei Zsolt¹ (*invited paper*)

¹*Department of Animal Breeding and Genetics, Faculty of Veterinary Medicine University of Belgrade, Belgrade, Serbia*

²*Department of Animal Breeding and Genetics, University of Veterinary Medicine, Budapest, Hungary*

³*Department of Food Hygiene and Technology, Faculty of Veterinary Medicine University of Belgrade, Belgrade, Serbia*

⁴*Department of Biology, Faculty of Veterinary Medicine University of Belgrade, Belgrade, Serbia*

⁵*Department of Animal Science, Faculty of Agriculture University of Novi Sad, Novi Sad, Serbia*

⁶*Institute of Meat Hygiene and Technology, Belgrade, Serbia*

⁷*Main Breeding Organization, Department of Sheep and Goat breeding and Genetics, Institute for Animal Husbandry, Autoput 16, 11080 Belgrade, Serbia*

Abstract

Lipe sheep is an autochthonous and endangered breed endemic to a limited area in Serbia, representing an important element of national livestock biodiversity. As a locally adapted genetic resource belonging to the Pramenka group of sheep, it holds ecological, cultural, and production-related value. However, population decline and exclusion from intensive breeding programs have made the population vulnerable to genetic erosion. This study evaluated sexual dimorphism of the Lipe sheep through body measurements, craniometric, and pelvimetric morphometry. A total of 19 adults (10 ewes and 9 rams, aged 2.5 to 3.5 years) were analyzed. Results showed highly significant differences between sexes for body traits ($p < 0.001$), with rams consistently exhibiting greater values. Significant dimorphism was observed in cranial traits, with rams displaying more pronounced characteristics. Rams also had yellowish to dark, large, triangular, and spirally twisted horns, a key feature of sexual dimorphism. In contrast, no significant differences were found for pelvic traits, suggesting a well-developed pelvis in ewes, ensuring easy lambing and uniform pelvic conformation. These findings indicate that the Lipe sheep exhibits stronger sexual dimorphism in body and cranial morphology while maintaining functional uniformity in pelvic structure. The study contributes to a broader characterization of the Lipe sheep and provides a foundation for targeted conservation and breeding strategies. Preserving this breed helps maintain agrobiodiversity and strengthens its role as a valuable genetic resource.

Keywords: autochthonous breed, genetic resource, Lipe sheep, morphometric analysis, sexual dimorphism

Strengthening the Sustainability of Sjenica Sheep

Elmin Taric¹, Zsolt Becskei¹, Nina Dominikovic¹, Branislav Vejnovic¹, Sara Kovacevic¹, Jelena Janjic¹, Bogdan Cekic², Mladen Raseta³, Snežana Paskas⁴, Mila Savic¹, Vladimir Dimitrijevic¹
(invited paper)

¹University of Belgrade, Faculty of Veterinary Medicine, Bulevar oslobođenja 18, Belgrade, Serbia

²Main Breeding Organization, Department of Sheep and Goat breeding and Genetics, Institute for Animal Husbandry, Autoput 16, Belgrade, Serbia

³Institute of Meat Hygiene and Technology, Belgrade, Serbia

⁴University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, Novi Sad, Serbia

Abstract

The Sjenica–Pešter Plateau, due to its natural characteristics, holds significant potential for sheep production. Breeding the Sjenica sheep in this region represents a key mechanism for securing livelihoods 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 of total fatty acids. 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 favorable lipid 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

Financial Support Measures of Endangered Local (Autochthonous) Breeds in Slovenia

Žan Metka¹, Bojkovski Danijela¹, Flisar Tina¹

¹University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Jamnikarjeva 101, 1000 Ljubljana, Slovenia

Abstract

The conservation of animal genetic resources for food and agriculture encompasses the biodiversity of genes and breeds of various animal species that contribute to food production and sustainable agriculture. This specific dimension of biodiversity is an important basis for agricultural development and global efforts to combat hunger. In Slovenia, 14 local, 14 traditional, and 27 foreign breeds are monitored, with particular emphasis placed on the preservation of local Slovenian breeds. Local (autochthonous) breeds are breeds that originated in the territory of the Republic of Slovenia, whereas traditional breeds are those that have adapted to the climatic and other conditions of a specific geographical area and have been subject to continuous breeding for at least 30 years, or at least 50 years in the case of horses and cattle. All other breeds are referred to as foreign breeds. Around the turn of the millennium, several populations of local breeds in Slovenia faced the threat of extinction. Through targeted professional measures and financial support from the national government, not only was the genetic heritage preserved, but the population size of many breeds also showed significant recovery. The most notable growth was observed in the Cika cattle breed, which increased 5.4 times during the monitoring period. Populations of the Krškopolje pig, Drežnica goat, and Posavje horse also more than doubled. Farmers who raise endangered local breeds receive financial support under the EU Common Agricultural Policy and the long-term national programme for the conservation of livestock diversity. The support is based on the number of purebred female and male breeding animals and the overall population size, which serves as the basis for assessing each breed's risk status. This article presents an overview of the scope and amount of financial support allocated to breeders for the conservation of endangered local Slovenian breeds in the period 2007–2024.

