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Danubian Animal Genetic Resources

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of Animal Breeds in the Danube Region
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Research articles

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Studies of colostrum and milk composition and quality of Gidran mares

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Abstract

Foals are born with intact but weakly functioning immune systems. Colostrum provides special immunoglobulins, which are essential for the development of passive immunity. The newborn foal is entirely dependent on antibodies from colostrum for protection against infection during the early neonatal period, meaning that after foaling, in a short time, they need to pick up a sufficient amount of colostrum in proper quality. The quantity and quality of colostrum cannot be influenced although it is well known that well-fed mares in good health typically produce enough antibody-rich colostrum for their newborn foals, the quality of colostrum can be determined by several methods. In 2022, I examined the gestation and lactation of 9 Gidran mares at Kismacs Experimental Station of Animal Husbandry of the University of Debrecen. I used a BRIX refractometer for measuring the colostrum IgG level, and a Lactoscan MCC Combo milk analyser for testing colostrum and milk.

Keywords: colostrum, immunoglobulins, foal, Gidran

Introduction

Successful breeding makes to a qualitative change. The goal is to make offspring to be better than their ancestry. Of course, this is influenced by a lot of factors, and it is a long way to go until we really know what a foal looks like. A few hours and weeks

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after foaling can be decisively influenced (NOVOTNI, 2002). In this study, we will discuss, among other things, the importance of achieving good health after foaling.
SZENCZI (2003) reported that 1 of 100 foalings require for human intervention. If there are no complications foaling is quick, but it is better to monitor the newborn from the first minutes. Adequate reproductive biology care, may reduce the risk of death or permanent damage to the foal's health.
Foals are born with an intact but weakly functioning immune system. Passive immunity in newborn foals decreases over time, as there is a process of developing active immunity. This is a temporary period when an immune deficiency state occurs, in this period foals have many difficulties. Colostrum provides special immunoglobulins, which are essential for the development of passive immunity. In the first months, almost no immune protein is produced, so their immunological functions are very weak. Mares circulation contain globulins. In the last 7-10 days of gestation, globulins take place in the mammary gland, and after that into the colostrum, this means an average of 1-2 litres of colostrum. Immunoglobulins, including immunoglobulin G (IgG), do not pass through the uterus from the mare to the fetus, foals depend on antibodies obtained from colostrum to be able to fend off pathogenic attacks. In order to assess the immune status of foals, the measurement of serum IgG is a quick, safe and obvious solution, therefore it is fundamentally decisive. In the mucous membrane of the small intestine of newborn foals there are special cells that actively and indiscriminately absorb large molecules, including immunoglobulins and bacteria through pinocytosis (KNOTTENBELT et al., 2004).
It is imperative to ensure protective immunity, so that the foal has timely access to high-quality colostrum. Serum IgG concentrations of at least 600 mg/dl are already adequate, with most foals above 800 mg/dL. Foals that do not obtain enough immunoglobulin from colostrum are considered to fail in passive transfer (FPT), with these foals having a higher risk of neonatal infection and death (McGuire et al., 1977). Thus, adequate immunity requires a serum IgG greater than 800 mg/dl, but not less than 600 mg/dl. Therefore, serum IgG between 400-800 mg/dl is called partial FPT (MORRIS et al., 1985).
The degree of absorption is significantly reduced from birth, in case of a problem it is important to intervene. A quarter of the foals born do not have access to adequate colostrum, which would protect them. Several things can cause this: the mare does not produce colostrum properly, the foal does not take it well, the absorption of colostrum from the intestines of the foal is inadequate.
The quantity and quality of colostrum cannot be influenced although it is well known that well-fed mares in good health typically produce enough antibody-rich colostrum for their newborn foals, but the quality of colostrum can be determined by several methods.
Controlling the immune status of foals is of great importance. Weak passive immunity does not necessarily immediately show symptoms, it can linger until the

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age of a few days, weeks. The health of the first days affects the subsequent performance, and thus the economic value of the foal.
My objectives were determination of IgG concentration and foal blood serum by farm methods. During my investigations, I sought answers to the following questions: whether IgG is influenced by the age of the mare, the gestation length of mare, the sex of foal. How to change: density, fat-, solids-non-fat, lactose-, and protein content of colostrum and milk during lactation.

Material and methods

In 2022, the gestation and lactation of 9 Gidran mares were examined at Kismacs Experimental Station of Animal Husbandry of the University of Debrecen. I used a BRIX refractometer for measuring the colostrum IgG level, and a Lactoscan MCC Combo milk analyser for testing colostrum and milk.
I marked the mares with G1-G9 codes. The examination of lactation lasted for 90 days. The results are the average of 2 times measured values.
I took the samples in 15 ml sampling tubes. Times of sampling: 0., 3., 6., 12., 18., 24., 36. hour, 2. day, 1. week, 1., 2., 3. month. I took a sample of at least 6 ml on one occasion, since the milk analyser machine measured with that much for sure, for the Brix refractometer it would have been enough for much less. After taking the sample, it was either tested fresh or stored frozen and thawed and measured later. I did not see any difference in the results of the two methods.
Brix refractometer shows the optical density, measures concentrations of solutes. The higher the colostrum antibody content the greater the light scattering. The scale of brix refractometer lasts from 0 to 32 %. 23 Brix% means 60 g/l IgG-concentration. High-quality colostrum can even be determined by visual inspection. Using Brix refractometer is quick and easy. Then the sample is yellowish, thick and sticky. Poor quality is white, thin, often watery.
With Lactoscan MCC Combo milk analyser we can measure fat-, solids-non-fat-, protein-, lactose content and density. The measurement went quickly with the milk analyser, as it examines a sample for 1 minute and then writes the results on the monitor.

Results and discussion

Length of gestations
According to literature, the length of gestation is 320-350 days, with an average of 336 days (BENE et al., 2013). Based on my own examination, I got a similar result. From the last fertilization average length of gestations were 335 days (Table 1.).

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Table 1. Gestation period of mares

Sample size	Mean (days)	Standard deviation	Minimum	Maximum
9	335.33	9.66	323	354

The gender of the newborns is 44.4% colt and 55.6% filly. Colts were born by 340 days and fillies were born by 331 days on average (Figure 1.).

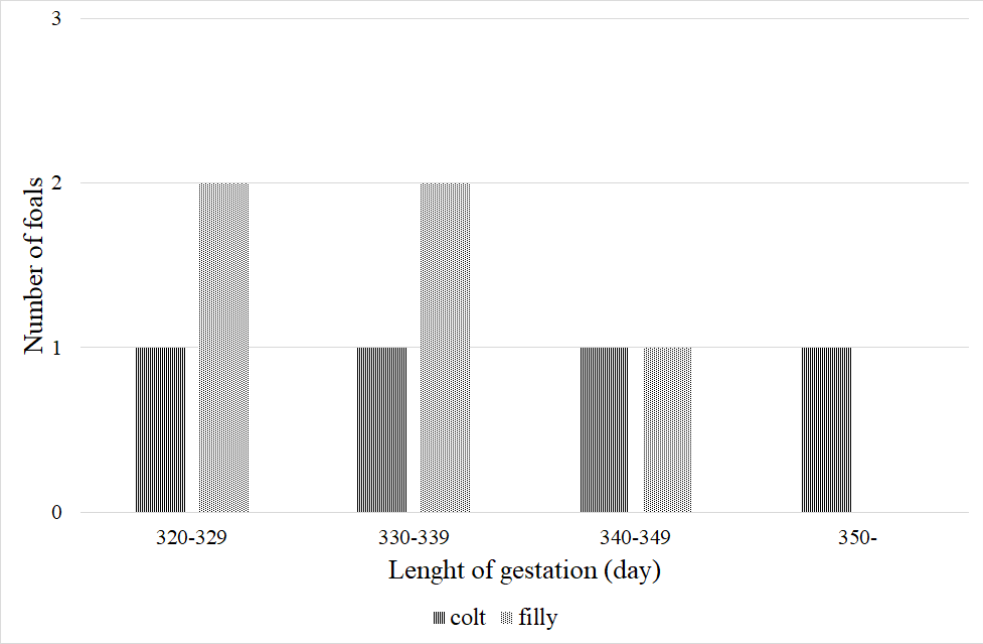

Figure 1. Gestation length and gender distribution of foals

The foals of mares fertilized in June and July foaling at 329, 331 days. Based on this, it is true that the gestation period of those fertilized between June and November is shorter. However, horses with active work tend to have a longer gestation period. However, the investigation did not prove that pre-abdominal, old mares and those that were fertilized between December and May would have a longer gestation period According to them, the gestation period can be influenced by the gender of the foal to be born and the month of fertilization.

Evaluation of colostrum by refractometer

Colostrum changes significantly by twelfth hour, by the twenty fourth hour it is transformed into milk. 23 Brix% corresponds to an IgG concentration of 60 g/l, which is already a good quality for colostrum supplementation (CHAVATTE-PALMER et al., 1998). 5 mares had colostrum above 23% (Figure 2.). G1, G3, G6,

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G8, G9 mares are also worth watching for the following foalings. If they show the same good results, it would be worth milking 200-250 ml of colostrum from them for the foals of mares with weaker colostrum. It is also worth freezing them, they can be safely used for 1-2 years. In the study, there was no correlation between the quality of the colostrum and the age of the mare, the length of gestation and the gender of the newborn.

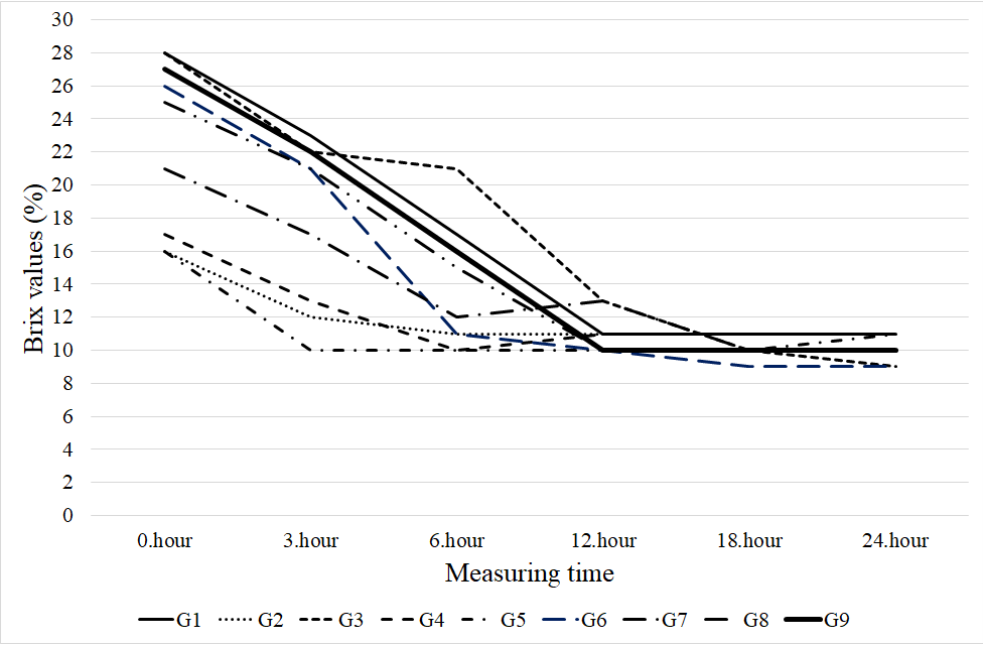

Figure 2. Brix% of colostrum after foaling

Assessment of colostrum and milk by density

The specific density of colostrum can also be used to determine its quality. Density of colostrum and milk were measured by MCC Combo Milkanalyzer. After Cash's studies (1999) 1070 kilogram/m^3 means 3000mg/dl IgG concentration. Therefore, based on this, the colostrum of G1, G3, G6, G8, G9 mares contains at least 3000mg/dl IgG concentration (Figure 3.).

