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Research articles

Difficulties in maintaining small horse breed populations, possible ways of decreasing the growth rate of inbreeding

MIHÓK, Sándor

Department of Animal Husbandry, Institute of Animal Science, Biotechnology and Nature Conservation, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen, H-4032 Debrecen, Böszörményi str. 138, Hungary
corresponding author: mihok@agr.unideb.hu

Abstract

Nowadays, the need for the survival and preservation of endangered breeds, including strains, genealogical lines, female families, has gained social acceptance. The primary objective within breeding conversation is to prevent genetic loss of the breed and to maintain genetic and phenotypic diversity. In simple terms: to avoid inbreeding. One of the conditions for the long-term maintenance of small breeds is to increase the population. The protection of mare families and genealogical lines is also an extremely important professional starting point. . The possibility of extending the persistence of the breed over time is helped by increasing the generation interval. In the case of endangered breeds, it is usually justified to deviate from the species-specific sex ratio when maintaining the breed, because a narrow sex ratio is beneficial for maintaining genetic diversity.

Keywords: gene preservation, livestock animals, endangered breeds

Nowadays, the need for the survival and preservation of endangered breeds, including strains, genealogical lines, female families, has gained social acceptance. A particular turning point in this respect was the Convention on Biological Diversity (CBD), adopted in Rio de Janeiro in 1992, which also emphasised the universal protection of domestic animal breeds having small populations within living systems.
The European Union has now established a financial support scheme for endangered breeds in two funding cycles (the third funding cycle is under preparation). The

support for gene conservation in the Member States of the European Union can be seen as an integral continuation of the "Rio Convention".

Moral and financial support is a very important part of the conservation of endangered breeds, but it is not in itself the solution to saving them. The biggest challenge in maintaining a breed is to meet the professional requirements for breeding according to the rules of genetic conservation. In short, it is the preservation of genetic diversity, the genetic stock of a breed that has been left to us.

The primary objective within breeding conversation is to prevent genetic loss of the breed and to maintain genetic and phenotypic diversity. In simple terms: to avoid inbreeding. Though, avoiding inbreeding is impossible for breeds with a closed pedigree and low population, such as the Gidran, the Shagya Arabian, the Hucul, the Nonius or the Mezohegyes Half-breed, due to the very limited possibilities for immigration. However, various breeding techniques can be used to delay or slow down the increasing of inbreeding, to preserve diversity, i.e. to reduce genetic variation to an acceptable level.

There is no doubt that maintaining small breeds requires a completely different mindset and action than performance-based breeding with large numbers. This is very hard to understand and even harder to accept.

One of the conditions for the long-term maintenance of small breeds is *to increase the population*. This makes sense, because a larger number of individuals increases the chances of selecting breeding stock candidates that are desirable in terms of utility, type and genetic value. Greater selection pressure makes real sense if selection is based on the need to meet the original use and to protect diversity at the same time. Selection should be made for all the traits that were once characteristic of the breed. In situ gene conservation, on the other hand, is about saving and preserving the existing population of the breed, which has been (partially) abandoned by the old use, while integrating the breed into the changed value system. The great challenge of gene conservation and the key issue for its survival is to find a rational use. In today's Hungarian equestrian society, the majority of people measure usefulness only in terms of success in equestrian sports, while the validity of such recognition of success for any breed under genetic conservation is questionable.

Gene conservation and new uses are professionally successful if they help to maintain the original gene combinations. In the case of the Hucul breed, the 'Hucul-path' can certainly be included, in the case of the Gidran, military can be brought into line with hunter riding, the endurance of the Shagya is certainly helped by its suitability for long-distance riding, but these cannot be exclusive.

By increasing the population, there is even some chance of increasing the essential genetic diversity. This can be explained by the fact that each offspring receives only

half of the genetic base of the parent. If more than one offspring of an individual (or parent pair) is bred, together they may pass on all the genetic material of the ancestor who is not bred to the next generation. Offspring from the same mating may have a distant genetic make-up, thus increasing genetic diversity. However, this cannot be detected by ordinary methods, but can be detected by molecular genetic methods (e.g. by assessing allele frequencies of DNA microsatellite loci).

Accepting the truth of the above, it makes sense to increase the number of small breeds if it is possible to attract as many breeders as possible to breed the breed. There is a limit to the size of the herd that can be bred by a single breeding farm or state stud. It is not possible to keep more breeding stock than a given number (usually not very large). There is not enough stable capacity, feed resources, service staff and the financial resources available to maintain the stud are limited.

For all in situ gene bank herds (almost regardless of the number of herds), the measurement of genetic diversity is the number and strict protection of the number of *mare families and stallion genealogical lines* (in the case of the Hucul, the Shagya Arabian, the number of strains, including the genealogical lines), and the balance among them. The availability and protection of all remaining mare families and genealogical lines is an extremely important professional starting point. (Causes of gene loss include war damage, anti-horse policies, lack of professional knowledge (e.g. the export of some Shagya mare families and stallions to the West), changes in the way they are used) It is considered a professional crime if the mare families and/or the stallion genealogical lines in any gene bank herd has been eroded. In this way the identity of the breed will be lost. The protection and maintenance of every available mare families is a much more important professional task than simply increasing the number of mares. The mare families are genetic merits that carry diversity. The number of mare families is a measure of the breed's potential for genetic renewal. Breeding should not be done with the offspring of the better-producing mare families, but all possible mare families should be maintained in balanced populations (approx. with the same numbers) in a gene bank flock. Of course, this is theoretical, because even under the most careful professional supervision, the numbers of mares in a family will vary, but we must strive to maintain a balance. The size of a mare family also affects the selection pressure, because a mare family with many individuals can allow more intensive selection than a family with few broodmares.

The situation is similar with stallion genealogical lines. There are genealogical lines 'A', 'B' and 'C' in the Gidran breed, while 'A', 'B'", 'C' and 'D' in the Nonius breed. In the Hucul and in the Shagya Arabian stallion strains are distinguished, and the maintenance of several separate lines of descendants from each is justified. In other words, the genetic diversity of the stallion park is also important for protecting

the diversity of the next generation. There is no reason, for example, to establish paternal half-siblings from a given genealogical line, unless they are surrounded by a line of other stallions belonging to the same genealogical line but not closely related to them. Otherwise, the genetic diversity of the population will decrease through their offspring. It is also not typical for breeding herds to be one-sided, with having 3-4-5 individuals from a genealogical line, whereas only one or none at all from other lines. The balanced use of stallions from different genealogical lines and strains is very important during the breeding work.

The inevitable increase in inbreeding from one generation to the next means that it is important to manage this threat over time. The possibility of extending the persistence of the breed over time is helped by ***increasing the generation interval***. It cannot influence the generation-to-generation change in genetic structure, but it can push out the same genetic loss over time. In the long run, it makes a difference whether age of breeding animals is between 15-20 years when they are the parents of the next generation or it is between 5-10 years. In gene conservation, the expected breeding progress from generation to generation is not a constraint, but the preservation of diversity is. Increasing the generation interval can help to successfully maintain a breed.

The lengthening of the generation interval is particularly useful in the case of large mare families, but it is preferable to shorten it in the case of small families (to take advantage of the small but still noticeable effect of the increase in numbers on the increase in diversity, and to protect the rare mare family from disappearing by increasing the number of mares).

In all but exceptional cases, increasing the generation interval of endangered horse breeds (livestock breeds) increasing the generation interval is in the breeders' best interest.

In the case of endangered breeds, it is usually justified to deviate from the species-specific sex ratio when maintaining the breed, because a narrow sex ratio is beneficial for maintaining genetic diversity, assuming that the stallions are not related to each other and to the mated mare population. In other words, the emphasis is on diversity in the case of stallions as well. Of course, it is very difficult to resolve the contradiction between the low number of offspring per stallion and the determination of its breeding value. (This is why the former national stud farm practice was genius, where young stallions were provided for the public breeding to prove their breeding value. This is no longer a reality for small breeds.)

Immigration is the most important factor in delaying the growth rate of related breeding. This is possible and most fortunate with intra-breed immigration, i.e. bloodlines, but the possibility of this is very limited. In the case of the Shagya Arabian, the "bringing back" of Shagya stallions and mares from Germany, Sweden

and Switzerland by Rombauer-Papócsi-Hecker after the foundation of the association is of great importance to this day. To a lesser extent, the Bábolna stud still does this today. The Nonius breed was also greatly helped by some breeding stock from the Őszény stud farm. Private breeders have also managed to obtain representatives of the mare families of the Mezőhegyes half-breed and Gidran, which are already extinct in our country. For many reasons, this is becoming more and more difficult for many endangered native breeds.

In the case of the Gidran (to a less extent in the case of the Mezőhegyes half-breed and the Nonius breeds), the possibility of other type of immigration is given or voluntarily offered by the type of the breed. The breed, in the first six decades of its development, was influenced by Arabic character and genetic background. Then, up to the turn of the 1900s, the English racehorse, later known as the English Thoroughbred, became increasingly important during the breeding. This is how the Gidran became an Anglo-Arabian breed, perhaps closer to the English half-breed character at the breed's peak and still is today. There is no professional misconception that the English Thoroughbred and the Arabian horse (including the Shagya Arabian, the Anglo-Arabian, and occasionally the Arabian Thoroughbred) are not considered to be strangers to the pedigree of the Gidran breed. For the other two, the English Thoroughbred can be taken into account.

It is characteristic of the half-breed horse that if from time to time there is no crossbreeding with the English Thoroughbred or the Arabian horse, signs of roughening will appear in the breed.

English Thoroughbreds were originally used to improve nobility, appearance defects and some (intrinsic) valuable traits (such as increased lung capacity and heart capacity, brighter blood temperament, better canter). Arabic was used to maintain the Anglo-Arabic character. It is no coincidence that both were regarded as improving breeds for Gidran.

Today the situation has changed. It is easy to see that both genotypes are increasingly losing their original breeding character, but their role has increased and they have become indispensable in maintenance of inbreeding. Not usually the breed, but the appropriate individual for this purpose!

All these small populations have mating difficulties. Maybe it is higher for Hucul and Gidran and maybe lower for other breeds. It is hardly possible to find mating partners that do not have common ancestors within the first three, often the first two generations. This raises not only questions of viability and reproductive biology, but above all it brings with it a major loss of diversity. As the rate of related breeding increases, so does the loss of diversity, which is the greatest obstacle to the effective maintenance of breeds that have declined to small numbers.

If, in addition to decreasing inbreeding, an English Thoroughbred or Arab half-breed can be used for crossbreeding that not only fits the breed type but also improves performance, this will be another "benefit".

In the last decades, there are many examples of English thoroughbreds or Arabian half-breeds in traditional Hungarian breeds that have not only maintained diversity but also improved performance. It is enough to mention the names of Direktor xx, Déva xx, Királyrét xx, Kegyúr xx, Hadik xx, Razbeg xx, Naum xx, Bob Herceg xx, The Bart xx, Masetta xx, Akitos xx, Francia xx and Délibáb. The Arabians were also excellent. In Hungary earlier Gazal III, Siglavy XII, O "Bajan, Mersuch XXII, Pap ox, Visbaden ox.