Keywords: animal genetic resources, biodiversity, endangered breeds, financial support measures

The Lipizzan Horse and its Fertility Characteristics in Hungary

Kovacs Máté¹, Gyúró Daniella², Posta János²

¹*University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management, Doctoral School of Animal Science*

²*Department of Animal Husbandry, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen, Böszörményi str.138, H-4032 Debrecen, Hungary*

Abstract

The Lipizzan breed has a strong connection to Hungary and its horse breeding traditions. Historically, the breed was improved by the Mezőhegyes stud and the Hungarian aristocracy, and it has been playing a key role in the success of Hungarian carriage driving sport. While several studies have examined the breed's genetic structure and quantitative traits, there is limited data on the fertility of Hungarian Lipizzan mares. This study aims to investigate fertility and reproductive characteristics of mares from several Hungarian studs. Equine fertility is influenced by various factors, including age, reproductive status, genetics, covering method and timing, and infections. Mare families also significantly affect reproduction, as some lines show declining population sizes. No international data exist on Lipizzan fertility by mare family, so this research addresses a gap in the literature. The study analyzed fertilization data of 220 mares from ten Hungarian studs, based on the OLIR database. Although the mares varied widely in age, age was not considered in evaluating conception rates. The population was divided into 24 age groups and assessed by breeding performance. Most coverings occurred in 2020 and 2022, with most foals born from 2019 and 2022 matings. The most successful breeding year was 2011, while 2020 and 2021 showed the lowest success. The mares belonged to 25 mare families, including Hungarian, classical, Fogaras-based, and Croatian lines. Fertility varied significantly among and within families.

Keywords: Lipizzan, fertility, mare families

Magnetic Nanopurification of Ram Spermatozoa Using Annexin V and Lectin-coated Nanoparticles to Improve Sperm Viability and Motility

Vašíček Jaromír^{1,2}, Svoradová Andrea², Baláži Andrej², Vozaf Jakub^{1,2}, Olexiková Lucia², Chrenek Peter^{1,2}

¹*Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Science, Tr. A. Hlinku 2, 94976 Nitra, Slovak Republic*

²*NPPC, Research Institute for Animal Production Nitra, 95141 Lužianky, Hlohovecká 2, Slovak Republic*

Abstract

Magnetic-activated cell sorting (MACS) is routinely used to remove defected spermatozoa from semen samples to enhance the overall quality of semen samples used for human reproduction or animal breeding. Mostly, annexin V conjugated nanoparticles are used to target apoptotic-like or moribund spermatozoa with deteriorated plasma membrane. Moreover, defective spermatozoa with disrupted acrosome have ligands for lectins on their surface, which can be also used for nanopurification process of animal spermatozoa. However, the combination of glycoproteins and annexin V conjugated nanoparticles has never been used to improve the quality of ram spermatozoa. Therefore, 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.

Key words: ram, spermatozoa, nanopurification, CASA, flow cytometry

Exterior and Production Characteristics of Brač Sheep

Ivan Vlahek¹, Jasna Kusanović¹, Velimir Sušić¹, Anamaria Ekert Kabalin¹, Sven Menčik¹, Aneta Piplica¹, Niko Popović¹, Maja Maurić Maljković¹

¹University of Zagreb, Faculty of Veterinary Medicine, Vjekoslava Heinzela 55, Zagreb, Croatia

Abstract

The Brač sheep is a locally adapted strain within the Dalmatian Pramenka breed, shaped by the specific environmental conditions of the Croatian island of Brač. Despite its cultural and agricultural significance, this sheep strain has not yet been fully described in scientific literature. This study aimed to record the exterior and productive traits of the Brač sheep to support its recognition and conservation as an autochthonous genetic resource. A total of 65 adult ewes and 13 adult rams from nine flocks were included in the study. The animals were kept primarily on pasture and used either for meat production or as dual-purpose animals. Body weight and seven morphometric traits were recorded. Measurements were conducted using a scale, Lydtin stick, measuring tape, and calipers. Lactation yield was assessed in five ewes after the weaning of lambs. Milk was measured every 30 days until drying off, and total yield was calculated using the Fleischmann formula. The average body weight of ewes was 41.08 kg, and that of rams was 54.55 kg. Rams were larger than ewes in all measured exterior traits. Withers height averaged 61.94 cm in ewes and 68.04 cm in rams, while pelvic height was 62.85 cm and 68.00 cm, respectively. The most notable variation was observed in pelvic width, with a coefficient of variation of 15.71% in rams and 8.11% in ewes. Average milk production during the lactation period was 68.7 liters per ewe. Average litter size was one lamb, with all ewes delivering singletons. The Brač sheep is characterized by a small body frame and modest productive performance, which are comparable to those of other Croatian Pramenka-type breeds.