The decrease continues until the 36th hour. The density of the colostrum decreased rapidly until the 12th hour (just like during the refractometer test) and from there, there was only a small change between the individual data. On average, the density of the colostrum was 1073.56 kg/m^3 in the 0th hour, which is already in the good category.

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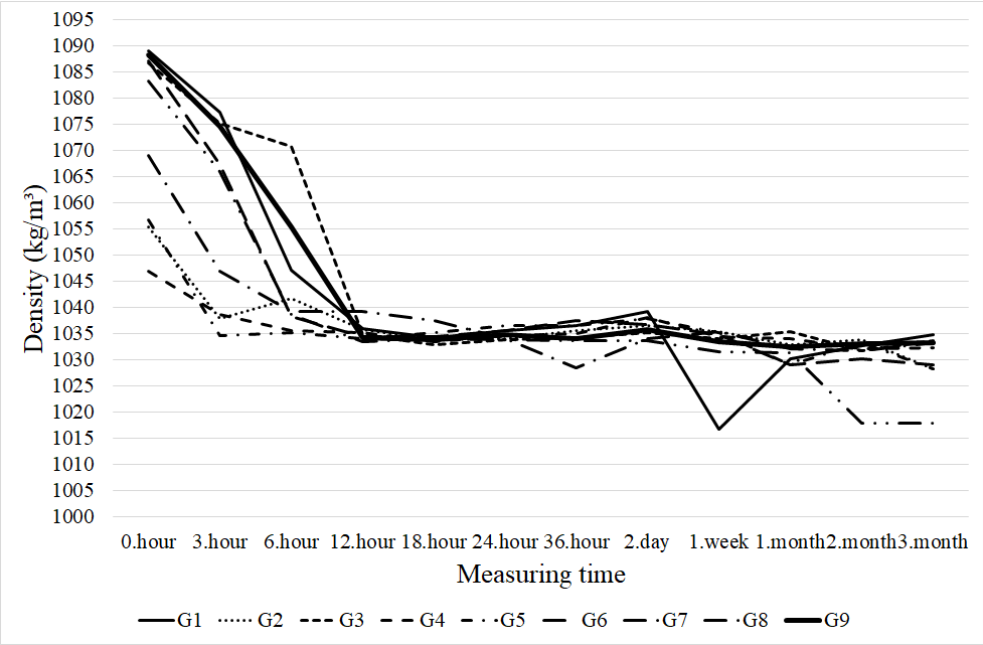

Figure 3. Changes in specific density of colostrum and milk

Fat content

There is the greatest error in sampling fat content. The fat content of mare's milk at the beginning of milking barely reaches 0,1%, at the end of milking the result can be 3-5 times, but even 10-20 times higher. The literature uses plenty of intervals because of this. There is a fact, fat content is decreased, when the energy level of feed is increased (CSAPÓ et al., 1995).

In average after foaling, the value rises, decreases in the 6th hour, and then rises again between the 12th and 24th hours. From the 36th hour to the 3rd month, the fat content of the milk continuously decreases (Figure 4.).

From the second day, the differences between individual values decreased.

Solids non-fat content

Since the Lactoscan milk analyser measures the solids non-fat content, I examined these data instead. Solids non-fat (SNF) means: protein, lactose and minerals together.

On average, the solids non-fat content immediately after foaling is 20,84%, and this value dropped spectacularly by 12th hour. We can see a small increase in the 24th and 36th hours, and from there until the end of the 3rd month, the SNF content in the milk decreases (Figure 5.).

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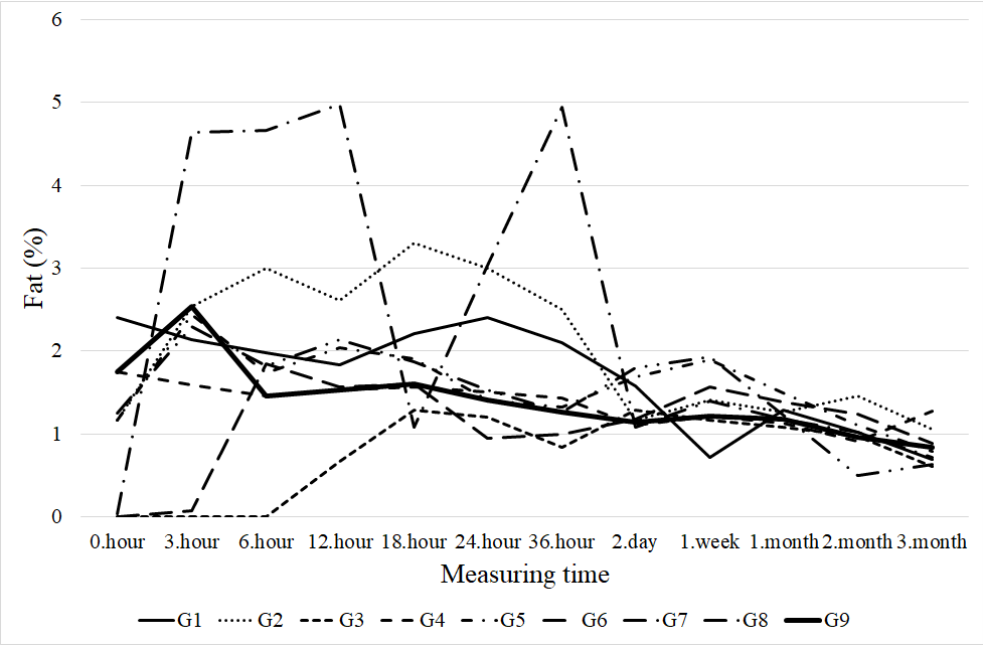

Figure 4. Changes in the fat content of colostrum and milk

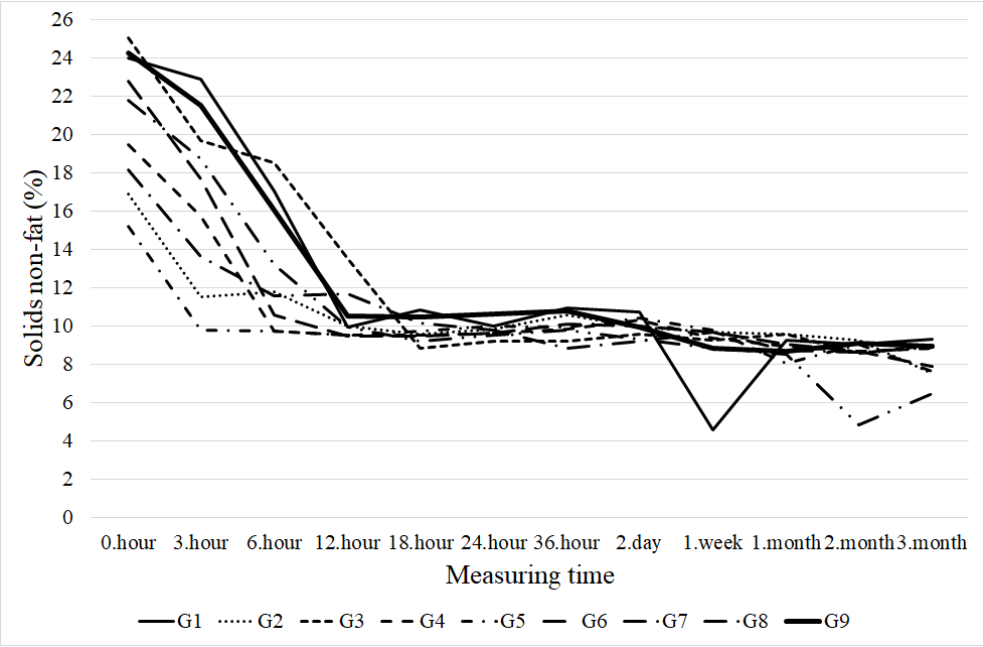

Figure 5. Changes in the solids non-fat content of colostrum and milk

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Lactose content

In the first measurements the variance of samples is high. During the study, the lactose content of the mares' colostrum varied between 6.73 -14.15% at the 0th hour (Figure 6.). It can be observed from the average values that the lactose content of milk decreases until the 36th hour, increases on the 2nd day, then decreases until the 2nd month and finally increases on the 3rd month. The biggest change can be observed up to the 24th hour. From 24th hour the values are similar, variance is low.