In addition to the importance of the English Thoroughbred and the Arabian for the maintenance of small horse populations, it should be emphasised that their immigration does not mean that the pedigree and phenotypic individuality of the breed is abandoned. Neither of them can be used unlimitedly, because that would mean adulterating the breed. Their use should follow the old Mezőhegyes breeding philosophy, according to which after English Thoroughbreds or Arabian stallions, first of all mare offspring should be bred, and the F_1 stallion that can be considered as a stud stallion must be of exceptional ability.

Even this is not the end of the principle of using English Thoroughbreds and Arabian half-breds to reduce the growth rate of inbreeding, because F1 mares must be mated with sires that can be traced back to the founder stallion. It is advisable to keep the proportion of English Thoroughbreds and/or Arabian half-breds in the gene bank herds in accordance with the principle of crosses for breeding. Constantly present, consistent target matings must be made.

It is also important to take into account that phenotype evaluation should never be carried out on F_1 mares. These individuals should be considered as transitional types of breeding, which are inevitable in the process of professionally correct breeding.

By breeding according to the rules of genetic conservation, by considering the possible variations, by managing the breed as a whole in a uniform way, the situation that is actually threatening can be overcome.

Optimization of protocol for stallion semen cryopreservation

KUŽELOVÁ, Lenka[1*] – VAŠÍČEK, Jaromír[1,2] – HALO Jr., Marko[3] – HALO, Marko[4] – CHRENEK, Peter[1,2]

[1]Slovak University of Agriculture in Nitra, Institute of Biotechnology, Tr. A. Hlinku 2, 94976 Nitra, Slovakia
[2]National Agriculture and Food Centre, Research Institute for Animal Production, Hlohovecká 2, 95141 Lužianky, Slovakia
[3]Slovak University of Agriculture in Nitra, Institute of Applied Biology, Tr. A. Hlinku 2, 94976 Nitra, Slovakia
[4]Slovak University of Agriculture in Nitra, Institute of Animal Husbandry, Tr. A. Hlinku 2, 94976 Nitra, Slovakia

*corresponding author: kuzeloval@gmail.com

Abstract

The aim of the preliminary study was to improve success of cryopreservation of stallion spermatozoa. The effect of different equilibration lengths and two techniques for freezing of stallion semen was evaluated, based on motility and viability parameters. Fresh semen was diluted with BotuCrio extender and divided into 3 groups equilibrated for 0.5h, 2h, and 4h. Subsequently, half straws of each group were cryopreserved in the vapours of the liquid nitrogen or in the automatic freezer. Results of stallion spermatozoa motility and progressive motility showed decreasing trend with increased time of equilibration. Moreover, a significant difference ($p < 0.05$) for the percentage of spermatozoa producing ROS was found between semen frozen in the vapours of the liquid nitrogen compared to semen cryopreserved by the automatic freezer in the same equilibration time. Nevertheless, results of this study are of preliminary character. Experiments using higher number of individuals need to be tested in order to find the best procedure for semen cryopreservation of Slovak national horse breeds.

Keywords: stallion, semen cryopreservation, ROS

Introduction

Generally, animal gene banks play an important role in agricultural production globally for the present and the future, and in sustaining the most of production systems and community livelihoods. The situation with animal genetic resources in the Slovak Republic is not satisfactory due to the fact that livestock semen doses stored in the gene bank are originated only from several Slovak breeds (CHRENEK et al., 2017). Therefore, if there is an opportunity to obtain biological material from valuable breeds, it is desirable to optimize specific cryopreservation process. The genetic selection of horses is purely based on desired phenotypic features, pedigree, and athletic achievements and rarely based on fertility (BRITO, 2007). Semen freezing studies aim to improve the performance of stallions, and favorable conditions for transporting semen, reduce disease transmission, and allow the use of genetic material for an indeterminate amount of time without affecting reproduction (AVANZI et al., 2006; AMAN and GRAHAM, 1992). Equilibration as the total period of spermatozoa contact with a cryoprotectant prior to freezing helps keep spermatozoa membrane integrity as well as their survival. The length of equilibration as well as the freezing curve type are responsible for many important physicochemical changes leading to different degrees of spermatozoa structure damage (FORERO-GONZALEZ et al., 2012) deteriorating spermatozoa characteristics after thawing. The objective of study was to evaluate the effect of different equilibration lengths and two techniques for freezing of stallion semen, based on motility and viability parameters and to find a suitable protocol for the cryopreservation of the stallion semen of genetically endangered species.

Material and methods

Semen Collection and Processing

Ejaculates were collected from clinically healthy Holsteiner breed stallion in Slovak University of Agriculture in Nitra. The frequency of semen collection was performed one time per week. The stallions were handled carefully in accordance with the ethical guidelines of the Animal Protection Regulation of the Slovak Republic RD 377/12, complying with the European Union Regulation 2010/63. Experimental protocols were approved by the committee at Slovak University of Agriculture in Nitra, Slovak Republic. Semen was obtained on a regular collection schedule using a lubricated pre-warmed artificial vagina (Colorado type, Minitube, Tiefenbach, Germany) on a dummy. Immediately after collection, semen was extended 1:1 with commercial extender BotuSemen® (Nidacon, Sweden) and transported to the laboratory. One aliquot of fresh semen was allocated for the assessment of the following sperm variables: motility, viability, apoptosis, plasma

membrane integrity, mitochondrial membrane potential, and ROS production.

Semen cryopreservation
The ejaculates were centrifuged at 600x g for 10 min at room temperature. The pellet was resuspended in BotuCrio (Nidacon, Sweden) and adjusted to 200×10 cells/mL. The samples were subsequently filled into 0.25 mL straws (Minitüb, Tiefenbach, Germany). After filling, straws were divided into 3 groups (n = 10 straws per group), immediately placed into a fridge (4–5°C), and equilibrated for 0.5h, 2h, and 4h. Subsequently, equilibrated straws were divided into 2 groups (n = 5 straws per equilibration length at least) and frozen using 2 freezing curves. One group of straws on the racks were placed 4 cm above the surface of the liquid nitrogen for 15 min and subsequently plunged directly into liquid nitrogen. Second group of straws were placed on a rack into the freezing chamber, cooled and frozen in the automatic freezer (IceCube, Minitube). The cooling ramps were −1°C/min from 20 to 5°C; temperature was maintained at 5°C for 5 min, followed by a −40°C/min ramp down to −140°C. After reaching −140°C, straws were removed from the chamber and plunged directly into liquid nitrogen. All straws were stored in liquid nitrogen until thawing and analysis.

Semen quality evaluation
The straws were thawed in a water bath at 37°C for 30 s and its content transferred to an Eppendorf tube pre-heated at 37°C. The same quality variables evaluated for fresh semen were assessed in frozen- thawed spermatozoa as described below.

Motility analyses
CASA (Computer Assisted Semen Analyzer) system with SpermVision software (MiniTüb, Tiefenbach, Gremany and the AxioScope A1 (Carl Zeiss Slovakia, Bratislava, Slovakia) were used to analyse spermatozoa motility. Five fields per sample were analysed for total motility (TM) and progressive motility (PM).

Flow cytometry analyses
Aliquots of semen samples from each group were adjusted to 10^6 cells/ml in Ca$^-$ and Mg$^-$free PBS (Biosera, France) and stained using selected chemicals for specific markers to identify different physiological cell attributes. The viability of spermatozoa was assessed using SYBR-14, a membrane-permeant nucleic acid green fluorescent dye (LIVE/DEAD® Sperm Viability Kit). YO-PRO-1 nuclear green dye was used to detect apoptotic-like changes in spermatozoa. The integrity of acrosome was inspected using PNA-FITC (*peanut agglutinin*). The activity of mitochondria was assessed through the mitochondrial membrane potential (MMP) using MitoTracker® Green FM and the measurement of reactive oxygen species (ROS) production was performed with CellROX Green Reagent. All chemicals were

purchased from Thermo Fisher Scientific (USA) unless stated otherwise. Fresh and post-thawed semen samples were incubated with the mentioned reagents either in accordance with the producer's manuals or as described previously (VAŠÍČEK et al., 2022). Reagents for staining were used at a final concentration in accordance with the producer's manuals or as follows: SYBR-14 (100 μM), YO-PRO-1 (100 μM), PNA-FITC (20 μM), MitoTracker Green FM (200 nM), and CellROX Green Reagent (2.5 μM). All samples were stained with a specific marker in combination with DRAQ7, a far-red fluorescent nucleic acid dye (BioStatus,Limited, Shepshed, UK), which stains nuclei of dead or membrane-compromised cells. Aliquots of stained samples were immediately analysed by flow cytometry using a FACSCalibur instrument (BD Biosciences, USA) and the FL1 (green) and FL3 (red) channels.

Statistical analysis

Experiments were repeated three times. Obtained results were evaluated using SigmaPlot software (Systat Software Inc., Germany) with one-way analysis of variance (Holm–Sidak method) and expressed as means ± SD. P-values at $p < 0.05$ were considered to be statistically significant.

Results and discussion

The data regarding effects of equilibration time and technique of freezing on the sperm quality parameters are included in Table 1 and Table 2. Freezing and thawing of stallion spermatozoa resulted in decreased values for sperm motion variables and viability parameters compared with fresh values (a vs. b, $p < 0.05$). No significant differences were found between time of equilibration, cryopreservation methods or their interactions with the variables as TM (total motility), PM (progressive motility), SYBR-14 (viable sperm), DRAQ7 (dead sperm), Yo-Pro-1 (apoptotic sperm), PNA (sperm with damaged acrosome), MT Green (sperm with high mitochondrial potential) of cryopreserved sperm. However, results of stallion spermatozoa motility and progressive motility showed decreasing trend with increased time of equilibration. Moreover, a significant difference ($p < 0.05$) for the percentage of spermatozoa producing ROS was found between semen frozen in the vapours of the liquid nitrogen compared to semen cryopreserved by the automatic freezer in the same equilibration time (14.02 ± 6.15 vs. 8.19 ± 4.97, 21.32 ± 5.19 vs. 9.83 ± 5.58, 26.18 ± 7.36 vs. 9.87 ± 5.32, respectively).