Keywords: Brač sheep, Dalmatian Pramenka, morphometry, production

Characterization of Reproductive Traits in the Buša Cattle Breed: Implications for Conservation and Productivity

Aneta Piplica¹, Maja Maurić Maljković¹, Anamaria Ekert Kabalin¹, Velimir Sušić¹, Ivan Vlahek¹,
Niko Popović¹, Sven Menčik¹

¹University of Zagreb, Faculty of Veterinary Medicine, Heinzelova 55, Zagreb, Croatia

Abstract

The Buša cattle, an autochthonous breed adapted to low-input and extensive breeding systems, represent a valuable genetic resource in the Republic of Croatia. Their reproductive performance, shaped by both genetic and environmental factors, is particularly important as it directly affects population viability, genetic conservation, and the economic feasibility of maintaining autochthonous cattle in smallholder systems. This study aimed to characterize key reproductive traits in Buša cows kept under extensive breeding conditions, including age at first conception and calving, service period, calving interval, and gestation length. Data were analyzed from the entire population of reproductively active cows during the period from 1992 to 2020. The interquartile range of age at first calving was between 22.0 and 31.2 months, corresponding to an age of two to three years, while the age at first conception ranged from 12.8 to 21.1 months. The duration of gestation length in the analyzed population was 280.8 ± 8.9 days. The median calving interval in the studied population of reproductive cows of the Buša cattle breed was 386.0 days, with the interquartile range from 341.0 to 518.0 days, and the duration of the service period ranged from 80.5 to 320.0 days. These results underscore distinct reproductive phenotypes reflective of adaptation to extensive breeding conditions, and potentially insufficient selection for reproductive traits.

Keywords: Buša cattle, reproductive traits, conservation, extensive breeding, autochthonous breed.

Buša – an Indigenous Cattle Breed in the Cow-Calf Production System

Niko Popović¹, Maja Maurić Maljković¹, Ivan Vlahek¹, Anamaria Ekert Kabalin¹, Sven Menčik¹,
Velimir Sušić¹, Aneta Piplica¹

¹University in Zagreb, Faculty of Veterinary Medicine, Heinzlova 55, Zagreb, Croatia

Abstract

The “cow-calf” system is a component of beef cattle production, in which the calf remains with the cow for an extended period or until slaughter. It is characterized as an economically viable and sustainable method of calf production, particularly in regions with limited resources for intensive agricultural production, such as certain parts of Croatia. This system utilizes grazing land and pastures with minimal investment in livestock housing and animal management. Additionally, extensive cattle keeping within pastoral systems is a longstanding tradition in these areas. Although beef breeds are typically favored in cow–calf systems, the use of indigenous breeds offers certain advantages. The buša breed, in particular, emerges as a promising choice due to its resilience, low maintenance requirements, and adaptability to the climatic and agricultural conditions of Croatia. The breed is especially common and abundant in Lika and inner Dalmatia, which are characterized by rocky and acidic soils, frequent droughts, and high and low temperatures—conditions that limit the potential for intensive agriculture but provide ample natural grazing areas suitable for extensive livestock production. Buša is a small-framed, late-maturing, and low-producing breed with ease of calving. Due to its modest growth traits, calves are typically slaughtered at around five months of age. Nevertheless, the breed’s population has been steadily increasing over the years. Additionally, buša represents a valuable source of genetic diversity and cultural heritage with potential for gastronomic positioning.

Keywords: “cow-calf” system, extensive livestock production, indigenous breeds

Indigenous Donkey Population in Croatia – Current Situation and Trends

Maja Maurić Maljković¹, Aneta Piplica¹, Niko Popović¹, Anamaria Ekert Kabalin¹, Sven Menčik¹,
Velimir Sušić¹, Ivan Vlahek¹

¹University of Zagreb, Faculty of Veterinary Medicine, Heinzelova 55, Zagreb, Croatia

Abstract

The donkey population in Croatia has drastically declined over the past decades, primarily due to mechanization in agriculture and transport, which rendered their traditional roles largely obsolete. In the mid-20th century, over 50,000 donkeys were present in the country; by the end of the century, only a few hundred remained. Croatia is home to three native breeds: the Littoral-Dinaric donkey, a smaller type traditionally kept in Dalmatia; the Istrian donkey, a larger and stronger breed from Istria; and the North Adriatic donkey, a medium type kept in the North Adriatic region, mainly the Kvarner islands. All breeds became critically endangered. However, over the past two decades, targeted conservation programs and increased public awareness have contributed to a positive trend in population recovery. Today, donkeys in Croatia are primarily used for milk production, valued both for human consumption and beauty products, as well as for meat in specific regions like Istria, and in tourism or cultural events. Local donkey breeds represent an important part of Croatia's Mediterranean heritage, supporting biodiversity, traditional practices, and the sustainability of rural communities. The long-term viability of these breeds requires further development of sustainable commercial strategies that align with their ecological and cultural roles.