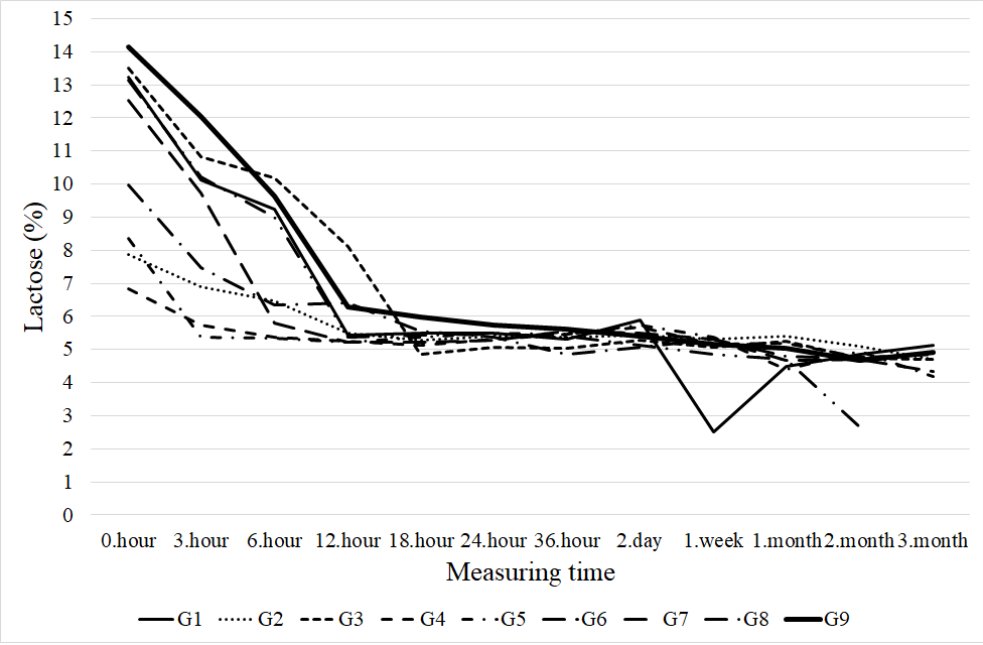

Figure 6. Changes in the lactose content of colostrum and milk

Protein content

Based on the literature, the highest protein content can be measured directly after foaling. Protein content is decreased, when the energy level of feed is increased. If mare has mastitis the protein content of milk is increased (CSAPÓ–SALAMON, 2018).

In the first measurements the variance of samples is high (Figure 7.). On average, the protein content of colostrum decreases on a large scale from hour 0 to hour 18. From eighteenth hour the values are similar, variance is low. The value increases on the 2nd day and the 3rd month.

The protein content varies in direct proportion to the lactose content and milk density (mostly this) in several mares. I did not experience any correlations between the mares.

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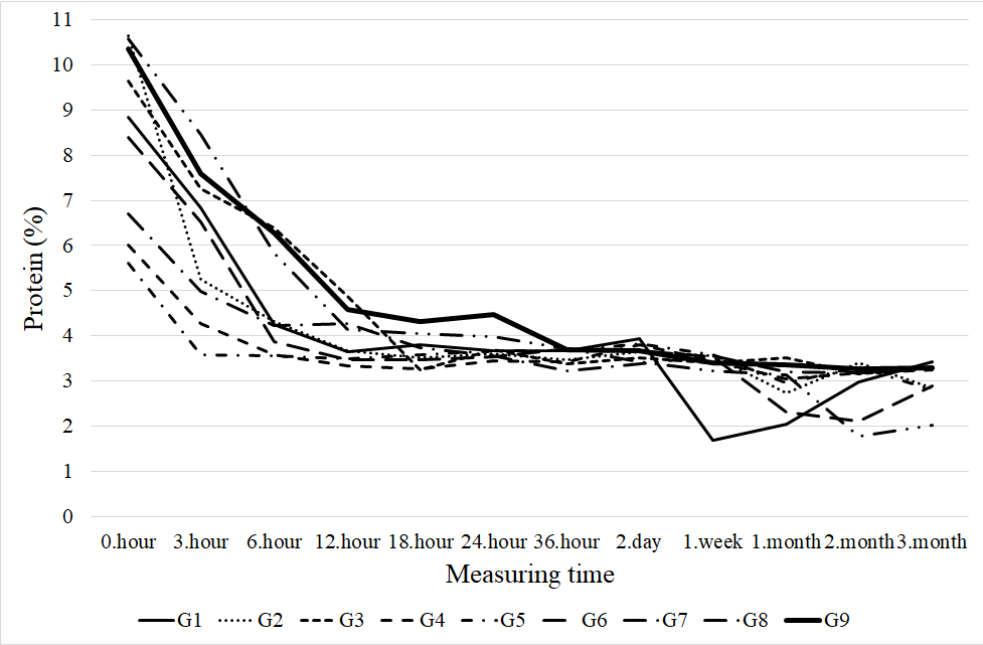

Figure 7. Changes in the protein content of colostrum and milk

Results summarized

Table 2. is made based on this experiment. The first two days contain more measurements, and the SNF was also introduced in my study. The average is moved by a prominent value in a certain direction, but the protein-lactose content and density in the aggregate table increase or decrease together.

Comparing several literatures, my values are different. Fat content is lower between 36. hour and 2. month. SNF content is lower in every measurements. Lactose content is higher to 18. hour, from 1.week is lower. Protein content is lower in 0. hour, from 1.week is lower.

Table 2. Composition of colostrum and milk of mares

Tested components	**Time after foaling**											
	hour								**week**		**month**	
	0	**3**	**6**	**12**	**18**	**24**	**36**	**48**	**1**	**1**	**2**	**3**
Density (kg/m)	1073	1058	1047	1038	1036	1035	1034	1036	1032	1032	1030	1030
Fat (%)	1.1	1.9	1.8	2.0	2.1	2.4	1.7	1.5	1.4	1.2	1.0	0.8
Non- fat-solids (%)	20.7	16.9	13.7	11.2	10.0	10.0	10.1	9.9	8.9	9.0	8.4	8.3
Lactose (%)	11.1	8.8	7.8	6.3	5.9	5.5	5.4	5.4	4.9	4.9	4.6	4.7
Protein (%)	8.3	6.2	5.0	4.2	3.8	3.8	3.6	3.6	3.3	3.0	3.0	3.0

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Conclusion and recommendation

The quality of the mare's colostrum is worth measuring with Brix refractometer, I would recommend using a colostrum replacement for foals in case of poor quality and feeding special pellet or muesli with mares. In the future, it would be worthwhile to also look at the blood serum level of the foals, supplemented with this data, a more accurate table could be prepared, in which the density, Brix% of the colostrum, the IgG concentration of the colostrum and the blood serum level of the foal are collected. This would facilitate immune status testing in field circumstances.

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Current status and future strategy for Istrian goat protection, conservation, and promotion

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Abstract

The Istrian goat is one of three Croatian autochthonous national breeds. It is white in color, with typically long horns and beards, and a large body frame. Due to the small number of living individuals, it is considered a critically endangered breed. It was reaffirmed and protected in 2013. Since then, several necessary steps for its revival have been taken, including gathering information on all existing animals and breeders, taking body measurements and genotypization of goats, and founding the breeding organization. In late 2022, a plan was started to revive, promote and commercialize the breed.

Keywords: Istrian goat, Croatian autochthonous breed, conservation

Introduction

Goats have been traditionally raised in Croatia for centuries. In the 19th century, around 750 000 goats were raised in Dalmatia alone. However, this number steadily declined towards the 21st century. In 2022 there were 71 872 goats in Croatia, of which the most numerous are foreign breeds Alpine and Saanen, and the Croatian autochthonous breeds Croatian spotted goat and Croatian white goat (MINISTRY OF AGRICULTURE, 2022.). The Istrian goat is the third Croatian Autochthonous

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breed of goats native to the Istrian peninsula. The population of Istrian goats suffered the heaviest decline in number, and at the end of the 20th century, around 100 Istrian goats were left. This prompted breeders, experts, and the local community to act to save this breed from extinction. This paper aims to provide information on the Istrian goat breed's current status and discuss the future strategy for its revival.

Description of the breed

According to the official data source (MINISTRY OF AGRICULTURE, 2022), Istrian goats have a big physical frame, strong constitution, and strong bones. The base coat colour is white, but grey or cream-colored hues are allowed. Beards and horns are present in does and bucks (Figure 1).

Figure 1. Istrian goat

Table 1. Body measurements and production characteristics of Istrian goats (MINISTRY OF AGRICULTURE, 2022)

Trait	Goats	Bucks
Height at withers (cm)	60-75	65-90
Body weight (kg)	55-80	70-120
Fertility (%)	150	/
Milk production (L)	300-400	/
Kid body weight at 4-6 months (kg)	20-30	

Large, rough, and coarse horns bent backward in bucks are desirable. Bucks are heavier and taller than goats. The body weight and height at withers vary greatly in both sexes (Table 1). Withers is clearly visible. The fertility of Istrian goats is similar

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to the fertility of Croatian white goats and Croatian spotted goats, and the milk production is higher. Kids reach 20 to 30 kg at 4 to 6 months of age (Table 1).

Current status

The historical importance of the Istrian goat is best illustrated by the fact that it is in the flag of the Republic of Croatia, representing the Istria region. However, this fact alone was not a sufficient motive for conserving the breed. The number of individuals declined to about 100 until 2012, when the Istrian goat was reaffirmed and protected as a third Croatian autochthonous goat breed (IVANKOVIĆ et al., 2014). The number of animals did not change significantly since then, and in 2022 there were about 120 Istrian goats (16 bucks and about 100 does) bred in 6 flocks of different sizes (PERSONAL COMMUNICATION, 2022). Most animals are kept for meat production (kids), with a tendency to shift to dairy production.

Several steps important for the revival of the breed were taken. MIOČ et al. (2013) researched the current exterior characteristics of different age and sex categories of Istrian goats. They recorded the following exterior measurements in adult goats: withers height (65.62 cm), trunk length (73.29 cm), chest width (19.48 cm), chest depth (33.55 cm), chest circumference (90.14 cm), leg circumference (8.40 cm), length of the horns (32.22 cm) and body weight (56.06 kg). Young females (6-18 months and 2 to 3.5 years) were also measured. It was concluded that at the average age of 31 months, young females reached 96.5%, 96.9%, 88.6%, and 89.4% of withers height, trunk length, chest width, and chest depth of adult animals, respectively. Measurements showed that this is the largest autochthonous goat breed in Croatia. IVANKOVIĆ et al. (2018.) conducted a genetic characterization of 29 Istrian goats to determine the nucleus and mitochondrial DNA variability of Istrian goats and their relationship to Croatian white and Saanen goats. The authors concluded that, despite declining numbers, Istrian goats maintained a high level of genetic diversity and significant genetic distance from the other two breeds. These results provided a basis for sustainable genetic diversity management of Istrian goats and the possibility for the development of economic utilization.

In 2022, a Reference Expert Network for the Conservation of Animal Genetic Resources (cro. Referentna ekspertna mreža za očuvanje animalnih genetskih resursa; REM AnGR) was founded in Croatia. The network's main aim is to monitor, coordinate, and give opinions and recommendations for the successful implementation of the National Program for the Preservation of Autochthonous and Endangered Breeds of Domestic Animals in the Republic of Croatia 2021-2025. One of the first tasks of the experts within the network is Istrian goat conservation and revival.

Also in 2022, the Association of Istrian goat breeders was founded. The organization gathers the remaining Istrian goat breeders with the common aims of breed

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standardization, increasing the number of individuals, and commercializing the Istrian goat products.