Table 1 Parameters (mean ± SD) evaluated in fresh and post-thawed semen of stallions in different equilibration time (0.5h, 2h, 4h) cryopreserved in the vapors of the liquid nitrogen (VLN)

Parameter	Fresh	VLN 0.5h	VLN 2h	VLN 4h
TM (%)	80.53[a] ± 1.52	56.93[b] ± 4.03	44.10[b] ± 4.46	40.33[b] ± 7.91
PM (%)	72.60[a] ± 2.77	48.72[b] ± 1.72	29.89[b] ± 3.97	26.07[b] ± 5.07
SYBR-14 (%)	75.02[a] ± 5.04	49.35[b] ± 5.87	32.42[b] ± 5.76	34.74[b] ± 15.56
DRAQ7 (%)	25.72[a] ± 6.23	48.62[b] ± 6.15	54.98[b] ± 11.13	60.68[b] ± 12.59
Yo-Pro-1 (%)	4.62[a] ± 3.07	30.67[b] ± 4.48	34.20[b] ± 10.63	40.24[b] ± 8.56
PNA (%)	7.72[a] ± 4.12	23.62[b] ± 11.03	46.85[b] ± 1.64	53.11[b] ± 12.94
MT Green (%)	77.19[a] ± 5.37	31.63[b] ± 6.36	24.19[b] ± 4.21	22.66[b] ± 8.53
CellRox (%)	3.02[a] ± 1,02	14.02[b] ± 6.15	21.32[b] ± 5.19	26.18[b] ± 7.36

Table 2 Parameters (mean ± SD) evaluated in fresh and post-thawed semen of stallions in different equilibration time (0.5h, 2h, 4h) cryopreserved by the automatic freezer (AF)

Parameter	Fresh	AF 0.5h	AF 2h	AF 4h
TM (%)	80.53[a] ± 1.52	64.77[b] ± 4.94	53.87[b] ± 10.66	51.61[b] ± 10.52
PM (%)	72.60[a] ± 2.77	54.24[b] ± 3.76	45.32[b] ± 9.73	44.32[b] ± 12.89
SYBR-14 (%)	75.02[a] ± 5.04	53.31[b] ± 4.12	46.35[b] ± 1.83	46.30[b] ± 7.59
DRAQ7 (%)	25.72[a] ± 6.23	46.14[b] ± 3.15	49.95[b] ± 3.95	52.95[b] ± 6.23
Yo-Pro-1 (%)	4.62[a] ± 3.07	26.71[b] ± 4.38	31.94[b] ± 12.17	34.41[b] ± 7.12
PNA (%)	7.72[a] ± 4.12	26.65[b] ± 5.14	34.96[b] ± 3.42	27.63[b] ± 5.69
MT Green (%)	77.19[a] ± 5.37	41.13[b] ± 6.56	36.19[b] ± 16.01	37.73[b] ± 9.56
CellRox (%)	3.02[a] ± 1.02	8.19[b] ± 4.97	9.83[b] ± 5.58	9.87[b] ± 5.32

Stallion sperm are extremely sensitive to cell alterations generated by freezing, osmotic changes induced during the process and osmotic stress resulting from exposure to hypertonic media (DEVIREDDY et al., 2002). Results of the present study are consistent with previous studies indicating that freeze-thawing procedures damage the plasma membrane and lead to decrease motility of stallion spermatozoa (ORTEGA-FERRUSOLA et al., 2008; HOFMANN et al., 2011). Similar to other studies, results of the present study indicate that cryopreservation was associated with increased in ROS production (NEILD et al., 2003; BALL, 2008). Osmotic shock (OS) has long been associated with and a major factor in sperm damage during cryopreservation; and while this statement still holds true, newer research demonstrates it is just one potential problem. The influx of hypertonic concentrations while freezing, and the hypotonic concentrations when thawing have been shown to

induce OS which has been shown to be detrimental to the integrity of sperm cells. Somatic cells have been well documented to show that OS is responsible for apoptosis, cell cycle arrest, DNA damage, oxidative stress as well as a variety of other actions (SALAMON and MAXWELL, 2000). This is especially true in stallions, as spermatozoa have a very limited osmotic threshold (DIETZ et al., 2007). Studies have shown that stallion sperm damaged during flash freezing and morphologically abnormal sperm generate greater amounts of ROS (PAGL et al., 2006). Present study showed that cryopreservation by automatic freezer didn´t rapidly increase ROS.

Conclusion and recommendation

Summing up the results, we concluded that automatic freezer and 0.5 h equilibration could be successfully use for cryopreservation of stallion semen. Nevertheless, results of this study are of preliminary character. Experiments using higher number of samples need to be tested in order to find the best procedure for semen cryopreservation of Slovak national horse breeds.

Acknowledgement

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Examination of the vitality of black-colour (Hortobágy) Hungarian racka lambs

OLÁH, János[1*] – POSTA, János[2] – ZOKOLICZA, József[2]

[1]Farm and Regional Research Institute of Debrecen, University of Debrecen, H-4032 Debrecen, Böszörményi str 138., Hungary

[2]Department of Animal Husbandry, Institute of Animal Science, Biotechnology and Nature Conservation, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen, H- 4032 Debrecen, Böszörményi str 138. Hungary

*corresponding author: olahja@agr.unideb.hu

Abstract

Hungary's sheep population is decreasing nowadays. On the first of December 2017, the herd consisted of more than 1.1 million sheep, and by June 2021, this number had barely reached 953 thousand. Based on data from the Central Statistical Office, on December 1, 2020, the sheep population numbered a total of 944 thousand individuals, of which 754 thousand were ewes (HCSO, 2020). Of this, native breeds account for 2.5%, and nearly half of this is accounted for by the Hungarian racka sheep (Hortobágy). This amounts to approximately ten thousand individuals (BODNÁR et al., 2016). For this small number of sheep, it is important to preserve their genetic values and avoiding inbreeding is even more important. This is particularly related to the fact that we need to examine the vitality of the lambs in order to produce even better breeding animals. It is important to monitor the behaviour of the sheep, the course of calving and the mother-lamb relationship, as this is of great importance in the development of technology. The observation of our farm animals influences feeding and husbandry technologies, thus we can create a better environment for them (BODNÁR et al., 2015).

Keywords: sheep breeding, lamb rearing, lamb vitality

Introduction

In sheep breeding, raising and selling lambs is very important, as this is one of the biggest sources of income in the sector. Particular attention must be paid to the vitality of the lamb. The indigenous breed that participated in the study was the Hortobágy racka and its lambs. The official name of our indigenous Hungarian sheep breed today is the (Hortobágyi) Hungarian racka sheep. It is a privilege to breed such a breed of sheep and raise its lambs, especially since it has outstanding national value. In Hungary, the preservation of the indigenous gene bank is extremely important.

Raising lambs requires a lot of time, persistence and different practices. Assisting with a difficult calving is essential, as this saves the life of the ewe and it's lamb. In these cases, we decide for ourselves whether the intervention is necessary or whether it will be economically profitable for us in the future. If the ewe is blessed with good lamb-raising skills and the lamb was born healthy, then there is no need for intervention.

In the case of triplets or more lambs, intervention is recommended and usually necessary. Ewes with low milk production have a harder time raising their lambs or cannot raise them at all. The intervention is also justified for lambs born with low birth weight and weak vitality, and even for mothers with poor nurturing qualities. Therefore, one of the most important goals is to examine the lamb's vitality, because we have to preserve the genetic values of our native national sheep breed, and another main breeding goal is to ensure that the good maternal qualities, high resistance and the strength of the breed are passed on to our breeding animals.

Material and method

The examined racka sheep were placed in harems on October 5, 2019 and were set until November 10. On average, forty sheep were assigned to a pedigree racka ram and a harem of ten was set up. They started calving on March 5, 2020, and the last calving took place on April 3. The lambing period took place in one month, and during this time the sheep were given scores. The difficulty of calving, the vitality of the lambs and their suckling ability were scored. During the experiment, 365 ewes and their lamb offspring were scored. Of course, the origin and age of each sheep is known. A total of 359 live-born lambs were scored. The ear number of the ewe, the ear number of the lamb and the date of calving were recorded. Table 1 shows the number of lambs according to gender and litter size. The gender marked with the number 1 is the ram and the gender marked with the number 2 is the ewe. A total of 176 rams and 183 ewe lambs participated in the study. As for the number of litters, litter size 1 was 329 and 30 twin lambs were born. As for the number of the litter, there were 329 with 1 litter and 30 twin lambs were born.

Table 1: Grouping of lambs by gender and litter size

	(1-ram; 2-ewe)	Number of animals
Sex of lamb	1	176 pcs
	2	183 pcs
Litter size	1	329 pcs
	2	30 pcs

Oestrus of the racka sheep is particularly seasonal, it ovulates once a year in the spring. Every year, the mating season starts at the end of summer and lasts an average of six weeks. Mating takes place in harems based on a program prepared in advance by the Hungarian Sheep and Goat Breeding Association. In general, 40-60 ewes can be placed in a harem. Thanks to these procedures, inbreeding can be avoided and the origin is known.

The data was analysed with Microsoft Excel 2016 and IBM SPSS Web Report statistical program. Analysis of variance was used to determine the differences between the data.

Ewes and their lambs were given scores based on the following table (Table 2).

Table 2: Scoring system for calving difficulty, lamb vitality and suckling ability

	Score: 0	Score: 1	Score: 2	Score: 3	Score: 4
Calving difficulty	Easy, unassisted, uncomplicated, short birth within 30 minutes.	Easy, unassisted, uncomplicated, longer, longer than 30 minutes.	Requires less help, is easy with assistance, does not require much effort, and does not take too long.	Difficult, protracted calving requiring greater intervention, even with assistance, with greater exertion.	Veterinary intervention is required.
Lamb vitality (recording within 5 minutes of lambing)	An extremely active and powerful lamb that stands up within 5 minutes of birth.	A very active and powerful lamb, able to stand up on its knees and hind legs shortly after birth.	An active sheep that can support its own body weight on the chest and knees.	A weak lamb lying on its side but able to lift its head.	A very weak lamb, lying on its side and unable to lift its head, shows very little movement.

Lamb's sucking ability	The lamb suckles well without assistance within 1 hour after birth.	The lamb suckles without assistance within 2 hours of birth.	The lamb requires assistance with suckling, which is required no more than 2 times in the first 24 hours after birth.	The lamb requires assistance with suckling, which is required at least 2 times and for more than 24 hours but less than 3 days after birth.	The lamb requires help with suckling for more than 3 days after birth.