Keywords: donkey, indigenous breeds, Istrian donkey, Littoral-Dinaric donkey, North Adriatic donkey

Morphological Measurements of Dogs: Practical Approach to Standardization of Autochthonous Breeds

Anamaria Ekert Kabalin¹, Maja Maurić Maljković¹, Ivan Vlahek¹, Maja Črnjević Diklić², Bojan Mataković³, Velimir Sušić¹, Aneta Piplica¹, Niko Popović¹, Sven Menčik¹

¹*University of Zagreb, Faculty of Veterinary Medicine, Heinzelova 55, Zagreb, Croatia*

²*Unconditional d.o.o., Radnička cesta 177, Zagreb, Croatia*

³*Croatian Kennel Club, Ilica 61, Zagreb, Croatia*

Abstract

Dog breed standards traditionally define general appearance, temperament, body proportions, coat characteristics, and acceptable variations. They also outline faults that reduce breeding value and disqualifying morphological or behavioral traits. However, while these standards provide a general framework, they often lack standardized morphometric data across sexes and age groups. To address this gap, we conducted research to develop a more precise morphometric framework for different dog breeds using systematic measurements. In this phase, two Croatian breeds (Tornjak and the Croatian Shepherd Dog) were examined. A total of 56 adult dogs (over 12 months of age) were included, with 28 individuals from each breed (both sexes). Twenty-three body measurements were applied to describe body structure, taken with a tape measure, Lydtin stick, and caliper. Parameters were selected based on breed standards and anatomical relevance. Preliminary analysis showed consistent sexual dimorphism in frame size, depth, width, and circumference—males generally had larger values, with some traits showing statistically significant differences. These findings highlight the value of detailed morphometric characterization in evaluating breed conformation. Identifying deviations in body proportions may help breeders select mating pairs that express desired traits more accurately. The study offers a replicable methodology for evaluating native breeds and refining standards based on empirical data.

Keywords: morphometric measurements, breed standards, Tornjak, Croatian Shepherd dog

The Water Buffalo (*Bubalus bubalis*) as an Autochthonous Genetic and Dairy Resource in Republic of Serbia

Dominiković Nina¹, Becskei Zsolt¹, Tarić Elmin¹, Kovačević Sara¹, Savić, Mila¹ – Dimitrijević, Vladimir¹

¹University of Belgrade, Faculty of Veterinary Medicine, Bulevar oslobođenja 18, Belgrade, Serbia

Abstract

Autochthonous breeds are considered valuable reservoirs of genetic diversity owing to their capacity to adapt to specific ecological and economic conditions. Among these, the water buffalo (*Bubalus bubalis*) holds particular importance in European agriculture, especially in Italy, Romania, Bulgaria, and parts of the Balkans. In Serbia, buffalo populations have traditionally been concentrated along major river basins. Most herds are found in central and southern Serbia, restricted to areas where traditional buffalo husbandry has been maintained due to geographic factors and cultural practices. However, according to FAO data, their numbers have markedly declined over the past decades, with some local populations approaching extinction. Over the past five years, the Serbian water buffalo population has ranged between approximately 1,100 and 1,600 head, peaking in 2022. According to the most recent records, the population now numbers just under 1,500 animals. In general, water buffalo are classified into Swamp and River subspecies, each with unique production traits and geographic distributions. Swamp buffalo are primarily kept in South and Southeast Asia, where they serve as draught animals and produce modest quantities of milk and meat. In contrast, River buffalo, which predominate in Europe, have been selectively bred for high milk yields and are the foundation of traditional dairy industries that produce specialty cheeses highly valued by consumers. Buffalo milk is characterized by its rich composition, containing 6–8% fat, elevated protein levels, and a high proportion of total solids, making it particularly suitable for processing into mozzarella and various traditional cheeses. In addition to their valuable milk traits, buffalo are recognized for their ability to thrive under suboptimal conditions, their effective feed utilization, and their resilience to various infectious diseases. Conservation of this genetic resource is fundamental for maintaining adaptive capacity and supporting the socio-economic sustainability of rural production systems. It is essential to implement selection and conservation programs and promote the valorization of buffalo-derived products in order to preserve this culturally valuable species in Europe.

Keywords: Water buffalo, milk fat, genetic resource, conservation

Breed History and Current State of Milking Tsigai Sheep in Hungary

Anna Mária Vásárhelyi¹, András Lévai², András Gáspárdy¹

¹*University of Veterinary Medicine Budapest, Institute for Animal Breeding, Nutrition and Laboratory Animal Science, István utca 2., 1078 Budapest, Hungary*

²*Hungarian Sheep and Goat Breeders' Association, Lőpotár utca 16., 1134 Budapest, Hungary*

Abstract

The dairy-type Tsigai is a breed variant of Tsigais native to Hungary and Serbia. The dairy Tsigai breed dates back to the 19th century with milk production being the focus of selection in some herds of historical Vojvodina (mostly in southern Bácska and Sirmium) aiming to enhance dairy productivity while retaining adaptation to local conditions. In Hungary, as a result of modern selection and Serbian imports, the current form of dairy Tsigai developed in the last decades of the 20th century. This breed is bigger in size than the native Tsigai variety, and exhibits a characteristic dairy conformation, with well-developed udders and average milk yields of 150–200 litres per lactation under semi-intensive keeping conditions. Reproductively, the breed displays traits such as late maturity and seasonal polyoestrous cycles, along with twinning and longevity supporting an efficient lamb production. With fewer than 300 breeding ewes, the dairy Tsigai is classified as critically endangered in Hungary. Conservation and genetic management efforts are ongoing, with future possibilities including organic dairy production and landscape management roles, ensuring the breed's survival and sustainable use. Conservation programs focus on genetic monitoring and controlled mating plans. Future perspectives include leveraging its milk quality for niche products and its potential role in sustainable, low-input farming systems. Previously, diversity testing involved the study of blood groups and protein polymorphisms, and revealed moderate within-population variability, but the narrow genetic base remains a concern. Direct genetic studies have been supplemented by the examination of STR polymorphisms. Today, the goal is to map the maternal lineage of the dairy Tsigai based on mtDNA.