Agency for Rural Development of Istria (cro. Agencija za ruralni razvoj Istre; AZRRI) proved to be a key link between Istrian goat breeders and experts. Guided by their success in coordinating the revival and commercialization of the Istrian Podolian Cattle breed, they intended to do the same with the Istrian goat. Therefore, in late 2022 they organized the first meeting to determine the Istrian goat's current status and plan future steps for its revival.

Future strategy

Several future steps are required and planned to revive the Istrian goat breed. The entire population of Istrian goats will be appropriately marked and registered. This will enable the precise monitoring of the number of animals and their movement between breeders. All bucks will be physically examined, and their semen will be collected, inspected, and stored. The biological samples containing DNA have been taken by the end of 2022. This will enable further molecular-genetic analyses.

One of the first tasks in *in situ* conservation is to increase the number of goats. However, pedigree data for the Istrian goat breed are incomplete. Thus, breeding plans aimed at increasing the number of individuals while maintaining genetic variability and reducing inbreeding are difficult to implement. The best option is to use the available pedigree data and the information provided by the breeders, of which the majority are familiar with the origin of their goats, especially bucks. Combining these two sources of information, a relatively sound breeding plan could be made. However, inbreeding will be impossible to avoid due to the small number of individuals. Instead, it will be kept at an acceptable level.

Production parameters will be recorded. This includes the birth weight of kids, average daily gain, body mass at weaning, duration of lactation, milk yield, and milk composition. These are the main prerequisites for the implementation of any breeding plan aimed at the increase in productivity. Milk quantity and quality traits, along with the udder conformation traits, should be prioritized.

Financial support from the Ministry of Agriculture and AZRRI should motivate breeders to be proactive in increasing the number of individuals and keeping records on their herds. Moreover, AZRRI intended to buy 20-30 young goats and „rent“ them to the breeders. That way it would be ensured that at least some goats won't be sold or slaughtered if the breeders decide to quit the job.

If everything above succeeds, the next step would be commercializing the breed and its products. Again, AZRRI, who has much experience in this area, should support the first steps. The majority of the Istrian goat breeders see their future in dairy production. Considering the Istrian environment, tourism, and specificity of the Istrian goat breed, small family farms with milk processing plants seem viable. A

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good model for dairy development might be the one implemented in Istrian sheep. Istrian sheep breeders persisted in quality over quantity. Today, the Istrian sheep cheese is the finest product of this breed, and it received transitional national protection for its origin in early 2022.

Conclusion and recommendation

The Istrian goat is the Croatian autochthonous, critically endangered breed. Its conservation and revival are important for national and cultural reasons and for preserving Croatia's animal genetic resources. Since 2013, significant efforts have been made to revive the breed. However, for long-term conservation and revival, the commercialization of breed-specific products is the best solution. Cooperation between breeders, experts, and the local community is necessary to achieve this goal.

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Sperm quality of Zemplin Rabbit and Liptov Bold-Spotted Rabbit breeds

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Abstract

Zemplin Rabbit and Liptov Bold-Spotted Rabbit are Slovak national breeds. Our aim was to characterize the sperm quality of these two breeds, as these characteristics are important for artificial insemination and cryopreservation as biodiversity conservation tools. For this evaluation, sperm samples of sexually mature Zemplin Rabbit (ZR) males (n = 6) and Liptov Bold-Spotted Rabbits (LR) (n = 4) were used. According to progressive motility (PM) data (CASA), samples were divided into two groups: A (>30% PM) and B (<30% PM). In addition to PM (ZR-A: 48.6±3.8%, ZR-B: 16±3.2%, LR-A: 35.38±2.6%, LR-B: 4±2.2%), total motility (TM) (ZR-A: 69.1±4.1%, ZR-B: 35.5±4.1%, LR-A: 59.1±3%, LR-B: 26.9±4.1%) and morphological abnormalities (ZR-A: 29.7±1.2%, ZR-B: 40±4%, LR-A: 34.3±4.3%, LR-B: 48.3±4.7%) were also assessed using the CASA system. The proportion of dead/live, apoptotic and oxidative damaged spermatozoa (Spz) was assessed by flow cytometry using fluorescent dyes: SYBR-14, Sytox Green, Yo-Pro-1 and CellROX Green, respectively. The results of flow cytometry correspond to the values of motility and morphometry. Sperm with PM less than 30% does not show proper quality values, while the sperm with PM higher than 30% is suitable for further analysis and use.

Keywords: rabbit, spermatozoa, CASA, flow cytometry

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Introduction

Biodiversity is one of the most important factors of sustainable agriculture. Farm animals, which in the past were mainly used for meat production, were developed on a relatively narrow genetic basis and the genetic management of genetic resources has been receiving attention recently. There are several major concerns regarding genetic resources. This is one of the most important reasons why the breeding of local breeds is so important for maintaining biodiversity.

Rabbit breeding has a long tradition in our region. Originally bred for meat, rabbits are currently also bred for sport and exhibitions (ALVES et al., 2015) as well as for specialized genotypes for biological research (TŮMOVÁ et al., 2011). Zemplin Rabbit (ZR) is a medium-sized breed, so that the Viennese Blue Rabbit, the New Zealand Red Rabbit and the Slovakian Grey-Blue Rex were particularly involved in its breeding. ZR has been an officially recognized breed since 1987. It is included in the European Breed Standards and is currently bred in the Czech Republic and Hungary in addition to Slovakia (CHRENEK et al., 2019). Liptov Bold-Spotted Rabbits (LR) is one of the youngest Slovak national rabbit breeds. The breed was recognized in 2005. The following combinations proved successful in its breeding: Vienna‘s wild-coloured and Dutch wild-coloured, or Vienna Wild-Blue and Dutch Pearl. It is a small or medium breed with good productivity and fertility. Several colour variations of LR are known: wild-coloured, black-and-blue and grey-blue (CHRENEK et al., 2019).

The CASA system (computer assisted sperm analysis) was used as an input analysis for the assessment of total and progressive motility and, in addition, for the evaluation of morphological changes. Standard sperm flow-cytometric analyses involved assessment of different spermatozoa characteristics, which affect the overall semen quality. The viability of spermatozoa basing on their plasma membrane integrity is commonly analysed either directly, using SYBR-14 dye, staining of live metabolically active cells, or in combination with dead cell dyes, entering cells via disrupted membrane (GARNER and JOHNSON, 1995). Sytox Green nuclear fluorochrome was used to evaluate dead cells. In addition to viability analysis, apoptosis-like changes in spermatozoa should be analysed, because such spermatozoa can be hidden within the live cell population. One of the most frequently used mechanisms for determining apoptotic cells is increased membrane permeability revealed by the nuclear dye - YO-PRO-1 iodide (PENA et al., 2005). Oxidative stress or damage, triggered by reactive oxygen species (ROS), may have unfavourable impact on spermatozoa fertilizing ability. There are several probes, which accumulate in cells and become fluorescent after oxidation and, thus, may be used for the detection of ROS in spermatozoa. In our case, CellROX Green was used as a probe that should bind DNA after oxidation (RILEY et al., 2021). Since rabbit

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ejaculate can contain a large number of granular bodies, we used a DRAQ5 far-red dye to distinguish nucleated cells from other events.
This work should serve as a basic evaluation of the microscopic sperm properties of the above-mentioned breeds. Following these results, it is possible to continue with more detailed analyses and then try to cryopreserve the samples for the purpose of biodiversity protection.

Material and methods

Clinically healthy and sexually mature males of ZR and LR were used in this experiment. The age of the individuals ranged from 10 to 18 months. Semen was collected two times per week through artificial vagina with warm water (50°C). Semen was immediately evaluated for volume, concentration, motility (initial checking) and divided into two groups: A (>30% PM) and B (<30% PM). A total of 32 ejaculates (16 from each breed) were used in our experiments.

Sperm motility
The motility and sperm movement were analysed by CASA (SpermVision™ software, Minitube, Tiefenbach, Germany) with light microscope (at the 200× magnification; AxioScope A1, Carl Zeiss Slovakia, Bratislava, Slovakia) and Makler counting chamber (Microptic, Barcelona, Spain). Samples were diluted by saline (0.9% NaCl; Braun, Nuaille, Germany) at ratio 1:20 (v/v). A drop of diluted semen (10 μL) was transferred to a counting chamber and analysed with manufacturer's pre-set parameters for rams. We mainly focused on total (TM) and progressive motility (PM).

Morphological changes
After motility analysis, an aliquot of samples was placed in the refrigerator and stored until the next day (approx. 24 h) to immobilize sperm for morphological analysis. Morphological abnormalities were measured by the same microscope as CASA was performed. In this analysis morphological abnormalities of sperm cells, e.g. detached flagellum from head, twisted flagellum, shortened flagellum, broken flagellum, cytoplasmic droplet flagellum, reduced or enlarged sperm head, or other pathological sperm were evaluated.

Flow cytometry
Individual ejaculate samples were washed in PBS($^-$) (phosphate buffered saline, Ca^- and Mg^- free; Biosera, Nuaille, France) (300x g, 5 min.). Each sample was divided into 5 tubes with DRAQ5 staining solution (100 μmol.ml^{-1}; BioLegend, San Diego, USA) to identify nucleated cells. The proportion of apoptotic cells was evaluated using YO-PRO-1 (100 μM; Molecular Probes, Lucerne, Switzerland), the proportion

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of dead cells was determined by Sytox Green staining solution (30 µmol.ml^{-1}; Thermo Fisher Scientific, Waltham, MA, USA) and oxidatively damaged sperm - using CellROX Green staining (Thermo Fisher Scientific, Waltham, MA, USA). We used staining with SYBR-14 (50 µg.ml^{-1}; Thermo Fisher Scientific, Waltham, MA, USA) to evaluate viability. The control sample (without staining solutions) was resuspended in 250 µl of PBS($^-$). The cells were incubated for 15 min at RT (room temperature) and in the dark (SYBR-14 - 15 min. in the dark at 37 °C). After the incubation period, the samples were washed in 1 ml of PBS($^-$) and analysed on a FACS Calibur flow cytometer (BD Biosciences, San Jose, CA, USA). At least 10,000 spermatozoa were analysed in each sample.

Results and discussion

Sperm motility of both breeds is shown in Figure 1. Progressive motility of B groups is below 20%, therefore it is considered unsuitable for fertilization, if we assume that the minimum PM value for successful fertilization is 30%. According to Theau-Clément et al. (2016), rabbit fertility is significantly higher, when total motility is above 80% and progressive motility is above 70%. Lavara et al. (2005) claim that insemination centres use a high percentage of total motile cells (more than 70%) as a selection parameter of ejaculates before their inclusion in the final pools and the use of heterospermic doses to perform inseminations.