Source: SheepNet-Tip&Trick, Scotland's Rural College (SRUC), United Kingdom (2019)

Results and conclusions

214 individuals performed exceptionally well and it can be said that the majority of the flock had easy lambing. The data support the easy calving characteristics of the Hungarian sheep. The frequency of lamb vitality 193 individuals from the studied flock have exceptionally good vitality. These data also confirm that the vitality of Hungarian sheep lambs is very good. Among the lambs born, 180 individuals performed very well in the examination of suckling ability. Lambs with higher vitality found the udder easier and faster than individuals with lower vitality. Rams show a value of 0.71a and jerks a value of 0.33b for sucking. In general, rams exhibited much less vital behaviour either after calving or during suckling.
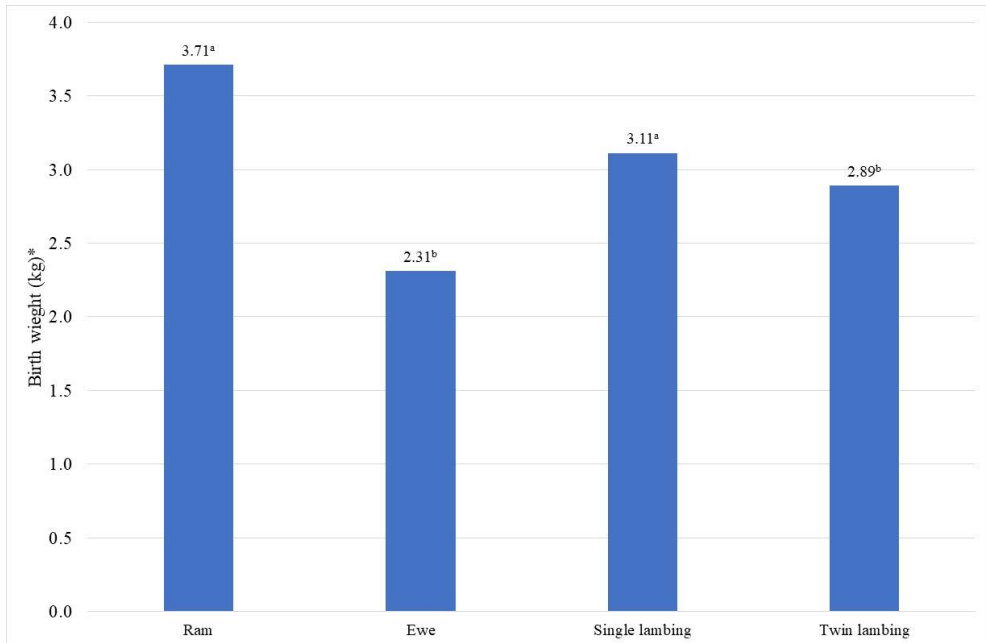
Figure 1: Correlation between birth weight and litter size

Figure 1 clearly illustrates the significance of birth weight and litter size. Ram lambs belonging to litter type 1 were born with a higher weight than ewe lambs. The significance value for rams is 3.71a, for ewes 2.31b. Litter number type 1 had a value of 3.11, litter type 2 had a value of 2.89. The gender of the lambs affects the vitality, as shown in figure 14. The significance value for rams is 0.93a, and for ewes 0.26.

Conclusions and recommendations

Based on the reproduction tests of the Hungarian racka sheep of black colour (Hortobágy), 306 out of 359 individuals calving easily, which is 85%. 8.3% of the herd are twin lambs, the rest are single lambs. The death rate was 2% in total, which can be reduced even more by continuous monitoring of the lambs. It is important for every sheep farmer to be able to raise as many lambs as possible and to keep them for breeding or to sell them. Therefore, lambs should be given due attention after calving and in the post-calving period. The weather plays a very important role in the calving season, as it can determine the day of a calving peak. You have to pay attention to the weather forecast in livestock breeding as well as in crop cultivation. I recommend that the start of the harem be brought forward by two weeks, so that the ewes start breeding sooner and those lambs that do not pass the evaluation of the breed characteristics can be sold at the lamb sale at Easter. It is also worth culling

the ram or rams whose lambs have lower vitality. Those lambs that had very good vitality and suckling ability in lamb age should be kept for further breeding, as these qualities can be inherited further.

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Bestimmung des Generationsintervalls in der Gyimeser Racka und seine Bedeutung bei gefährdeten Schafrassen

SCHÜTZ, Lea[1] – GULYÁS, László[2] – WAGENHOFFER, Zsombor[1] – SÁFÁR, László[3] – BECSKEI, Zsolt[4] – GÁSPÁRDY, András[1] – KÁRPÁTI, Edina[5]*

[1]Institut für Tierzucht, Tierernährung und Labortierkunde, Veterinärmedizinische Universität Budapest, István Str. 2, 1078 Budapest, Ungarn
[2]Institut für Tierwissenschaften, Albert-Kázmér-Fakultät von Mosonmagyaróvár, Széchenyi István Universität, Vár Platz 2, 9200 Mosonmagyaróvár, Ungarn
[3]Verband für Ungarische Schaf- und Ziegenzüchter, Lőportár Str. 16, 1134 Budapest, Ungarn
[4]Lehrstuhl für Tierzucht und Genetik, Tierärztliche Fakultät, Belgrad Universität, Bulevar Oslobođenja 18, 11000 Belgrad, Serbien
[5]Antal Wittmann Multidisziplinäre Doktorandenschule für Pflanzen-, Tier- und Lebensmittelwissenschaften, Széchenyi István Universität, Egyetem Platz 1, 9026 Győr, Ungarn

*Korrespondenzautor: edina.karpati@gmail.com

Zusammenfassung

Die Idee, gefährdete Haustiere zu erhalten, ist nicht neu. Trotzdem haben viele dieser Rassen dokumentierte Abstammungen, die nur wenige Jahrzehnte zurückreichen. Das ungarische Stammbuch der Schafrasse Gyimeser Racka wurde 2005 gegründet. Ziel der Autoren ist es, anhand des Pedigrees die Länge des Generationsintervalls bei dieser Rasse zu bestimmen und mit entsprechenden Ergebnissen anderer Rassen zu vergleichen. Den längsten Generationsabstand wurde in der Mutter-Lamm-Beziehungen (4,13 und 4,63 Jahre) gefunden. Dies der Wert für die Widder produzierenden Mütter war 4,00 Jahre, aber interessanterweise liegen die Mütter, die mit weiblichen Nachkommen zur Fortpflanzung beitragen, nicht viel darüber (4,14 Jahre, P>0,05). Der kürzeste Abstand zwischen aufeinanderfolgenden Generationen konnte zwischen den Zuchtböcken und ihren werdenden Zucht- und Nutz-Lämmernachkommen ermittelt werden (3,28 bzw. 3,69 Jahre). In der Beziehung zwischen den Vatertieren und ihren Zuchtsöhnen war dieser Wert noch niedriger (3,14 Jahre). Väterlicherseits bekommen wir niedrigere Werte, weil die Böcke häufiger ausgewechselt werden und kürzer in der Zucht bleiben. Mütter hingegen

bleiben ein Jahr länger in der Zucht (P<0,05). Innerhalb der oben genannten Routen gab es jedoch keinen nachgewiesenen Unterschied (P>0,05) in Abhängigkeit vom Geschlecht der Lämmer mehr. Sowohl niedrigere als auch höhere Werte als diese finden sich in der Literatur, hauptsächlich abhängig davon, wie die Beziehungen zwischen Eltern und Nachkommen definiert wurden. Bei gefährdeten Haustieren unter Genschutz ist es vorteilhaft, wenn das Generationsintervall lang ist, da hier der jährliche durchschnittliche Rückgang der genetischen Vielfalt nicht signifikant ist.

Schlüsselwörter: Generationsintervall, Rassenerhaltung, Stammbaumanalyse

Einleitung

Im südlichen Teil der Karpaten entwickelte sich zu Beginn des 19. Jahrhunderts eine spezifische Variante der Racka-Gruppe. Aufgrund seines Fells war dieses Mischwollschaf kleinwüchsig und widerstandsfähig gegen das kalte Bergwetter. Es war ein charakteristischer Dreinutzungstyp (DRĂGĂNESCU und GROSU, 2010). Rumänische Hirten hielten einzelne dieser Schafe in Teilgebieten der Walachen, welche als Turcana (türkische Schafe) bezeichnet wurden, während Tiere, von den Szeklers in Siebenbürgen gehalten, Gyimeser (Havasi) Racka genannt wurden. Als Valaska (Walachenschaf) wurden die Tiere bezeichnet, welche von Vlachschen Hirten an den nördlichen Ausläufern der Karpaten angesiedelt wurden.

Die Wurzeln des Gyimeser Racka reichen in unserem Land Hunderte von Jahre zurück. Die Rasse wurde Anfang der 1990er Jahre in Ungarn eingeführt, um diesen historischen ungarischen Rassen zu erhalten. Die Ziele zur Erhaltung der Rasse sind daher nicht so ausgereift wie bei anderen einheimischen ungarischen Rassen. Dieser Zeitraum ist zu kurz bemessen, um aus den bisher gesammelten verlässlichen Daten weitreichende Schlüsse für die Zucht zu ziehen (FÖLDI et al., 2017). Heute besteht die Herde der Gyimeser Racka-Mutterschafe aus fast 1.000 Individuen. Es hat charakteristisch breit gebogene Spiralhörner, die bei Widdern länger sind (GÁSPÁRDY, 2011). Ihre Mischwolle ist schmutzig weiß, nicht selten mit bunten Flecken gesprenkelt. Der Kopf und die Enden der Beine sind meist schwarz mit kleinen Flecken, oft bildet sich um die Augen herum ein schwarzer Fleck (Brillen; KOPPÁNY, 2002).

Stammbaumdaten können auf verschiedene Weise analysiert werden, eine davon ist die Bewertung des Generationsintervalls. Das Generationsintervall (GI) ist das Durchschnittsalter der Eltern bei der Geburt der Nachkommen (LUSH, 1945). Dies ist, unter Berücksichtigung des Gentransfers, genauer gesagt das Durchschnittsalter der Eltern bei der Geburt der Nachkommen, die als Zuchttiere an der Entstehung der nächsten Generation beteiligt sind. Als Bezeichnung der ersten Deutung haben

MCMANUS et al. (2019) das Durchschnittsalter der Eltern (mean age of parents) vorgeschlagen, und den zweiten das Generationsintervall (generation interval). Falls das Ziel in einer Zucht auf Erhalt der Population ausgerichtet ist, ist ein langes Generationsintervall als sinnvoll zu erachten (GROENEVELD, 2009).

Die Literatur liefert unterschiedliche Angaben zur GI von Schafen. GoYACHE et al. (2003) berichten Generationsintervalle zwischen 2,67-2,87 Jahren auf der väterlichen Seite und 3,11-3,23 Jahren auf der mütterlichen Seite bei gefährdeten Xalda-Schafen. Bei Nilagiri-Schafen in Indien haben VENKATARAMANAN et al. (2013) das GI anhand von Stammbaumdaten aus 48 Jahren analysiert. Sie definierten seine Dauer mit 2,55 Jahren in der väterlichen Beziehung und 4,15 Jahren in der mütterlichen Beziehung. Ein zusätzlicher Nutzen dieser Arbeit besteht darin, dass die Autoren auf die beobachtete Abnahme des GI der mütterlichen Seite im Laufe der Zeit aufmerksam machten. Im Gegensatz dazu haben ORAVCOVÁ und KRUPA (2011) einen Anstieg des GI (von 4,70-5,36 auf 6,84-6,94 Jahre) in der Referenzpopulation der gefährdeten Rasse Valaska am Ende des Verarbeitungszeitraums festgestellt. RAFTER et al. (2022) untersuchten den GI in Bezug auf Lämmer, die im angegebenen Jahr (2021) geboren wurden. Sie kamen zu dem Ergebnis, dass bei modernen Rassen, unabhängig von der Rasse, das GI zwischen Vater und Nachkommen immer am kürzesten ist. Das GI zwischen Vater und Nachkommen pro Rasse lag zwischen 2,0 Jahren (Texel) und 2,9 Jahren (Charollais), während das Generationsintervall zwischen Mutter und Nachkommen zwischen 3,2 Jahren (Belclare) und 3,8 Jahren (Galway) lag. Stammbaumdaten von mehr als 20 Jahren (1988 und 2011) wurden von MOKHTARI et al. (2015) über iranische Moghani-Schafe analysiert. Das durchschnittliche GI betrug 4,48 Jahre, das sich wie folgt näher aufschlüsselt: Vater-Sohn = 4,51 Jahre, Vater-Tochter = 4,05 Jahre, Mutter-Sohn = 4,94 Jahre und Mutter-Tochter = 4,43 Jahre. Die höhere Zahl auf der weiblichen Seite deutet darauf hin, dass Muttertiere im Allgemeinen länger in der Zucht verbleiben als Böcke.