Keywords: breed preservation, dairy-type, genetic diversity

The black gold of the Danube River. Sturgeons in Bulgaria: life history, present status and future challenges

Alexander Atanasoff¹, Galin Nikolov², Dimitar Spasov²

¹*Trakia University, Faculty of Veterinary Medicine, Student campus, Stara Zagora, Bulgaria*

²*Trakia University, Faculty of Agriculture, Student campus, Stara Zagora, Bulgaria*

Abstract

For decades, sturgeons have been classified as the most endangered fish species on the globe. The combination of their slow growth rate and overutilization has resulted in natural population declines for many species around the world. To date, the vast majority of related work on sturgeon species has focused on conservation by preventing and counteracting poaching and illegal trade in caviar. While the protection of natural populations will provide further conservation benefits, the focal point of this paper is also on sturgeon aquaculture in Bulgaria as an alternative approach. The present study provides a topical overview of the natural population of sturgeon in the Bulgarian part of the Danube River and sturgeon aquaculture production in our country, highlighting their life history, present status, and future challenges.

Keywords: Danube River, Sturgeons, Immunomodulator, Immunity, Sturgeon

Current Trends and Future Perspectives in Gidran Horse Breeding in Croatia

Jelena Ramljak¹, Branko Kovačić², Sanja Jakrlin², Josip Trogrlić², Nikica Prvanović-Babić³

¹*University of Zagreb, Faculty of Agriculture, Svetošimunska cesta 25, Zagreb, Croatia*

²*Croatian Association of Gidran Horse Breeders, Franje Vidivića 59F, Čazma, Croatia*

³*Faculty of Veterinary Medicine University of Zagreb, Heinzelova 55, Zagreb, Croatia*

Abstract

The breeding of Gidran horses in today's Croatia dates back 140 years, more precisely to 1885. A significant revitalization began around 2009 with the purchase of 20 horses from Hungary, which formed the breeding stock and marked a new era for Gidran breeding in Croatia. As of today, the population comprises 137 registered Gidran horses. The current trend in breeding focuses on developing a modern sport horse, with primary use in the endurance discipline. However, Gidrans are also successful in show jumping. These horses are actively involved in events that celebrate traditional and cultural heritage. Special attention is directed toward preserving the lines of stallions and mares to maintain "breed structure" and genetic variability. Advanced assisted reproduction technologies are used, including oocyte collection (OPU), ICSI, freezing embryos in a gene bank, and successful transfer into donor mares. To preserve a mare line, embryo transfer was carried out for the first time in Croatia in 2025 for the Gidran breed. This act served not only to preserve the mare family but also to advance knowledge and the use of modern assisted reproduction technologies in equine breeding. Looking ahead, the strategic goal is to guide Gidran breeding toward producing a versatile horse suitable for different equestrian disciplines, recreation, and therapeutic riding, while maintaining genetic diversity and ensuring the continuation of systematic breeding.

Keywords: Gidran, breeding, assisted reproduction, future perspective

Partial Correlations Between Various Inbreeding Coefficients of Horse Breeds Developed in Mezőhegyes

Renáta Klein¹, János Oláh², János Posta¹

¹*University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management, Böszörményi street 138., Debrecen, Hungary*

²*University of Debrecen, Institutes for Agricultural Research and Educational Farm, Böszörményi street 138., Debrecen, Hungary*

Abstract

Maintaining genetic diversity is a crucial part of conservation efforts. In horse breeding, pedigree data provides accurate and long-standing information about the animals' ancestry and genetic relationships. The Mezőhegyes Stud in Hungary was founded in the late 18th century, by Joseph II, Holy Roman Emperor. Nowadays it is one of the oldest state-owned stud farm in Europe. Three historical horse breeds (Gidrán, Nonius, Furioso-North Star) originated here. Since Wright introduced the first inbreeding coefficient in 1922, more complex measures have been developed to assess inbreeding in populations. In recent decades, alternative coefficients—such as Ballou's ancestral inbreeding coefficient and Kalinowski's original and new coefficients—have become increasingly popular tools for evaluating inbreeding levels more comprehensively. In this study, we conducted a partial correlation analysis among the results obtained from various inbreeding coefficients in three horse breeds from Mezőhegyes. The different methods used were: Wright's inbreeding (F_{Wright}), Ballou's ancestral inbreeding coefficient (F_{Ballou}), and Kalinowski's original (F_{Kal}) and Kalinowski's new ($F_{\text{Kal_new}}$) inbreeding coefficient. As expected, the historical indicators (F_{Ballou} , F_{Kal}) show a strong correlation with each other.