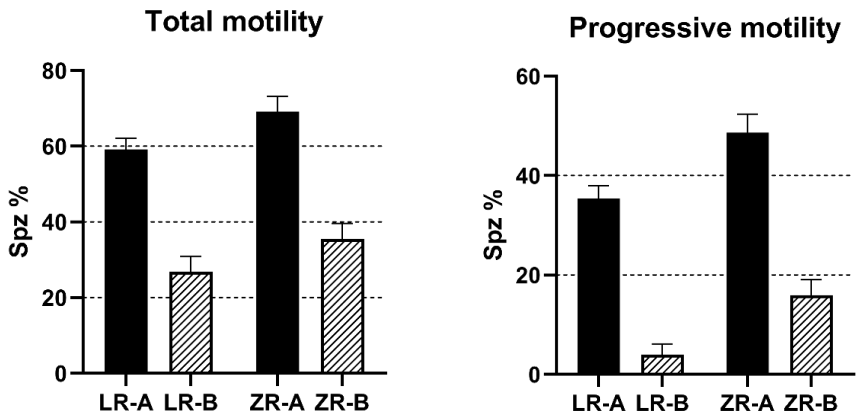

Figure 1. Motility parameters of analysed rabbit semen samples. The data are expressed as the means ± SEM.

The total value of morphologically abnormal sperm (Figure 2) is over 20% in both breeds and groups, which is not within the range of commercial insemination dose standards (malformation rate ≤ 20%). The most common malformation in both

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breeds and groups was a cytoplasmic droplet. An increase in the incidence of abnormalities in spermatozoa and abnormal trajectories has been observed when semen samples presented a high incidence of cytoplasmic droplets in rabbits (FAUSTO et al., 2001). The percentage of sperm cells with normal morphology is an important indicator of semen fertility (BARTH and OKO, 1989) and the sperm characteristic is most highly correlated with fertility in humans (MORTIMER and MENKVELD, 2001).

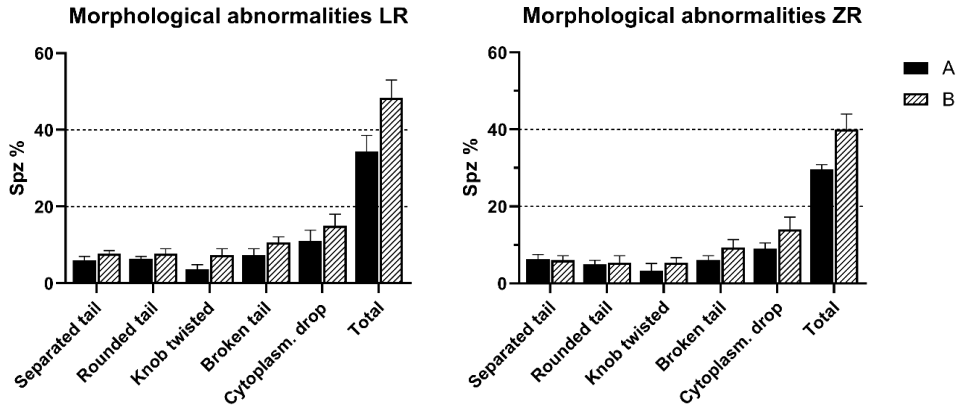

Figure 2. Percentage of individual abnormalities and all abnormal spermatozoa. The data are expressed as the means ± SEM.

Beside the motility and morphological abnormalities, viability was also evaluated. The proportion of live rabbit sperm was assessed by the SYBR-14 probe, which is most widely used marker of live cells, mainly in combination with PI (Propidium iodide),7-AAD (MARTÍNEZ-PASTOR et al., 2010) or DRAQ7 (VAŠÍČEK et al., 2022). Only sperm that maintain an intact acrosome can take part in fertilizing an oocyte. Therefore, the percentage of sperm with damaged acrosome should be low in order to maintain high fertility levels. Sytox Green, a green dead cell dye, was used in combination with the DRAQ5 red fluorescent dye to analyse dead sperm. This dye has been previously reported to be useful for sperm analysis either alone, or in combination with other specific probes, e.g. Annexin V or DHE (VARUM et al., 2007; DE IULIIS et al., 2006). In addition, a green apoptotic-like changes dye, Yo-Pro-1, was used for apoptosis assessment (KUŽELOVÁ et al., 2017). All the above-mentioned parameters were evaluated by flow cytometry. Although in oxidatively damaged cells we detected negligible values in both groups A and B, other markers corresponded to motility parameters because the proportion of apoptotic and dead cells was higher in group A (Figure 3).

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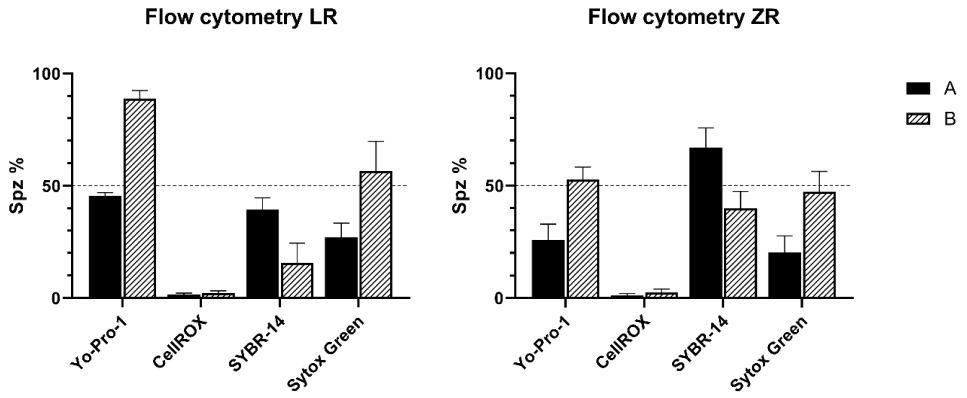

Figure 3. Evaluation of rabbit sperm quality parameters by flow cytometry. The data are expressed as the means ± SEM.

Conclusion and recommendation

Our results show inappropriate quality of group B in both breeds. Individuals from this group should not be used for artificial insemination. Group A could be used for insemination, but it is likely that these sperm samples would not be suitable for cryopreservation. However, we cannot confirm this claim until further analysis is carried out using a wider panel of markers or cryopreservation itself.

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Estradiol and testosterone hormones as a method for sex determination of Siberian sturgeon (*Acipenser baerii*) from Zhrebchevo Dam Lake, Bulgaria

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Abstract

Determination of the gender in sturgeon is very important in fish farmers, as sex is one of the main factors that determine aim of cultivating them. One of the method for maturation monitoring of sturgeons is steroid hormone analysis. On this base the current study used estradiol and testosterone hormones to determine the gender of 2-3-year-old Siberian sturgeon (*Acipenser baerii*) cultivated in sturgeon broodstock farm located in the Zhrebchevo Dam lake, Bulgaria. The serum concentrations of circulating reproductive hormones found in the both sex were similar to the hormonal profiles in previously reports. The results were confirmed also by histological examination, which showed pre-vitellogenic stage (Stage I) in female and maturity (Stage V) at the male individuals. The findings indicate the force of current steroid hormones as a method for sex determination. In conclusion, analysis of circulating reproductive hormones may be a useful method in early determining the sex of Siberian sturgeon.

Keywords: determination of gender, 17β-estradiol, sturgeon, testosterone

Introduction

In Bulgaria, sturgeon production started since the end of the last century (NIKOLOVA et al., 2018). Based on export data from the Convention on International Trade in Endangered Species of Wild Fauna and Flora and production

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data from Food and Agriculture Organization of the United Nations for the period 2016-2020, Bulgaria produced mainly Siberian sturgeon (22%) (EUMOFA, 2023). Early differentiation of the gender in this species is very important in fish farmers, as sex is one of the main factors that determine cultivating them for caviar or meat in the future (RUSENOV et al., 2019). Generally, females are raised until they start to produce caviar, which can take at least six years (JAHRL, 2013). In contrast males are usually bred within 3-4 years mainly for meat and an insignificant part for breeding (Park et al., 2020). In this regard, several invasive and non-invasive methods have been developed to early determine the sex of these sturgeon species. The most common of them include endoscopy (MUNHOFEN et al., 2014), laparoscopy (FALAHATKAR et al., 2011), ultrasound (RUSENOV et al., 2019), vitellogenin mRNA expression (Park et al., 2020) and steroid hormones (ABBASI et al., 2016),

Therefore the purpose of this study was to examine serum steroid hormone levels and also gonad development as evidence of gender in Siberian sturgeon.

Material and methods

Fish samples

In present study, twelve Siberian sturgeon (*Acipenser baerii* BRANDT, 1869) aged < 3 years and in the early stage of sexual maturation (weight 2210.82±31.41 g) were obtained from sturgeon broodstock farm, located in the Zhrebchevo Dam Lake, Bulgaria (42.587996, 25.898514), where were reared in net-cages reflecting the ambient condition of the reservoir.

Blood samples and hormone analysis

Blood samples were drawn from the vena caudalis using a needle (20G). The collected samples (approx. 1 ml) were centrifuged at 3000 rpm for five minutes (Ohaus FC5515, Ohaus Corp., USA) at room temperature. After coagulation, the obtained serum was immediately removed, placed into a clean Eppendorf microcentrifuge tube and stored at -20°C until analysed. Serum 17β-estradiol (E2) and testosterone (T) analyses were conducted with Elisa analyser, HumaReader HS (Human GmbH, Wiesbaden, Germany) and its measurement AccuBind®Elisa kit (Monobind, Inc., USA).

Determination of sexual maturity stages

Gonad biopsy samples were taken during an ultrasound examination in order to determine the gonad stage. The samples of gonad tissue were fixed in 10% buffered formalin and processed for paraffin embedding. Paraffin embedded sections, 4-5 µm thick, were stained with haematoxylin and eosin (H&E) and examined by light microscopy (ALLEN, 1992). The sexual maturity stage of the gonad was identified

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according to WILDHABER et al. (2007). The mean oocyte diameter (μm) was measured by Olympus C-mount camera adapter (U-TV0.63XC, Olympus Ltd, Co., Japan).

Statistical analysis

The statistical evaluation of the data obtained from the experiment was made using the software Statistica v.10 (STATSOFT INC., USA, 2010). The results were presented as mean and standard deviation of the mean (Mean ± SD).

Results and discussion

The present study used a non-lethal approach to determine the sex and reproductive stage of Siberian sturgeon by using some of steroid hormones, to wit 17β-estradiol and testosterone. In sturgeon species, the steroid hormones are unrecognizable until sex differentiation occurs (YOUNESZADEH-FASHALAMIET al., 2018).