Das Ziel dieser Forschung war es, die Länge des Generationsintervalls basierend auf den Stammbaumdaten der Gyimeser Racka zu bestimmen. Die gewonnenen Ergebnisse sollen die Züchter dabei unterstützen, die angestrebten Generationsabstände verschiedener „Zuchtpfade" zum Erhalt der genetischen Vielfalt zu erreichen.

Material und Methode

Wir haben die Datenbank des Verbandes für Ungarische Schaf- und Ziegenzüchter (MJKSZ) (2005-2020) verwendet, um den Generationsintervall der Gyimeser Rackapopulation zu bestimmen.

Das Generationsintervall (weit und eng interpretiert) wurde berechnet, indem wir die Geburtsdaten der registrierten Individuen (Eltern und ihre Nachkommen) in alle vier Pfade (Vater-Sohn, Vater-Tochter, Mutter-Sohn, Mutter-Tochter) unterteilt haben. Die Länge des Generationsintervalls gemäß den Pfaden wurde durch einfaktorielle Varianzanalyse bestimmt. Die verifizierte Differenz zwischen den Mittelwerten wurde mit dem Tukey HSD (honest significant difference) Test für ungleiches N untersucht (TIBCO Software Inc., 2020).

Ergebnisse und Auswertung

Der Stammbaum des Gyimeser Rackabestandes (zwischen 2005 und 2020) enthält die Daten von insgesamt 16.947 Individuen, einschließlich der Gründertiere, deren Geburtsdatum unbekannt ist. Als Ergebnis haben wir 15.308 Zuchtpfade gefunden, bei denen das Geburtsdatum sowohl der Eltern als auch der Nachkommen bekannt ist.

Die Tabelle 1 zeigt, dass das Generationsintervall, welches das Durchschnittsalter der Eltern angibt, bei der Geburt ihrer gesamten Nachkommen ca. 4 Jahre beträgt. Die detaillierten Eltern-Nachkommen Verbindungen, je nach Geschlecht der Eltern und der Nutzung des Nachwuchses, weisen bemerkenswerte Unterschiede in der Länge der Generationsintervalle auf. Diese sind einerseits in dem Vater-Nachkommen Pfad kürzer als in dem Mutter-Nachkommen Pfad, andererseits sind diese in der Eltern-Zuchtnachkommen Beziehung auch statistisch nachweisbar kürzer als in der Eltern-Nutznachkommen Beziehung. Der längste Generationsabstand wurde in der Mutter-Nutzlamm-Beziehungen (4,63 Jahre) gefunden. Der kürzeste Abstand zwischen aufeinanderfolgenden Generationen konnte zwischen den Zuchtböcken und ihren werdenden Zuchtlämmernachkommen ermittelt werden (3,28 Jahre).

Veränderte Tendenzen können festgestellt werden, in der Länge der Generationsintervalle in Bezug auf die Pfade Eltern zu Nachkommen, welche für die Weiterzucht genutzt werden. Hier zeigt sich, dass es einen signifikanten Unterschied in der Entwicklung des Generationsintervalls nach dem Geschlecht des Elternteils gibt, dieser jedoch beim Geschlecht der Nachkommen naturgemäß nicht festzustellen ist $P > 0,05$. Der Wert für die Widder produzierenden Mütter war 4,00 Jahre, interessanterweise liegen die Mütter, die mit weiblichen Nachkommen zur Fortpflanzung beitragen, nicht viel darüber (4,14 Jahre, $P > 0,05$). In der Beziehung zwischen den Vatertieren und ihren Zuchtsöhnen war dieser Wert noch niedriger (3,14 Jahre). Väterlicherseits erhielten wir niedrigere Werte, weil die Böcke häufiger

ausgewechselt wurden und kürzer in der Zucht verblieben, wohingegen mit Muttertieren ein Jahr länger gezüchtet wurde (P<0,05).

Tabelle 1: Generationsintervalle der Rasse Gyimeser Racka nach Pfaden

Eltern – NK Pfaden	N	Mittelwert	-95% untere KL	+95% obere KL	Standardfehler des Mittelwerts
Eltern – NK	15308	4,04	4,01	4,07	0,015979
Vater – Zucht-NK	1168	3,28[a]	3,20	3,36	0,042906
Vater – Nutz-NK	7410	3,69[b]	3,65	3,73	0,020194
Mutter – Zucht-NK	921	4,13[c]	4,00	4,26	0,065120
Mutter – Nutz-NK	5809	4,63[d]	4,57	4,69	0,028661
Eltern – Zucht-NK	2089	3,66	3,58	3,73	0,038529
Vater – Zuchtsohn	99	3,14[a]	2,88	3,40	0,129071
Vater – Zuchttochter	1069	3,29[a]	3,20	3,38	0,045326
Mutter – Zuchtsohn	80	4,00[b]	3,59	4,41	0,205985
Mutter – Zuchttochter	841	4,14[b]	4,01	4,28	0,068590

NK – Nachkommen, KL – Konfidenzlimit

[a, b, c, d] – P<0,001 Tukey HSD (honest significant difference) für ungleiches N

ORAVCOVÁ und KRUPA (2011) haben in ihrer Untersuchung der slowakischen Valaska-Rasse (Stammdaten 1986-2010) Werte von 3,52 (Vater-Sohn) und 2,64 Jahre (Vater-Tochter) auf der väterlichen Seite erhalten. Auf der Mutterseite betrug die Länge des Generationsabstands 4,70 (Mutter-Sohn) und 5,36 Jahre (Mutter-Tochter). Unsere Werte waren auf der väterlichen Seite ähnlich, auf der mütterlichen Seite jedoch höher.

Besonders in der Population der Romanov-Nukleus-Population zeigten DANCHIN-BURGE et al. (2010) einen signifikanten Unterschied in der Länge des Generationsabstands nach Geschlecht der Nachkommen (Vater-Sohn = 1,9 Jahre, Vater-Tochter = 4,0 Jahre, Mutter-Sohn = 2,0 Jahre und Mutter-Tochter = 3,8 Jahre). Die Ähnlichkeit der mütterlichen und väterlichen Pfade ist von großem Vorteil bei der Aufrechterhaltung einer seltenen oder gefährdeten Rasse. ARKENBERG (2014) beschrieb eine andere alte ungarische Schafrasse, die Zigaya (Cigája), bei der die Pfade wie folgt waren: Vater - Nachkomme 4,10 und Mutter - Nachkomme 4,46 Jahre (P=NS).

Schlussfolgerungen und Empfehlungen

Unsere eigenen Ergebnisse zeigen, dass die zur weiteren Zucht verbleibenden Nachkommen von jüngeren Eltern geboren werden, d.h. jedoch aus Sicht der

Erhaltung der Rasse und der Charaktere ist der GI als ungünstiger zu betrachten. Diese Werte sind in Zukunft zu erhöhen, wenn eine mögliche jährliche genetische Veränderung verhindert werden soll. Wir halten eine Strategie für denkbar, bei der Nutztiere aus früheren, jüngeren Lammungen (1-2) genommen werden und Zuchttiere aus späteren Lammungen (ab 3-4 Jahren).

Wir erachten es als wichtig, diese Ergebnisse auch Züchter zukommen zu lassen. Je kleiner eine Rasse und je ärmer sie an genetischer Vielfalt ist, desto sinnvoller ist es, das Generationsintervall zu verlängern.

JOAKIMSEN (1969) hatte zuvor eine grafische Darstellung des Alters der Eltern zum Zeitpunkt der Geburt ihrer Nachkommen präsentiert und auf deren schiefe Verteilung aufmerksam gemacht. Bei seiner Bearbeitung hat er die Daten jedoch nicht normalisiert, soweit wir wissen, hat dies kein anderer Autor getan.

Für die Beschreibung der Eigenschaften, bezogen auf die Länge der Generationsintervalle, nach ARKENBERG (2014) ist der Median zuverlässiger als der Mittelwert. Das Alter der Elterntiere war in der von uns untersuchten Population der Gyimeser Racka ähnlich, daher empfehlen wir in Zukunft, vor der Datenverarbeitung den Versuch einer Normalisierung der Basisdaten zu unternehmen.

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Determination of the generation interval in the Gyimes Racka and its importance in endangered sheep breeds

Abstract

The idea of preserving endangered domestic animal breeds is not new. Despite this, many of these breeds have documented ancestry dating back only a few decades. The Hungarian stud book of the Gyimes Racka breed of sheep was established in 2005. The aim of the authors is to use the pedigree to determine the length of the generation interval in this breed and to compare it with the corresponding results from other breeds. The longest generational gap was found in mother-lamb relationships (4.13 and 4.63 years). This value for the ram-producing mothers was 4.00 years but interestingly the mothers producing female offspring lag behind contribute to reproduction, not much above (4.14 years, P>0.05). The shortest distance between successive generations was found between the breeding rams and their breeding and non-breeding progenies (3.28 and 3.69 years, respectively). In the relationship between the sires and their breeding sons, this value is even lower (3.14 years). On the father's side we get lower values because the rams are changed more often and remain in breeding for a shorter time. Mothers, on the other hand, stay in breeding one year longer (P<0.05). However, within the paths mentioned above, there was no longer any proven difference (P>0.05) depending on the sex of the lambs. Both lower and higher values than these can be found in the literature, depending mainly on how parent-offspring relationships have been defined. In the case of endangered domestic animals, it is advantageous if the generation interval is long, as here the annual mean decrease in genetic diversity is not significant.