Keywords: horse breeding, genetic diversity, inbreeding, correlation analysis

Changes in Production Parameters of Indigenous Tsigai

Bácsi Eszter Ilona^{1,2}, Klein Renáta³, Egerszegi István⁴, Bodnár Ákos⁴, Oláh János¹

¹University of Debrecen, Institutes for Agricultural Research and Educational Farm, Farm and Regional Research Institute of Debrecen, 4032 Debrecen, Böszörményi 138.

²University of Debrecen, Doctoral School of Animal Science, 4032 Debrecen, Böszörményi 138.

³University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management, Institute of Animal Science Biotechnology and Nature, Department of Animal Husbandry, 4032 Debrecen, Böszörményi út 138.

⁴Hungarian University of Agriculture and Life Sciences, Institute of Animal Sciences, Department of Animal Husbandry and Animal Welfare, 2100 Gödöllő, Páter Károly 1.

Abstract

Native and protected breeds of local importance play a crucial role in the utilization of pastures, as these animals are best adapted to the local climatic conditions and natural conditions. They also contribute significantly to preserving genetic diversity and local traditions. The Kismacs Animal Breeding Experimental Farm of the University of Debrecen plays an extremely important role in the genetic preservation and breed maintenance of indigenous Hungarian animal breeds, including native poultry, sheep and horse breeds. In the case of the indigenous sheep breeds, the primary objective is also to preserve genetic variation and the stability and uniformity of production parameters. Studies measured growth performance in lambs from the indigenous Tsigai flock on the farm to determine the weight gain of this breed. Measurements were taken from lambs born between 29 January 2024 and 29 February 2024. Body weight at birth was recorded for each lamb, and weight gain was continuously monitored until January 2025, when the sheep were certified for the year. The results of these measurements are evaluated in this article. The average body weight at birth of ewe lambs was $4.442 \text{ kg} \pm 0.878 \text{ kg}$, and for rams it was $4.873 \text{ kg} \pm 1.055 \text{ kg}$. The average daily weight gain was $260 \text{ g/day} \pm 59 \text{ g/day}$ for rams and $277 \text{ g/day} \pm 80 \text{ g/day}$ for ewe lambs. Body weight corrected to 60 days was $19.59 \text{ kg} \pm 4.42 \text{ kg}$ for ewe lambs and $21.2 \text{ kg} \pm 5.34 \text{ kg}$ for rams for the two genders. This indicates that there were no significant differences in the body weight and body weight gain of rams and ewe lambs.

Keywords: Tsigai, weight gain, lambs

Cryopreservation Studies to Establish a Sperm Bank of Hungarian Giant Rabbit

Éva Váradi Kissné¹, Árpád Drobnyák¹, Zsuzsa Szabó¹, Éva Török¹, Barbara Végi¹

¹ National Centre for Biodiversity and Gene Conservation, Isaszegi út 200, Gödöllő, Hungary

Abstract

For the safe gene conservation of our native rabbit breed, the Hungarian Giant, we aim to create an in vitro sperm bank in addition to the existing nucleus colony in our institute. In recent years, we are working to develop an efficient freezing protocol. In this study, the first step was to find the effective DMSO concentration for the best protective effect during cryopreservation. In the second step, the efficiency of Lake extender supplemented with glycerol and DMF was compared with BotuCrio. In Experiment 1, the collected sperm was diluted 1:1 with modified Lake extender containing 6–8–10% DMSO, filled into 0.25 ml straws, and after equilibration, static freezing (5 cm above liquid nitrogen for 15 min) was applied. Thawing was carried out at 37 °C for 30 sec. In Experiment 2, part of the sperm was diluted with BotuCrio in a 1:4 ratio, while the other part was diluted with Lake diluent in the same ratio, using 1% glycerol and 4% DMF as CPA. The freezing and thawing were carried out in the same way as in Experiment 1. In the fresh and frozen/thawed sperm samples, motility (subjective scoring and CASA), the ratio of live/dead cells, and sperm abnormalities were checked using eosin–aniline blue vital staining. In testing DMSO ratios, neither the ratio of live, intact spermatozoa (13.5% vs. 17.7% and 14.8%) nor total motility (7% vs. 8.4% and 8.6%) showed any significant variation. During the trial of two different extenders, modified Lake diluent (with 1% glycerol and 4% DMF) produced a significantly higher ratio of live, intact cells (13.8 vs. 23.4) and total motility (12.8 vs. 21.4) than BotuCrio. Although the results of in vitro freezing are below the data reported in the literature, it is necessary to perform AI tests to check the real fertilization ability of frozen/thawed Hungarian Giant sperm.