In the current investigation, there were differences in serum 17β-estradiol concentration among pre-vitellogenic (stages I) and cortical alveoli (stage II). No serum E2 was found in stage I, while female in stage II was recorded (0.35±0.09 ng/ml) (Figure 1). However, E2 was found to be higher in female juvenile sturgeon than in male. This may be due to the fact that unlike mature male, females in the pre-vitellogenic stage do not secrete gonadotropin hormone (GTH). The GTH is thought to be responsible for inducing vitellogenesis, as well as at a later stage to regulate final ovarian maturation and ovulation. Hence, it can be concluded that the female individuals at the stage I reproduction process still have relatively low or lack pituitary concentrations and functional receptors of GTH. In other hand, female vitellogenin (VTG) starts to produce in response to increasing estradiol levels during development of oocytes, while in male is low or lacks VTG. These data are supported from earlier reports in other sturgeon species, where plasma or serum17β-estradiol level increased from the pre-vitellogenic to the vitellogenesis stage and reached its highest level in the post-vitellogenic stage (WEBB et al., 2002; WILDHABBER et al., 2007; HOSSEINZADEH et al., 2013; Carolyn et al., 2016; YOUNESZADEH-FASHALAMI et al., 2018). Actually, the lack of huge differences in E2 values between the both sex was expected, as immature individuals are known to have the lowest basal levels (WHEELER et al., 2016), in contrast to female Siberian sturgeon in stage IV, which correspond to the maximum incorporation of vitellogenin (PELISSERO et al., 1989).

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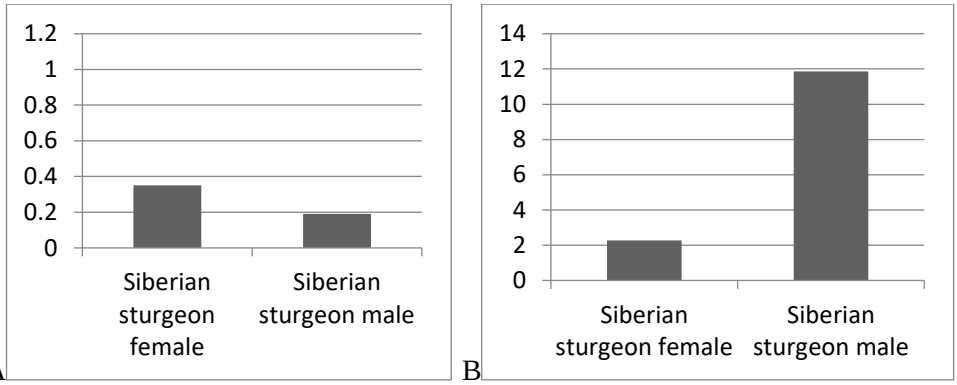

Figure 1. Serum 17β-estradiol (**A**) and testosterone (**B**) in Siberian sturgeon (Mean ± SD).

The testosterone plays the main role in spermatogenesis and therefore may be used to differentiate between immature female and male Siberian sturgeon, but only in the case the concentration in blood above 4 ng/ml (ABBASI et al., 2016). The mean serum testosterone concentration of Siberian sturgeon was found to be higher in males (11.85±0.71 ng/ml) than in females (2.28±0.02 ng/ml). Like 17β-estradiol, serum testosterone value showed an increasing trend with the stage. In study again with female sturgeon in pre-vitellogenic stage treated with testosterone implants was determined higher levels of gonadotropin. It could be suggested that it had strong positive feedback between testosterone and pituitary but without further effect on ovarian development (MOBERG et al., 1995).

The histological data on oocytes showed that the Siberian sturgeon, like the bester sturgeon, white sturgeon, shortnose sturgeon, sterlet and many other sturgeons, has a heterogeneous asynchronous ovarian development in the early stage of reproduction (FALAHATKAR, 2015). In the current study, histological analysis confirmed that females were at the pre-vitellogenic stage (Stage I), according to the classification of WILDHABER et al. (2007). Oocytes (average diameter 200-300 μm) were located at the periphery of the ovarian lamellae (Figure 2). Furthermore, the development of oocytes was accompanied by a decrease in the concentration of testosterone and an increase in the concentration of 17β-estradiol. This is in complete agreement with a previous study in the same species (MOSYAGINA and ZELENNIKOV, 2016).

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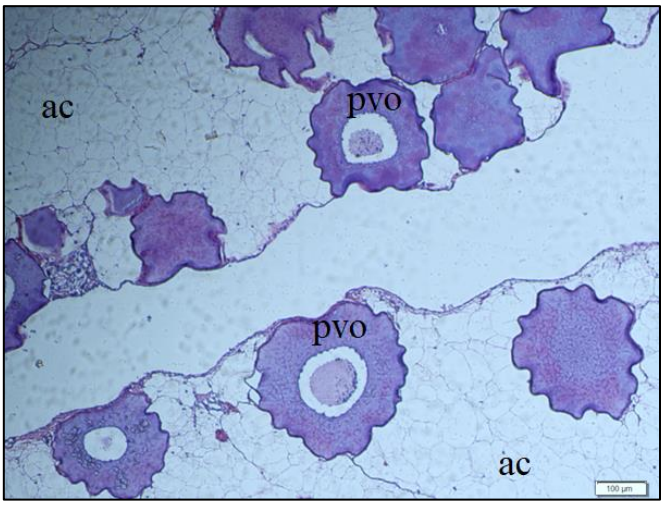

Figure 2. Histology of Siberian sturgeon ovarium. Pre-vitellogenic oocytes (Stage I) at the periphery of lamellae. **(ac):** adipocytes **(pvo):** pre-vitellogenic oocytes

Separately, the ooplasm surrounding thick basal lamina showed strong basophilia and the nucleus contained many nucleoli close to the nuclear membrane (Figure 3).

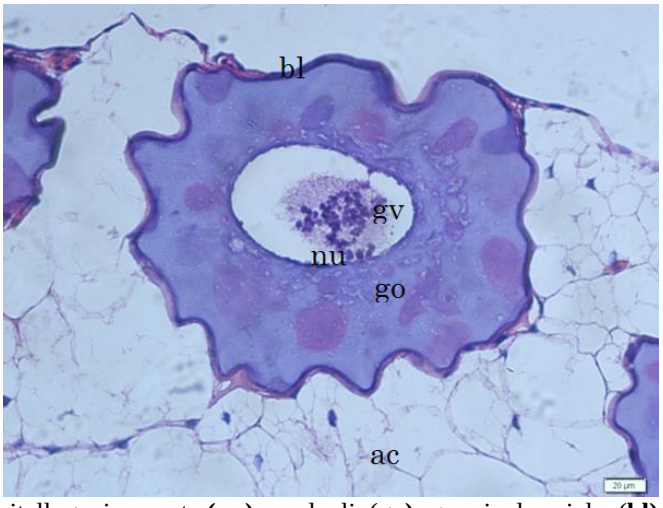

Figure 3. Pre-vitellogenic oocyte **(nu):** nucleoli, (**gv):** germinal vesicle, (**bl):** basal lamina, (**go):** granular ooplasm, **(ac):** adipocytes

The histological section of Siberian sturgeon testis showed maturity stage V. Large testicular tubules were filled by elongate, intensely basophilic heads of mature spermatozoa (Figure 4). In the Siberian sturgeon male, the intensification of the activity of steroid secretory cells was indirectly determined by the stage, which is

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connected by an increase in the concentration of testosterone in blood (MOSYAGINA and ZELENNIKOV, 2016).

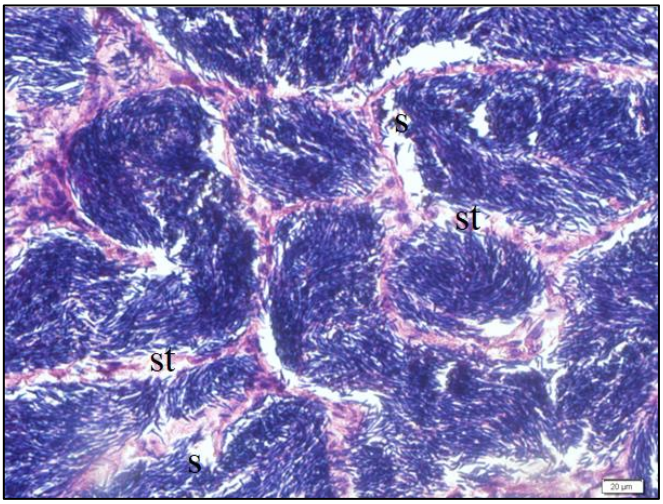

Figure 4. Histology of genderin Siberian sturgeon testis. The rod-likeheads of abundant mature spermatozoa (stage V) (**arrow**) filling large seminiferous tubules (H&E) (**st**): seminiferous tubule, (**s**): spermatozoa

Using serum E2 and testosterone concentrations, we were able to identify gender in Siberian sturgeon with high accuracy similar to other methods in same species such as endoscopy (MUNHOFEN et al., 2014), laparoscopy (FALAHATKAR et al., 2011), ultrasound (RUSENOV et al., 2019).

In conclusion, results of the present study indicate that the determination of steroid hormone levels may be indicative of early gonadal maturation, and subsequent histological examination are useful and effective methods for determining the stage of reproduction in 2-3-year-old Siberian sturgeon.

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Testing of microsatellite markers for individual identification of fallow deer

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Abstract

The fallow deer (*Dama dama*) of Hungary has excellent value to our country due to its game meat and antler trophies. As an attempt to aid law enforcement against illegal activities, such as poaching, illegal trading, and in other cases like traffic accidents, we aimed to develop a genetic marker set suitable for individual identification. During our research, 28 microsatellite markers on 15 fallow deer samples from two different populations were tested. Four microsatellites were found to be polymorphic, each with two or three alleles. Based on our current results, Hungarian fallow deer populations show low genetic diversity. This is in agreement with previous studies conducted on the species and is probably a direct result of the species' past extinction from the most of Europe during the Pleistocene and later its human-mediated reintroduction to most of its current range. The low number of polymorphic markers presents the need to include additional markers.

Keywords: fallow deer, microsatellite, individual identification

Introduction

Fallow deer (*Dama dama*) has an estimated population of approximately 40 000 individuals in Hungary. The game meat and antler trophy make the animal valuable for the hunters and the country. The hunting of the species is regulated by strict laws regarding hunting season, hunting permits and weapons usage. If any of those regulations are not met, then it is considered illegal hunting which depending on the

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severity can come with a penalty ranging from the confiscation of equipment and a fine to prison sentence.