Key words: generation interval, breed preservation, pedigree analysis

Looking for added value of milk products of autochthonous sheep breeds

BECSKEI, Zsolt[1]* – PASKAŠ, Snežana[2] – SAVIĆ, Mila[2] – TARIĆ, Elmin[2] – GÁSPÁRDY, András[3] – PAJIĆ, Marija[4] – RAŠETA, Mladen[5] – LAZAROVA, Mariya[6]

[1]Department of Animal Breeding and Genetics, Faculty of Veterinary Medicine University of Belgrade, Belgrade, Serbia
[2]Department of Animal Science, Faculty of Agriculture University of Novi Sad, Novi Sad, Serbia
[3]Department of Animal Breeding and Genetics, University of Veterinary Medicine, Budapest, Hungary
[4]Department of Veterinary Medicine, Faculty of Agriculture, University of Novi Sad, Novi Sad, Serbia
[5]Institute of Meat Hygiene and Technology, Belgrade, Serbia
[6]Department of Animal Husbandry - Ruminants and Dairy Farming, Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria

*corresponding author: beckeizolt@gmail.com

Abstract

In order to determine the added value of the product, characterization of sheep milk and cheese samples from Sjenica sheep were assessed and compared to British milk sheep. The analyses were carried out according to standard chemical procedures. A comparative study showed that the milk from the Sjenica sheep was higher in fat and protein and had a lower casein/fat ratio. It also had higher total and somatic cell counts than British milk sheep. Sjenica cheese had significantly higher dry matter and fat content. However, both cheeses were classified as soft full-fat cheese with brine. The study showed that sheep's milk contains many essential components for cheesemaking and distinguishes sheep's cheese from other types of cheese, but standardisation of cheese production is still needed. To preserve the autochthonous sheep breeds of Serbia, promoting cheese production with added value is crucial and together with increased production could contribute to the sustainable development of rural areas.

Keywords: sheep, autochthonous breed, added value product, sheep cheese

Introduction

There is a growing awareness of the need for high levels of biodiversity and its important role in improving the sustainability of low-input dairy production systems (POPOVIĆ-VRANJEŠ et al., 2018). Protecting local products means not only preserving the different local ecosystems at different levels: animals and plants (breeds and local varieties), but also microbial ecosystems, landscapes and dairy products (BERARD and MARCHENAY, 2006). Although many laws and measures have been adopted in Serbia for the protection of autochthonous breeds and genetic resources, many of them are still at risk of complete disappearance (POPOVIĆ-VRANJEŠ, 2018). Due to its compositional characteristics, sheep's milk is well suited for cheese production (POPOVIĆ-VRANJEŠ et al., 2017), and in Serbia sheep's milk is mainly used for cheese production. Autochthonous breeds do not produce large volumes of milk, but they have good quality milk with a desirable protein-fat ratio and small, naturally homogenised fat globules (POPOVIĆ-VRANJEŠ, 2018). The average size of fat globules is therefore the smallest (< 3.5 μm) in sheep milk (PARK et al., 2007). Milk quality depends on many factors, and each component of milk influences cheese yield and quality (POPOVIĆ-VRANJEŠ et al., 2018). SOBRINO et al. (2018) found that, except for the casein/fat ratio, the season was the greatest source of variation in bulk tank milk characteristics of Manchega sheep. The relative stability of the C/F ratio made the sheep's milk more standardised, which is necessary for cheese production. Conversely, the farming system (semi-intensive and intensive) did not affect the chemical composition and physicochemical properties of sheep's milk in the study by KASAPIDOU et al. (2021).

Production of sheep's cheese in Serbia is usually at the farm level or in small local dairy units, with only about 370 tonnes of sheep's cheese produced in 2016 (FILIPOVIĆ, 2019). Traditionally associated with high-quality products such as lamb and Sjenica cheese, the Sjenica Pester plateau is characterised by a specific floristic composition of pastures (SAVIĆ et al., 2017). Sjenica sheep cheese is the best-known cheese from this area and is a protected designation of origin (PDO) product (FILIPOVIĆ, 2019). Sjenica cheese is made from either cow's or sheep's milk and belongs to the group of white cheeses. Producers of this cheese face a number of problems. The main challenges for the export of Sjenica cheese are the insufficient certification of this food product for the European Union market and the lack of a standardised production process, which results in variations in the quality of the product. Although white brine cheese is the most consumed type of cheese in Serbia (PASKAŠ et al., 2020), there is a lack of awareness of Sjenica sheep's cheese among regional consumers due to insufficient communication efforts (FILIPOVIĆ, 2019).

This study aimed to investigate the influence of different sheep breeds on the chemical composition and hygienic quality of milk, as well as its suitability for cheese production. A study of the chemical composition of white brine cheese was also carried out.

Material and methods

Milk sampling and analysis
From March to October 2022, samples of raw sheep's milk (n=16; n1=8; n2=8;) were collected from local farms. The farms differed in the breed of sheep reared. On the first farm Sjenica sheep was reared and on the second farm British milk sheep. On each farm, bulk milk samples were collected once a month (at regular intervals) and analysed on the same day. Both flocks were milked by hand twice a day and samples were taken after the morning milking. The standard analysis of the chemical composition of the milk was carried out with the MilkoScan FT+ analyser using the FTIR technique. The MilkoScanTM FT+ techniques comply with the ISO 9622: ISO 9622/IDF 141:2020 standard and the official AOAC method 972.16. FossomaticFT FC (ISO 13366-2:2008) and BactoScanTM FC+ (ISO 16297:2020) were used to determine the somatic cell count and total bacterial count of raw milk.

Cheese sampling and analysis
Cheese samples (n=10; n1=5; n2=5) were collected from the same producers and analysed for chemical composition. Dry matter was determined using a standard method for measuring weight loss after drying (AOAC 926.08-1927). Protein was determined by the Kjeldahl-Van Slyke method for total N (AOAC 2001.14) using a Kjeltec Auto Analyzer and multiplying by 6.38. Fat content was determined according to IDF (2008) and ash was determined by dry ashing at 550°C (AOAC 935.42). Phosphorus was determined using a spectrophotometer (PG Instruments, type T80+) (AOAC 995.11), while flame photometry (Sherwood, type M410) was used for Ca and Na. The NaCl content was determined by the Volhard method (FIL-IDF, 1988).

Statistical analysis
Results were statistically analysed and presented as mean, standard deviation, range and coefficient of variation. A t-test at $p < 0.05$ was used to determine a significant difference between the means. Statistical analysis was performed using Statistica 10 (StatSoft STATISTICA 10.0).

Results and discussion

Table 1 shows the chemical characteristics and hygiene parameters of the sheep milk samples. There was a statistically significant difference in the average fat content between milk samples from two different sheep breeds. The total protein content of Sjenica sheep milk was 5.58 and the fat content was 7.23g/100g. These results are in line with the range previously reported for Sjenica sheep milk (SAVIĆ et al., 2017). Compared to the results of SAVIĆ et al. (2014), the fat content was higher, but the protein and dry matter content were lower. There were no significant differences (p<0.05) in the casein/fat ratio between milk samples from the two different breeds. Both breeds showed a higher variation in the ash content and in the C/F ratio, which can negatively affect cheese production. The results related to the hygienic parameters of milk SCC and TBC showed that the milk from both breeds was of good quality. No difference was found in the hygienic parameters (SCC and TBC), but the results of the milk samples varied greatly in milk from both breeds. Monitoring SCC is a good tool for assessing the hygienic and sanitary quality of milk, but in sheep's milk, it is necessary to consider non-pathological factors that cause large variations in SCC and are different from those in cow's milk (RAYNAL-LJUTOVAC et al., 2007). This study shows the opposite results to those of TONAMO et al. (2020) for the SCC count of British milk sheep. They also differed from the SCC and TBC results in Lacaune sheep milk (MERILIN JUNIOR et al. 2015). SCC in milk affects cheese yield and cheesemaking performance. Milk with a low SCC can yield more than 4% more protein in cheese production than milk with a high SCC (RAYNAL-LJUTOVAC et al., 2007).

Table 1. Physicochemical composition of milk from two different breeds of sheep

Parameters (g/100g)	SJENICA SHEEP MILK			BRITISH MILK SHEEP MILK		
	$\overline{x} \pm$ SD	range	CV (%)	$\overline{x} \pm$ SD	range	CV (%)
Dry matter	17.89±1.06	16.54-19.77	5.93	17.26±0.73	16.09-18.66	4.26
SNF	10.64±0.58	9.79-11.5	5.42	10.99±0.61	10.21-12.20	5.58
Fat	7.23[a]±0.69	6.57-8.5	9.49	6.34[b]±0.34	5.88-7.0	5.31
Protein	5.58±0.49	5.13-6.7	8.80	5.50±0.61	4.33-6.38	11.09
Lactose	4.05±0.37	3.48-4.52	9.03	4.28±0.43	3.6-4.88	10.11
Casein	4.44±0.41	4.06-5.37	9.22	4.47±0.57	3.40-5.16	12.78

Ash	1.03±0.22	0.82-1.46	21.56	1.10±0.19	0.83-1.38	17.56
C/F	0.62±0.07	0.49-0.72	11.71	0.70±0.10	0.46-0.82	14.73
SCC (*1000/ml)	1499.62±457.82	872-2031	30.53	1384.62±484.59	465-2065	34.78
TBC (CFU*1000/ml)	224.87±101.15	124-392	44.98	129.12±76.09	35-240	58.93

SNF-solids non-fat; C/F-casein/fat; SCC-somatic cells count; TBC-total bacteria count; $x \pm$ sd-arithmetic mean + standard deviation; cv-coefficient of variation; Means with different letters are significantly different (p<0.05

Table 2. Chemical composition of white-brined cheese from two different breeds of sheep

Parameters	SJENICA SHEEP CHEESE			BRITISH MILK SHEEP CHEESE		
	$\overline{x} \pm$ **SD**	**range**	**CV (%)**	$\overline{x} \pm$ **SD**	**range**	**CV (%)**
Dry matter (g/100g)	45.77a±1.10	44.80-47.06	2.41	43.77±0.83	42.80b-44.90	1.89
Fat (%)	24.85[a]±0.73	23.89-25.74	2.93	23.71[b]±0.74	22.98-24.68	3.10
Protein (%)	15.45±0.77	14.63-16.25	4.98	14.45±0.86	13.38-15.32	5.95
Total ash (%)	2.35±0.11	2.21-2.51	4.76	2.42±0.12	2.23-2.56	5.18
FDM	54.28±0.72	53.33-55.23	1.33	54.18±1.25	52.62-55.80	2.30
MFFB	72.15[a]±0.90	71.10-73.08	1.25	73.71[b]±0.80	72.75-74.80	1.08
NaCl (%)	3.34[a]±0.40	2.85-3.74	11.88	2.75[b]±0.32	2.22-3.01	11.83
S/M (%)	6.17[a]±0.80	5.18-7.06	12.95	4.88[b]±0.55	4.03-5.34	11.24
Ca (%)	0.38±0.07	0.31-0.48	18.27	0.43±0.06	0.37-0.51	13.34
P (%)	0.24±0.04	0.19-0.28	15.45	0.26±0.03	0.22-0.29	10.24

FDM-fat on a dry matter basis; PDM- a protein on a dry matter basis; MFFB-moisture on a fat-free basis; S/M (%)-salt in moisture; $\overline{x} \pm$ SD-arithmetic mean + standard deviation; cv-coefficient of variation; Means with different letters are significantly different (p<0.05).