Keywords: cryopreservation, Hungarian Giant Rabbit, sperm bank

Slovak - Hungarian Interreg Project from the Perspective of the Hungarian Partner

Barbara Végi, Eszter Várkonyi, Éva Váradi, Árpád Drobnyák, Kitti Buda, Bence Lázár, Nóra,
Pálinkás-Bodzsár, Csilla Eiben, Krisztina Liptói

National Centre for Biodiversity and Gene Conservation, Gödöllő, Hungary

Abstract

Everyone is aware of the importance of cooperation in research and gene preservation, as well as in all areas of life. The Hungarian NBGK came into contact with the Slovak NPPC through the DAGENE 2023 conference. It quickly became clear that experts from both countries share similar views and consider it extremely important to preserve the genetic material of native species. Their joint application, “Support for cross-border biodiversity conservation in ex situ conditions,” was awarded funding under the Interreg VI-A Slovakia-Hungary Cooperation Programme (HUSK/2302/1.2/018). Objectives of NBGK: Hungary's only native rabbit breed, the Hungarian Giant rabbit is critically endangered, so it is extremely important to protect the species using both *ex situ in vivo* and *in vitro* methods. The effectiveness of sperm cryopreservation protocols for our indigenous poultry breeds is often below the current results around the world. Therefore, further research is needed to identify the causes and improve the methods used. In the case of birds, *in vitro* preservation of female gametes faces numerous obstacles due to their reproductive biology. However, embryonic stem cells or day-old gonadal tissue may be suitable for long-term preservation of the female genome. One of the project's goals is to create stable male and female cell lines from the native Slovakian Oravka breed, which can be stored in the gene bank after *in vivo* and *in vitro* testing. Deep freezing of gonadal tissue has already been used for several breeds, and the aim is to expand our institute's gene bank samples while transferring and introducing this method to the Slovak gene bank. Through mutual support, the partnership enables continuous knowledge exchange and insights into managing Interreg projects.

Keywords: biodiversity, gene conservation, SK-HU collaboration

Indigenous Carpathian Cattle Breeds – the Basis for the Scientific and Transboundary Aspects of the Implementation of the “Carpathian Convention” and Its Protocols

Terpai Vasyl

Association of the Brown Carpathian Cattle Breed, street Mukachivska 2, Mukachevo city, 68600, Ukraine

Abstract

In the author's opinion, indigenous cattle breeds of the Carpathians are the basis of scientific and transboundary aspects of the implementation of the “Carpathian Convention” and protocols thereto. These indigenous cattle breeds are also the national heritage of the country in which these breeds are bred. The publication provides data on the distribution of 19 local breeds in the Carpathian region and the possibility of restoring officially extinct ones. Based on the results of the study and analysis of scientific works, their list was compiled, the number of breeding stock and the average number per farm are given. A number of differences in territorial distribution and their different genesis have been established; this is due to the long history of development. A limited number of breeding stock has been identified, and a clear trend towards decline is observed, which indicates the threat of extinction. The succession of breed composition that is currently taking place in the region is indicated. The results obtained are important for continuing further research based on cooperation between various institutions of the united countries in the Carpathian Euroregion, studying the features of cattle breeding and the impact of the industry on the functioning of landscapes, preserving biodiversity, restoring the transformed meadow ecosystem, and ensuring the sustainable development of communities in the Carpathians. They indicate the expanded functions of aboriginal cattle breeds. It is emphasized that one of the forms of wildlife protection is the organization of the preservation of indigenous breeds of cattle, which act as a matrix of farm animal populations and are based not only on ecological and biological principles, but also cover the entire spectrum of ideological, economic, socio-cultural, ethical, and ethnic beliefs.

Keywords: biodiversity, ecology, indigenous breeds, sustainable development, transboundary cooperation

Sperm Freezing Studies in Honey Bee (*Apis mellifera carnica*)

Árpád Drobnyák, Éva Kissné Váradi, Zsuzsa Szabó, Éva Török, Tímea Rácz, Tamás Sipos, Edit
Zajách, Barbara Végi

National Centre for Biodiversity and Gene Conservation, Gödöllő, Hungary

Abstract

One important tool for preserving genetic diversity is sperm cryopreservation, as an *ex situ*, *in vitro* method, that can also be used for honey bees. Based on the literature, we are working on a freezing process that will enable the effective preservation of Carnica bee semen in the NBGK sperm bank. In Experiment 1 was tested the effectiveness of 10% DMSO and 10% DMA as cryoprotectant. In Experiment 2 the straws were divided into two parts. One part was frozen 1 cm above the surface of liquid nitrogen for 30 min (one-step method) and the other part was frozen 5 cm for 15 min and 1 cm for another 15 min (two-step method). The thawing was carried out in all of two experiments at 5 °C water bath in 12 sec. The motility was determined by subjective scoring and the live/dead sperm ratios were established by aniline blue-eosin staining method. Were instrumental inseminated (IIS) 5 queens from one-step group and 11 queens from two-step group with frozen sperm. One part of IIS queens were used for histological sampling of spermatheca, while the other part IIS queens were reintroducing to colony respectively. According to our results, DMA and DMSO protected cells to the same extent. In Experiment 2 after thawing no significant differences were observed between the groups in motility (one-step: 1.69 vs. two-step: 1.26) or live cell percentage (one-step: 38.62% vs. two-step: 35.89%). Histological examination confirmed the presence of sperm in the spermatheca of two queens, while three reintroduced queens failed to begin egg-laying within 10 days after insemination. The inadequate results necessitate further development of freezing thawing methods and testing of queen fertility to develop a more effective method for the *in vitro* gene bank of *Apis mellifera carnica*.