Unfortunately, illegal hunting poses an increasing problem to wildlife in Hungary (ELEK 2019), together with traffic accidents threatening the fallow deer population. Because of these reasons, the development of genetic identification methods is in demand to aid law enforcement in solving the cases that arise. If there was a method suitable for this purpose it would serve as a dissuasive to potential poachers as well. The development of methods like this is the goal of forensic animal genetics which has a more than 20 years long history in Hungary (PÁDÁR et al. 2019, PÁDÁR et al. 2020) but there is an increasing need to further expand the list of species which marker sets are available for (ZENKE et al. 2015, ZENKE et al. 2017, PÁDÁR et al. 2022, PÁDÁR et al. 2022). For several wild and domesticated animals, like deer species, big cats, wild boars, pigs, bears, and canines, microsatellite marker sets constructed for individual identification are already available (SIM et al. 2021, SINGH et al 2004, LORENZINI et al. 2020, MEREDITH et al. 2019, MEREDITH et al. 2005, ZENKE et al. 2011). Fallow deer, however, is not among those species as a marker set has not yet been tested and validated either in Hungary or in other countries. Our aim is to develop such a method by selecting and testing tetrameric microsatellite markers from closely related species.

Material and methods

We received samples from 15 fallow deer legally shot between 2019 and 2022 during the hunting season from two populations (Pilis=7, Isaszeg=8). DNA was extracted by using FavorPrep™ Tissue Genomic DNA Extraction Mini Kit. Quality and quantity control was done by agarose gel electrophoresis and Qubit® 2.0.

Table 1. Data of the tested 28 tetranucleotide microsatellite markers: source species (name of the marker groups marked with bold), locus name, and references.

Species	**Microsatellite marker**	**Publication**
red deer (*Cervus elaphus*) DeerPlex	C01, C229, T26, T108, T123, T156, T172, T193, T501, T507	SZABOLCSI et al. 2014
mule deer (*Odocoileus hemionus*) Ohe	OheB, OheC, OheD, OheE, OheF, OheG, OheH, OheI, OheJ, OheK, OheM, OheN, OheO, OheP, OheQ, OheR, OheS, OheV	JONES et al. 2000

The tetrameric microsatellites were selected from two publications (JONES et al. 2000, SZABOLCSI et al. 2014) because without a whole genome sequence marker designing was not possible. Twenty-eight markers were chosen, ten tested on red deer (*Cervus elaphus*; DeerPlex markers) (SZABOLCSI et al. 2014) and 18 designed

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to mule deer (*Odocoileus hemionus*; Ohe markers) (JONES et al. 2000) (Table 1). The selection was based on the knowledge that markers developed for a species tend to work in closely related species as well, furthermore, from cross-species testing of the DeerPlex markers we already had confirmation that most of them give products in fallow deer.

PCR protocols were available for both marker sets, thus the first tests were concluded using those parameters. If the PCR did not result in an adequate quantity and quality of product the original protocols were changed accordingly.

The separation of the alleles was done using capillary electrophoresis (ABI Prism3500 GeneticAnalyzer) after that allele sizes were visualized with the help of OSIRIS software. The probability of identity (PI), observed (Ho), and expected heterozygosity (He) were calculated based on the results.

Results and discussion

Out of the 28 markers, two failed to amplify during PCR (T26 and OheD) while one had shown too many non-target PCR products (OheS) leaving 25 markers for further testing.

Altogether four markers were proven to be polymorphic (13%), out of which C229 and T156 had two alleles each (C_1/C_2; T_1/T_2) while OheF and OheQ had three alleles respectively ($F_1/F_2/F_3$; $Q_1/Q_2/Q_3$) within the 15 samples (table 2). In the case of OheQ and T156, only one population had heterozygotes, the first was monomorphic in the Isaszeg population samples while the latter was monomorphic in the Pilis population samples. Observed heterozygosity of all 15 samples was 0.133 (OheQ and T156) and 0.267 (OheF and C229). Expected heterozygosity varied from 0.115 (OheQ) to 0.232 (C229). The calculated PI was 0.22. The interpretation of this is that out of 100 fallow deer on average 22 would have the same genetic profile.

All these statistics indicate low genetic diversity among Hungarian fallow deer. For comparison, the DeerPlex markers in Hungarian red deer were all polymorphic (sample size=303). The marker with the lowest number of alleles had seven alleles (C229) and the marker with the highest number of alleles had 27 alleles (T156) (FRANK et al. 2022). Other studies regarding fallow deer showed similar results, suggesting the potential causes to be the Pleistocene extinction, bottleneck effect, human-mediated reintroduction, and other anthropogenic effects (BAKER et al. 2017). While this reasoning seems sound and well-established, we cannot completely discard the possibility that the markers we tested are not suitable for fallow deer and thus we cannot draw conclusions regarding the Hungarian population solely based on these markers, especially with the small sample size (n=15) and only two sampled populations. However, the low genetic diversity in all other European populations indicates that the former explanation is more likely.

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Table 2. Statistical data of the four polymorphic microsatellite markers: locus name, number of alleles, observed heterozygosity (Ho), allele frequencies, expected heterozygosity (He) examined fallow deer samples, sample size (n), and locations: Pilis (P), Isaszeg (I), total sample size (Σ).

Locus	**Number of alleles**			**Ho**		
	P (n=7)	I (n=8)	Σ (n=15)	P (n=7)	I (n=8)	Σ (n=15)
C229	2	2	2	0.286	0.250	0.267
T156	1	2	2	0	0.250	0.133
OheF	3	2	3	0.286	0.250	0.267
OheQ	3	1	3	0.286	0	0.133
Locus	**Allele frequencies**			**He**		
	P (n=7)	I (n=8)	Σ (n=15)	P (n=7)	I (n=8)	Σ (n=15)
C229	C_1=0.142 C_2=0.856	C_1=0.125 C_2=0.875	C_1=0.133 C_2=0.877	0.243	0.219	0.226
T156	T_1=1.000	T_1=0.125 T_2=0.875	T_1=0.067 T_2=0.933	0	0.219	0.125
OheF	F_1=0.070 F_2=0.070 F_3=0.860	F_1=0.125 F_3=0.875	F_1=0.003 F_2=0.010 F_3=0.087	0.251	0.219	0.232
OheQ	Q_1=0.070 Q_2=0.070 Q_3=0.860	Q_1=1.000	Q_1=0.030 Q_2=0.030 Q_3=0.940	0.251	0	0.115

Similarly, low genetic diversity was observed in other wild animals, such as marsh deer (*Blastocerus dichotomus*). Several genetic markers were tested in this species as well (proteins (OLIVEIRA et al. 2005), mitochondrial DNA (MÁRQUEZ et al. 2006) and dimeric microsatellites (OLIVEIRA et al. 2008)), and they all showed low genetic polymorphism just like in fallow deer. The causes in this species are mainly inbreeding and their polygamous mating system (OLIVEIRA et al. 2005).

Conclusion and recommendation

In conclusion, Hungarian fallow deer showed to have a low allele polymorphism and genetic diversity. Out of 25 markers, only four were polymorphic and even those only had two or three alleles. Both the expected and observed heterozygosity were low and based on the PI value the marker set in its current state does not have the required power of discrimination for forensic investigations.
The inclusion of further markers is necessary as well as the increase of sample size and number of sampled populations. We are planning to test all available markers with tetrameric structure from closely related species in the future.

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Slovenian Learners` Knowledge about Slovenian Native Livestock Breeds

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Abstract

In this paper, we present the results of the study on Slovenian learners` knowledge of native livestock breeds in Slovenia. We surveyed a total of 164 young learners, aged 12 to 15, from three elementary schools in rural areas using a questionnaire. They were asked if they had ever heard the term native breed and what the term Slovenian native breed meant. On the list of 23 livestock breeds, they had to tick the Slovenian native breeds. Finally, learners were asked to list their most common sources of information about Slovenian native breeds. The results of the study show that most learners had already heard the term native breed. Moreover, the learners know best that the Lipizza horse and the Carniolan bee are Slovenian native breeds. However, they do not know that the Bosnian mountain horse is also a native breed on Slovenian territory. Few learners recognised the Drežnica goat and the Cika cattle as Slovenian native breeds. Television and Internet are the most important sources of information about native breeds, while school education obviously does not play a role.

Keywords: Slovenian Native Breeds, Knowledge, Learner

Introduction

Autochthonous (native, indigenous) breeds are livestock populations that have adapted to local conditions, including traditional agricultural production systems and environments. They originate from specific geographic regions, are adapted to the environmental conditions of those regions, and are commonly used there (FAO, 2012). In recent decades, the number of native breeds has declined due to the

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demands of intensive livestock farming and global economic development. It is estimated that nearly 30% of the world`s native breeds are endangered (FAO, 2021). In Slovenia, the conservation of animal genetic resources (AnGR) is included in the various sectoral strategies, plans and programmes at the national level. The long-term programme for the conservation of AnGR biodiversity serves as a strategic document that contains the priority actions needed to protect AnGR, with a focus on Slovenian native breeds (Program …, 2016). National legislation recognises 14 livestock breeds as native to Slovenia: five sheep breeds, four horse breeds, one cattle breed, one pig breed, one goat breed, one chicken breed, and one subspecies of the western honey bee. Education and awareness of the important role of native breeds is carried out through various social media, publications, agricultural fairs and promotional materials for various groups and school classes.

Elementary school learners have various relationships with animals in their daily lives (KNOBLOCH et. al, 2007). They learn about animals in both formal and informal settings. The Experience obtained in interactions with pets, domestic, livestock, and exotic animals, as well as on school field trips to museums, farms, zoos, and outdoor/nature centres, have a significant impact on learners` attitudes and beliefs about animals. Integrating agriculture into elementary and middle school curricula brings learning to life. Elementary and middle school teachers believe that schools play an important role in teaching about agriculture, food, and natural resources. Teachers` beliefs and perceptions about agriculture (biodiversity – native livestock breeds) likely influence what and how they include agriculture in their lessons.

Educators have suggested that integrating agriculture into the general curriculum would help learners, based on the arguments of experiential learning, a community-based curriculum, and authentic or applied learning in real-life situations. Interdisciplinary education is the key to engaging people to think deeply about agriculture (biodiversity) and its role in society. Teachers' beliefs and past experiences influence what and how they teach (BURROWS et al., 2020).

Knowledge of Slovenian native livestock breeds varies widely among elementary school learners. It depends mainly on the modern learning-objective and process-oriented curricula of elementary science education. As far as we know, there are no data on knowledge of Slovenian native livestock breeds among elementary school learners in Slovenia. Therefore, the aim of this study was to investigate which breeds learners recognize as Slovenian native breeds.

Material and methods

A total of 164 learners aged 12 to 15 years from elementary school in rural areas were interviewed using a questionnaire: 80 girls and 84 boys.