Table 2 shows the chemical composition of white-brined cheese from the two different breeds. It was found that dry matter and fat were significantly lower in British sheep's cheese. In a study by JANDRIĆ and SAVIĆ (2019), after 30 days of ripening, Sjenica sheep's milk cheese contained an average of 28.31 g/100 g of milk

fat, while at the beginning of the process, it contained 23.43 g/100 g. The research results for Sjenica cheese were within the mentioned ranges. A statistically significant difference was also found for NaCl content, MFFB and S/M ratio. The salt content of the cheese depends on the salt content of the brine. As the salt concentration in the brine increased, the salt content in the cheese also increased, as did the total ash, fat and protein content (RAHIMI et al., 2013). No difference was found in protein and FDM content, although the milk was not standardised. The dynamics of fat in dry matter showed a tendency of a balanced increase throughout the ripening period, from 49.44% to 52.72% in Sjenica sheep cheese (JANDRIĆ and SAVIĆ, 2019). The values obtained in this study were higher than those mentioned for both cheeses. According to the FDM content, the cheeses were classified as full-fat cheeses, and according to the MFFB, they belonged to the group of soft cheeses (SERBIAN REGULATIONS, 2014). The Ca and P contents of the two sheep cheeses were the most variable components. Ca and P directly influence cheese texture by participating in the protein network that forms the structural matrix of cheese (RAHIMI et al., 2013). Therefore, it was expected that the effect of different Ca and P levels on cheese texture would also vary, which could have an impact on cheese sensory properties.

Conclusion and recommendation

The results of this study showed the favourable composition and properties of Sjenica sheep's milk, with significantly higher fat and dry matter contents compared to British milk sheep's milk. The results also confirmed the good quality of Sjenica white cheese. By adhering to higher standards and good manufacturing practices, sheep's milk and cheese can be produced safely without compromising quality. In particular, the production of Sjenica cheese could help to preserve the genetic diversity of domestic animals. To conserve local species and breeds, more breed-level data is needed and a knowledge base on livestock biodiversity needs to be developed.

To preserve the autochthonous sheep breeds of Serbia, promoting cheese production with added value is crucial and together with increased production could contribute to the sustainable development of rural areas.

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Maternal diversity of the Yellow-faced sheep of Kecskemét based on the mtDNA control region

TULLY, Eilish[1] – HARMAT, Levente[2] – MARÓTI-AGÓTS, Ákos[1] – ZENKE, Petra[1] – KOVÁCS, Endre[1] – GÁSPÁRDY, András[1]*

[1]Institute of Animal Breeding, Nutrition and Laboratory Animal Science, University of Veterinary Medicine Budapest, István utca 2, 1078 Budapest, Hungary
[2]Experimental Farm, University of Veterinary Medicine Budapest, Dóra-major, 2225 Üllő, Hungary

*corresponding author: gaspardy.andras@univet.hu

Abstract

Regarding the Yellow-faced sheep of Kecskemét or Sand-sheep, known as a variant of Yellow-faced Berke sheep, very little research has been undertaken to date and the small population, which is mainly found in the southern region of Hungary, is at risk of extinction. In this study blood samples from Yellow-faced sheep of Kecskemét were selected for Control Region (CR) sequencing and subsequent analysis. These were compared with CR data available from GenBank resources and other indigenous Hungarian breeds in order to compare and contrast the differences and similarities between these. A total of 40 individuals from 2 flocks in the South of Hungary were sampled in 2020. Investigations were performed based on the total number of sites (1174 bps) of the CR. It was revealed that the relative genetic diversity within the Yellow-faced sheep of Kecskemét (haplotype and nucleotide diversity 0.950 and 0.01635, respectively), in comparison to other indigenous breeds, albeit from a limited population, cannot be deemed a narrow genetic pool. The values of the Tajima D test, Fu's Fs statistic, Fu & Li's D*- and F* tests were found to be non-significant (in each case P > 0.10). Statistical evaluation does not indicate a lack of alleles. This study demonstrated three haplogroups within the Yellow-faced sheep of Kecskemét population – A, B and C. Haplogroup B was the most prevalent, which is typical for European sheep breeds, given an understanding about their arrival into Europe from the Near East. This is comparative to other Hungarian breeds, such as the Cikta and Polled Racka, and also similar to the native breeds of neighbouring countries. Regarding Haplogroup A, which was found to a minor frequency, being lower than that found in the Cikta or Polled Racka, but to a

greater extent than Tsigai. To date, haplogroup C, which is typically demonstrated in sheep from Central Asia, had only been discovered in one native Hungarian breed: the Cikta, however its presence was also discovered in the Sand-sheep. The examination of the haplogroups seems to confirm the fact that the sheep came to Hungary not only from Asia Minor, but also from the interior of Asia in the past, and the mitochondrial genetic information of these latter animals maintained in some representatives of today's breeds, surviving the effects of crossbreeding and selection.

Keywords: Yellow-faced sheep of Kecskemét or Sand-sheep, mtDNA control region, maternal origin

Introduction

The yellow-faced sheep from Kecskemét is not an independently accepted breed, it is currently included in the herd book of the yellow-faced Berke (yellow-headed Tsigai) breed together with the Kovásznai (variant of Covasna) sheep in Hungary. Today, this joint official herd book collects the breeding and production data of a total of 80 dams, the same number of hoggets and 12 qualified rams of one state institute and four private farms.

The Tsigai sheep first came to the territory of Hungary at the end of the 18th century (1792) (RODICZKY, 1904). Its colour variants can be separated based on the colour of the fleece and the short hairs. Individuals with black, dark brown, light brown, yellowish-red, white and variegated (spotted) heads and legs were distinguished among the white-fleeced Tsigais. In the course of history, two basic colour variants of the white-wooly Tsigai, also belonging to the mountain type, spread in the Transylvanian parts. One of them is the brown-faced and small-legged, so-called Covasna Tsigai (ruğine, rusty in Romanian). The first, most classic and almost yellow individuals of this version were the Tsigais from Hétfalu. Later, HAMMOND et al. (1961) also mention the reddish head but white or variegated fur and the completely black Romanian Tsigai. In the last decade, the yellow-faced (called also as red-faced) colour variants of the Tsigai native to Covasna (Kovászna) and Harghita (Hargita) counties have entered our country. Since 2016, they have been treated as an independent breed under the name yellow-headed Berke by the Hungarian Sheep and Goat Breeding Association. The other one, to SZENTKIRÁLYI's (1923) unifying proposal, is the variant with a completely black head and legs. The latter spread in other Hungarian historical areas. Further sub-variants were isolated in the Southern Region. The triple-purpose but more meaty csókai (variant of Čoka), the dairy zombori (variant of Sombor with a milk production of over 100 litres), as well as the ancient version, which is now extinct

and most reminiscent of Transylvanian type, is the árpatarlói (variant of Ruma) (ULMANSKI, 1922) and the large-bodied, also good-milking doroszlói (variant of Doroslovo) (KOVACS, 2000) was. All of them have chocolate brown or black head and legs, and white wool. In the territory of today's Hungary, it is worth distinguishing two sub-varieties according to their origin: one is the lowland one, which shows the greatest similarity with the csókai variant, and the other is the mountain one, which entered Jákotpuszta from the Highlands (Slovakia) three decades ago. Almost without exception, they have white fur and dark brown or black heads and legs.

According to the study of PÓCZOS (1934), there is no significant correlation between the colour of the head and the shearing weight and the amount of milk. Between the two world wars, based on the colour of the short hair on the head and legs, 46% of the mothers in the herd of Kisszállás Estate JSCo., originating from the southern region, were chocolate brown, 33% shiny black, 12% brown with snow and 9% reddish (yellow) found it. So, individuals with yellow heads and legs were regularly found in Tsigai flocks. This colour is still spreading today, and we should pay more attention to maintaining it.

The Yellow-faced sheep of Kecskemét, also known as the Sand-sheep, was a well-known and bred sheep throughout the Danube-Tisza region, but in recent decades it can only be found in the sandy wastelands around Kecskemét. Today, their total population in 8 farms can be estimated at around 850. Hornless individuals can be characterized by a uniform yellowish brown (rust brown) colored head and legs. Their skin is pigmented, their claws are slate gray, steely and hard. Their eyes are lively, their udders are well developed, and their ears are particularly long, which can also be linked to their selection for milk production. The neck is usually dewlap-free, although there is a small degree of longitudinal flap of skin, which may be the result of a former Merino effect. Their bones are strong, and their bellies are bulky (HEGEDŰS, 2021). Their fur is white consisting of larger staples, and black fibers may also occur in this. Their wool covers the neck and trunk, sometimes the wool also spreads to the forehead, where it appears as a white spot (rosy forehead). Most of their lambs are brown or dark brown (similar to calf or dog) at birth, but all of them turn white with time. The shepherds of Kecskemét considered only the one with a black spot on some part of the body to be a real Yellow-faced sheep (Figure 1). A lot of emphasis is placed on this even today.

Figure 1. Lamb of Yellow-faced sheep of Kecskemét with a spot on the shoulder

The aim of our study is to characterize the maternal background and maternal genetic diversity of the Yellow-faced sheep of Kecskemét. For this, we use the nucleotide sequence of the control region (CR) of the mitochondrial genome (mtDNA). We assume that as an inclusion variant, it can serve with a specific genetic pattern left over from the past. With our results, we would like to contribute to the genetic identification of the haplotypes (families) of that overlooked variety and its successful maintenance. The control region also carries important phylogenetic information, so our second goal is to place that variant in the kinship network formed by the gene bank control sequences and the sequences of the Hungarian indigenous breeds examined so far.

Material and methods

Presentation of the farms and sampling
On February 18, 2020, we collected biological samples from a total of 36 female and 4 male breeding animals in the herds of László HEGEDŰS in Kunbaracs (Bacsó farm) and then on March 10 in Mihály NÉMET's herd in Szentes (Bokros farm). This represents 50% sampling coverage of the officially registered stock. These two sampled farms purchased their individuals from the same seed stock. Although rams do not pass on the mitochondrial genome, their samples are suitable for assessing the maternal background and the diversity of the breed variant.

DNA purification, amplification and sequencing of the mitochondrial control region
These steps are described in detail in TULLY's (2022) dissertation on the subject.

Evaluation of sequences
The mutations were evaluated with the test developed by FU and LI (1993), then the D-test proposed by TAJIMA (1989) was used as a population genetic evaluation method to analyze the detected mutations. The number of polymorphic base sites of the entire test sample was determined using the DNAsp 6.0 software, and then the average nucleotide difference within and between farms was calculated (ROZAS et al., 2017). We used the method of JUKES and CANTOR to determine the corrected base substitutions within sequences (JUKES, 1990). The distribution of haplotypes was plotted using Network 10.2.00 software (fluxus-engineering.com) (BANDELT et al., 1999). The samples were sorted into haplogroups based on the GenBank reference samples (HIENDLEDER et al., 2002; MEADOWS et al., 2005). Furthermore, we also selected a common representative haplotype sequence for each haplogroup from the samples of the Hungarian indigenous sheep breeds that we have typified so far (Cikta, Tsigai and Lowland Polled Racka) (KOVÁCS et al., 2020; GÁSPÁRDY et al., 2021 and 2022).