Keywords: honey bee, sperm cryopreservation, gene bank

Pedigree Based Analysis of Population Structure in Hungarian Sheep (Racka Sheep)

András Gáspárdy¹, András Lévai²

¹*University of Veterinary Medicine Budapest, Institute of Animal Breeding, Nutrition and Laboratory Animal Science, István utca 2. Budapest, Hungary*

²*Hungarian Sheep and Goat Breeders Association, Lőportár utca 16, Budapest, Hungary;*

Abstract

The Hungarian sheep (Racka sheep) is the best known and most characteristic native sheep breed of Hungary, which has been bred in the Carpathian Basin for centuries. Regular pedigree data collection has been ongoing since 1994. The black and white colour variants are bred separately and registered in closed studbooks, and although they are similar in appearance, they are genetically distinct. Thanks to subsidies, the breed is currently flourishing, but the processing of studbook data and population genetic analysis are essential for its long-term, secure maintenance. The white Racka herd numbers 4,314 living animals currently, of which 2,362 are registered in the main herd book, while the black Racka herd numbers 3,905 animals, of which 2,143 are registered in the main herd book. The white Racka variant is kept by 48 breeders and the black one by 43 breeders, with an average number of 90 and 91 individuals per farm, respectively, with a very large variation. There are few large flocks (with more than 200 ewes), six for each colour. Therefore, it can be said that the stock is fragmented, which is favourable for preserving the genetic diversity of the breed, as more rams are used than in the case of modern breeds at large operations. The sex ratio is advantageous, 1:24 for white and 1:21 for black variant. The current breeding situation therefore appears favourable. However, we have found that despite the seemingly favourable data, genetic narrowing is occurring in both colours. In the white colour, this process is particularly noticeable in the male sex. Here, the number of lines has decreased from 103 to 42 in 30 years. In the black colour, this process is visible in both sexes. The number of lines has decreased from 88 to 41, and the number of families from 3163 to 1128. These data show that the long-term sustainability of the breed is at risk. The effective population size exceeds 2,200 individuals for both colour variants, but this seemingly favourable data alone is not sufficient for a correct assessment of the situation. Taking into account external factors (the high selection pressure applied to rams, the strong dependence of breeders on subsidies, the fragmentation of breeding farms, and the low willingness of breeders to cooperate), it can be said that new methods need to be developed to ensure the sustainability of the breed. Our main goal is to preserve the original characteristics of the Hungarian sheep, a breed that has defined Hungarian shepherding for centuries, and to maintain its original genetic diversity. To this end, we have processed the pedigree data of 110,000 individuals recorded over the past 30 years. As part of this, the ram lines and maternal families have been identified and coded. Going forward, we aim to use this knowledge to propose a breed maintenance program based on the within-family selection.

Keywords: Hungarian Racka sheep, within-family selection, maternal lineages.

ABOUT “DAGENE”

By the 1980s, the loss of this animal genetic heritage was a recognized problem across the continent, spurring calls for concerted conservation efforts. The International Association for the Conservation of Animal Breeds in the Danubian Region, known as DAGENE, was founded in 1989 in Budapest, Hungary. Established in the wake of decades of industrialization and changing agricultural practices, the organization set out to protect the rich, yet threatened, genetic diversity of the Danube River region's domestic animals.

Responding to this growing concern, several individuals and organizations across the Danube valley began collaborative efforts to prevent the extinction of their native livestock. In 1990, just one year after its founding, DAGENE established the DAGENE Gene Preservation Bank at Üllő Dóra-Major, near Budapest. This facility served as a crucial hub for collecting, storing, and managing the genetic material of endangered breeds, serving as a backstop against extinction.

Over time, countries within the region, such as Croatia, also began to implement their own national programs, which were often supported by or aligned with DAGENE's broader mission. As an international association, DAGENE has always emphasized cooperation among the countries that share the Danube basin. While headquartered in Budapest, the association organizes and facilitates research across its member states, which include countries like Switzerland, the Czech Republic, Moldova, Serbia, and Romania. This network approach ensures that local challenges and successes are shared across borders.

To promote a scientific approach to conservation, DAGENE founded the journal *Danubian Animal Genetic Resources* (DAGR) in 2016. The journal serves as a platform for sharing research on breed preservation, with a specific focus on the unique issues facing the Danubian Valley.

The association's yearly conferences bring together researchers and students to discuss the latest findings and strategies in animal genetic resource conservation. These meetings have historically fostered new collaborations and helped train the next generation of conservationists.

Today, DAGENE is a recognized part of a larger European conservation network, including entities like the European Union Reference Centre for Endangered Animal Breeds (EURC-EAB). Its work has evolved to address contemporary challenges, such as the effects of climate change on livestock and the importance of traditional breeds for rural identities, biodiversity, and agritourism. From its grassroots origins in 1989, DAGENE has matured into an essential organization for protecting the unique animal genetic resources of the Danube region, ensuring that this vital part of the area's natural and cultural heritage is not lost.