Results and discussion

The length of the aligned CR sequences was 1183 bp. During the alignment, data was lost at 9 base positions, so excluding these gaps, we evaluated the mtDNA control region based on a total of 1174 base pairs. In the CR region of the entire study sample, the number of monomorphic base sites was 1061, while the number of polymorphic base sites was 113 with 115 mutations. In the case of the latter, there was a singleton mutation in 25 places and a parsimony mutation in 88 places (with 2 triple mutant versions).
The mean of the nucleotide diversity (π) in the studied herd of Sand-sheep is $16.35*10^{-3}$, and the standard deviation (SD) is $2.31*10^{-3}$. The corrected value of nucleotide diversity according to JUKES and KANTOR is π(JC) $16.63*10^{-3}$. The value of the haplotype diversity (H_d) is 0.950, its standard deviation is $17*10^{-3}$.
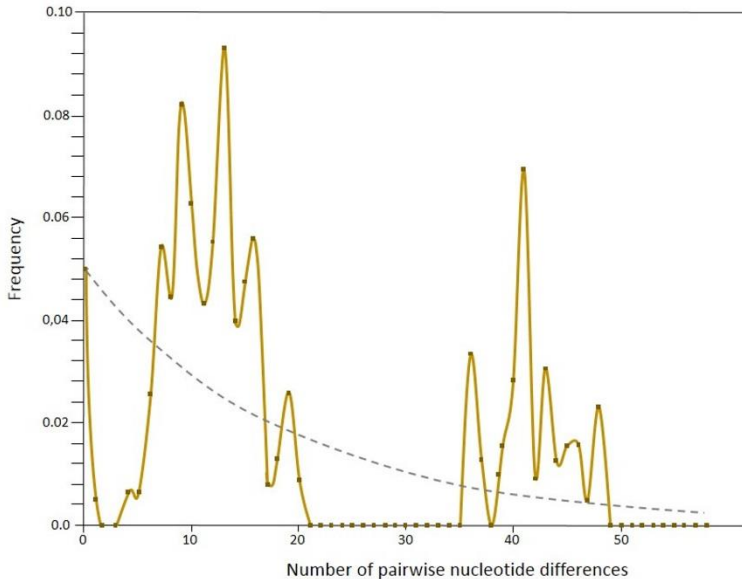
Figure 2. Frequency distribution of the number of sequence mismatches between pairwise combinations of Sand-sheep CR haplotypes

The average number of nucleotide differences between populations was 16.990. The average pairwise nucleotide difference (k) is 19.192. Nevertheless, the nucleotide difference is around 10 in the majority of the herd, and around 40 in the rest (Figure 2). The dashed line represents the expected distribution for a constant stock size; its course is moderate. Points connected by a solid line reflect a bimodal observed distribution. The distinct peaks indicate that there are two dominant groups of haplotypes associated with a relatively constant population size of the Sand-sheep over time.

The average number of nucleotide exchanges per site between plants (D_{xy}) is 14.47×10^{-3}, and the number of net nucleotide exchanges per site between populations (D_a) is 0.80×10^{-3}. Table 1 refers to the DNA divergence between the two sampled populations. More mutations occurred in the Bokros farm, which has a larger number of individuals. Here, the average number of nucleotide differences (k) and nucleotide diversity (π) were higher than in individuals from the Bacsó farm (21.611 versus 10.489 and 18.41×10^{-3} versus 8.93×10^{-3}). There were 26 shared mutations between the populations.

Table 1: DNA divergence between the two farms

Parameter	Bacsó farm	Bokros farm
Number of sequences	10	30
Number of polymorphic sites	30	109
Total number of mutations	30	111
Average number of nucleotide differences (k)	10.489	21.611
Nucleotide diversity (π)	$8.93*10^{-3}$	$18.41*10^{-3}$
Shared mutations	26	

The D* and F* tests of FU and LI performed on the entire study sample did not give significant results, $53.62*10^{-3}$ (p > 0.10) and -0.41194 (p > 0.10), respectively. Similarly, the value of the FU Fs statistic 0.806 was not significant (p = 0.029), just like the result of the TAJIMA D test was -1.06609 (p > 0.10).

The haplogroup and haplotype distribution is shown in Figure 3. The number of CR haplogroups identified in the Sand-sheep was three. The most populous of the haplogroups was B, followed by A, with 34, and 4 individuals, respectively. Furthermore, 2 Sand sheep individuals were included in haplogroup C.

Based on the CR region of the mtDNA of the samples, there are 3 haplogroups and 21 haplotypes. Haplogroups A, B and C were identified. Haplogroup A has 1 haplotype, haplogroup B has 19 haplotypes, while haplogroup C has 1 haplotype. Figure 3 reveals the taxonomic location of the individuals of the Sand-sheep in relation to each other and to the individuals used as controls. The 34 individuals of the Sand-sheep (on both farms) are closely related to the previously defined haplogroup B of the sheep. The mouflon also belongs to this haplogroup. It can be seen that the native breeds Cikta, Tsigai and Polled Racka also show individuals that are in this haplogroup. Four individuals (from both farms) belong to haplogroup A, which is genetically significantly distant from it. Haplogroup C was detected in the Sand-sheep with 2 individuals (only on the Bokros farm). So far, C has only been determined in the Cikta of our domestic sheep. However, it was not possible to detect haplogroups E and D in the Sand-sheep. Urial and Argali, included as out-groups, as distant relatives of the domestic sheep, are clearly separated from all of them.
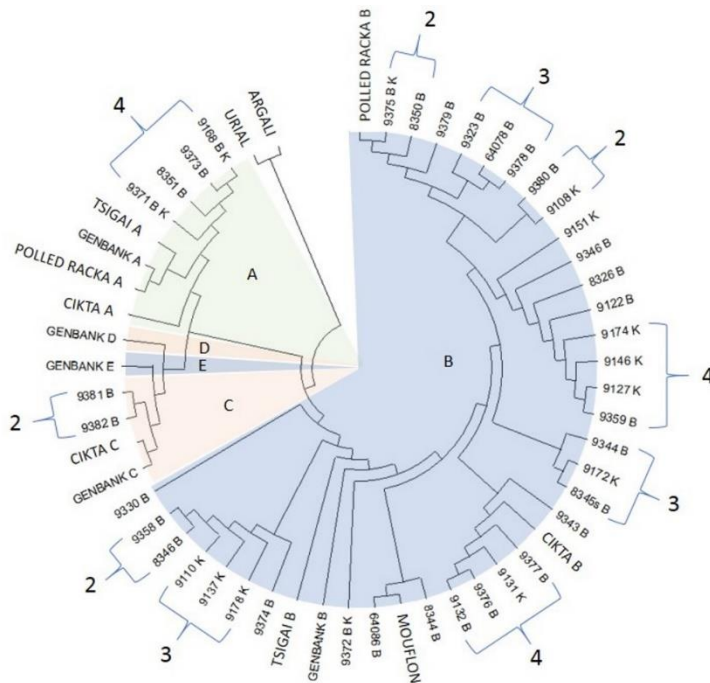
Figure 3. The composition of the Sand-sheep according to CR haplotype and haplogroup. The figure also shows the location of Argali, Urial, Mouflon and the five GenBank haplogroups (A-HM236174, B-HM236176, C-HM236178, D-HM236180, E-HM236182 (MEADOWS et al., 2005); O. musimon Mouflon HM236184, O. ammon Argali HM236188, O. vignei Urial HM236186 (HIENDLEDER et al., 2002), as well as the most common haplotypes of Tsigai, Cikta (KOVÁCS et al., 2020; GÁSPÁRDY et al., 2021) and Polled Racka (GÁSPÁRDY et al., 2022). The capital letters indicate the haplogroups, while the colours associated with the letters indicate that which individual belongs to the given haplogroup. Additional numbers show individuals belonging to the same haplotype, where more than one individual represents a haplotype.

Conclusion and recommendation

Based on the above indicators, it can be concluded that the sand sheep show sufficient maternal mitochondrial (CR) genetic diversity and at the same time a relatively constant population size over time, taking into account the situation of other protected native sheep. If the pairwise nucleotide differences give a unimodal distribution curve, it would indicate that the herd has recently undergone a demographic expansion; a sudden expansion with foreign individuals.

Despite the fact that the breed has bred in itself, it cannot be considered to have a narrow genetic stock in terms of molecular microevolution either. The TAJIMA D test, FU's Fs statistic, FU & LI's D* and F* test values are not significant (in each case p > 0.10). Our statistical evaluation does not indicate a lack of alleles, nor does it indicate a narrowing of the genetic diversity occurring in the history of the population.

Haplogroup B is the most common among Sand-sheep specimens (34.85%). This haplogroup is characteristic of European sheep domesticated in the Middle East, and correspondingly also of other native Hungarian sheep (97% in Tsigai, 81% in Cikta and 86% in Polled Racka). Investigations carried out in the neighbouring countries of the Balkan Peninsula pointed out that the origin of the Eastern Adriatic sheep breeds (FERENCAKOVIC et al., 2013) and the Romanian breeds (Turcana, Tsigai and Black-headed Ruda) (DUDU et al., 2016) is unanimously attributed to haplogroup B mothers. In our case, haplogroup A appeared with a lower frequency (4.10%). In the case of Cikta and Polled Racka sheep, the proportion of haplogroup A is 12% and 14%, respectively (KOVÁCS et al., 2020; GÁSPÁRDY et al., 2022), while this haplogroup is also present in Tsigai, but much less typically (3%) (GÁSPÁRDY et al., 2021). The haplogroup B is also dominant in the Western Balkan Pramenka sheep (ĆINKULOV et al., 2008), Dubrovnik Ruda sheep and Istrian sheep (FERENCAKOVIC et al., 2013), but also few individuals are separated from it as belonging to haplogroup A. In our study, it can be considered a very special result that we observed the occurrence of haplogroup C in Sand-sheep (2.5%). This haplogroup is characteristic of Inner Asia (GANBOLD et al., 2019; CHEN et al., 2006; SULAIMAN et al., 2010) and the Indian subcontinent (77%) (LV et al., 2015) and has so far only been detected in Cikta (9%) of the Hungarian native breeds. In Europe, haplogroup C has only been found in the Iberian Peninsula (Portugal (PEREIRA et al., 2006) and Spain (PEDROSA et al., 2007)), Italy (MARIOTTI et al., 2013) and the southern countries of the Balkan Peninsula (Albania and Greece (PARISET et al., 2011). The presence of haplogroup C supports the view that the Sand-sheep or Yellow-face sheep of Kecskemét has a complex maternal background. The examination of the haplogroups seems to confirm the fact that sheep came to Hungary not only from Asia Minor, but also from the interior of Asia in the past, and the mitochondrial genetic information of the latter survived the effects of crossing and selection and was preserved in the representatives of today's breeds.

However, the narrowing of genetic diversity resulting from inbreeding can generally be predicted for that breed variant. Therefore, as a future plan, we intend to set the further characterization of the Yellow-faced sheep of Kecskemét with the inclusion of nuclear markers and microsatellites, as well as its comparison with the Yellow-faced sheep of Covasna, in addition to joint pedigree registration, to what extent the mutual use of breeding rams can be recommended in terms of blood refreshment of the two variants.

Acknowledgement

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