The questionnaire contained the following questions:

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- Have they ever heard of the term native breed? If so, what does this term mean?
- Do they know a native breed and if so, write it down.
- Identify native and non-native breeds
- From what sources of information did they get most of their knowledge about Slovenian autochthonous breeds?

In the survey form we entered all 14 native Slovenian livestock breeds recognized by national legislation, and in addition we included other non-native breeds bred in Slovenia. When including other breeds, we focused on breeds that have the adjective Slovenian in their name, but are not native breed.

The teachers distributed the questionnaire to the learners and they answered the questions themselves. It took about 10 minutes to complete the questionnaire.

A survey was conducted in June 2022 among the learners of the last triad of elementary school in rural areas.

Results and discussion

A total of 164 learners aged 12-15 years were surveyed (Figure 1). The breakdown of the total number of boys and girls is shown in the graph. Most learners were in the 13-year-old age group (61 learners) and the fewest learners were 12 years old (25 learners). In terms of gender breakdown, 81 girls and 83 boys were surveyed in all four groups.

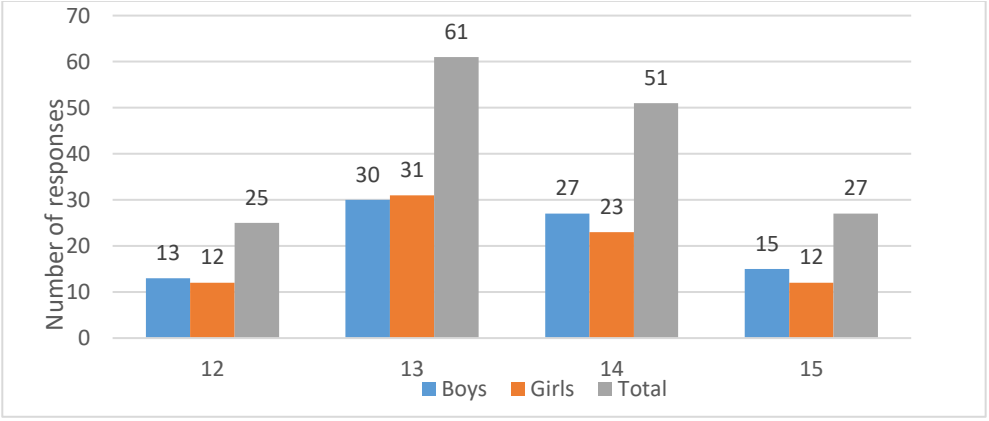

Figure 1: The number of learners who responded the survey, grouped by age and gender

In each age group, the majority of learners had heard the term native livestock breed (Figure 2). Similarly, the majority of learners indicated that they knew at least one of the Slovenian native livestock breeds (Figure 3). However, only a few of them

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wrote down the name of the breed or animal (Lipizza horse, Carniolan bee, Karst shepherd, human fish, bear, wolf …).

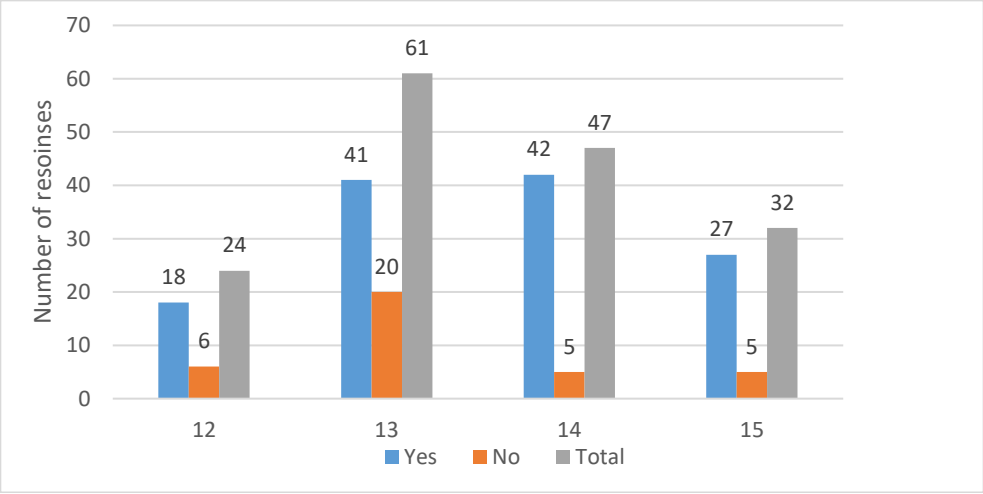

Figure 2: Have you ever heard the term native breed?

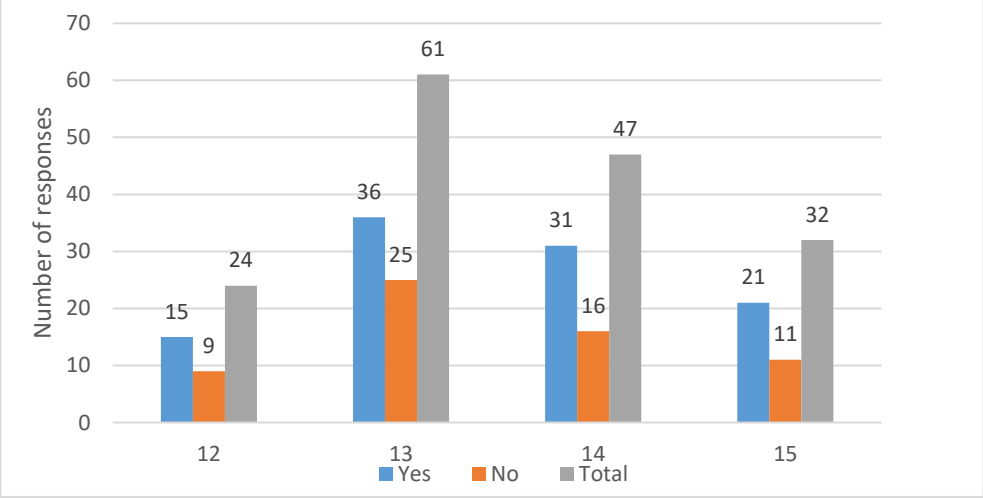

Figure 3: Do you know any of the Slovenian native breeds?

All Slovenian native livestock breeds are shown in Figure 4. The results of the answers where the learners recognized the breeds as Slovenian native livestock breeds are shown in proportions (%).

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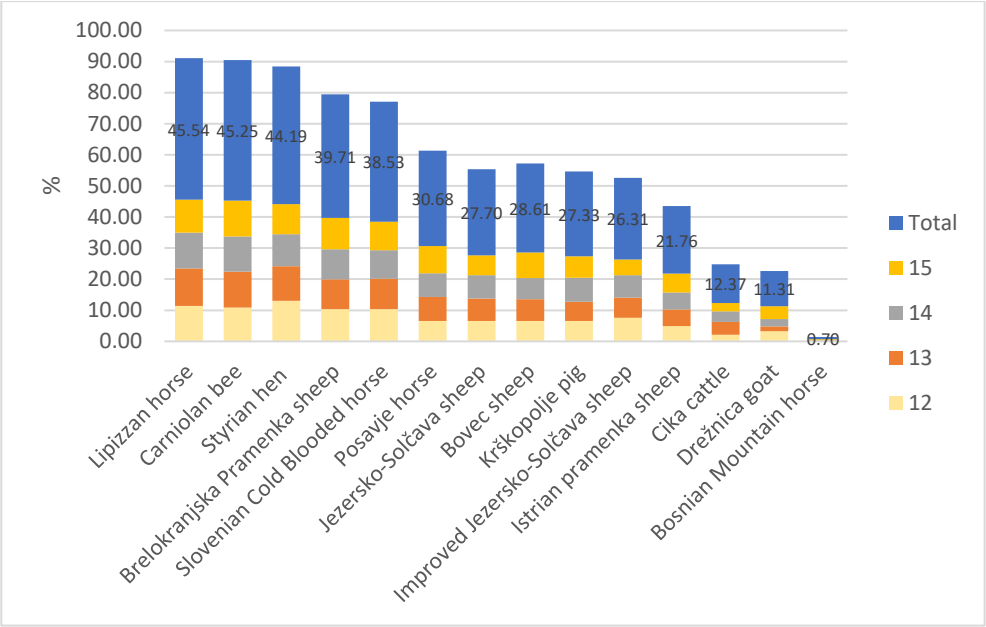

Figure 4: The results of the answers if the learners recognized the breeds as Slovenian native livestock breeds

It is clear that the Lipizzan horse and the Carniolan bee are best known by the learners. These results are very interesting, but not surprising. The Lipizzan horse is one of the oldest bred horse breeds in Europe and the world and has been bred since its foundation in 1580 at the Lipica stud farm in Slovenia. Lipizzan horses are very much associated with Slovenia, although they are also known in Austria through the Spanish Riding School in Vienna. The story of the Lipizzan horse in Slovenia is very popular. The Lipizzan horse is often used for promotional purposes to present Slovenia for tourism. Of all the Slovenian native livestock breeds, the photo of the Lipizzan horse is the most frequently published in the public media.

The Carniolan bee has a similar history of popularity as the Lipizzan horse. Slovenian beekeepers are very active in promoting the Carniolan bee. As part of the "Traditional Slovenian Breakfast" project, which takes place every year on one day in November in kindergartens and elementary schools, beekeepers raise awareness among learners about the important role of bees in successful gardening at home and in the global production of food and in agriculture. In addition, there are many other projects to promote the Carniolan bee: opening of the House of the Carniolan bee, production of an anatomical model of the Carniolan bee in 3D 30 cm… In 2022, both breeds, the Lipizzan horse and the Carniolan bee, were included in the prestigious list UNESCO of intangible cultural heritage. Almost the same result as for the Lipizzan horse and the Carniolan bee was obtained for the Styrian hen (45 % of learners recognized it as a Slovenian native breed). This could be due to the

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adjective "Styrian" in the name, as the same name is used for a region in Slovenia (Styria region).

It is not surprising that, practically all learners did not know that the Bosnian mountain horse is a native breed on Slovenian territory (officially recognized in 2021). In addition, the Drežnica goat and the Cika cattle were classified as a Slovenian native livestock breeds by only 11% and 12% of the learners, respectively. Unfortunately, this means that elementary school learners hardly know these two breeds, which have been bred on Slovenian territory for centuries.

It is evident that television and the Internet together outperform the other responses in all age groups of learners (Figure 5). These two sources are followed by documentaries, school lessons, and others. The graph shows that educational workshops play a minor role in educating learners about Slovenian native livestock breeds.

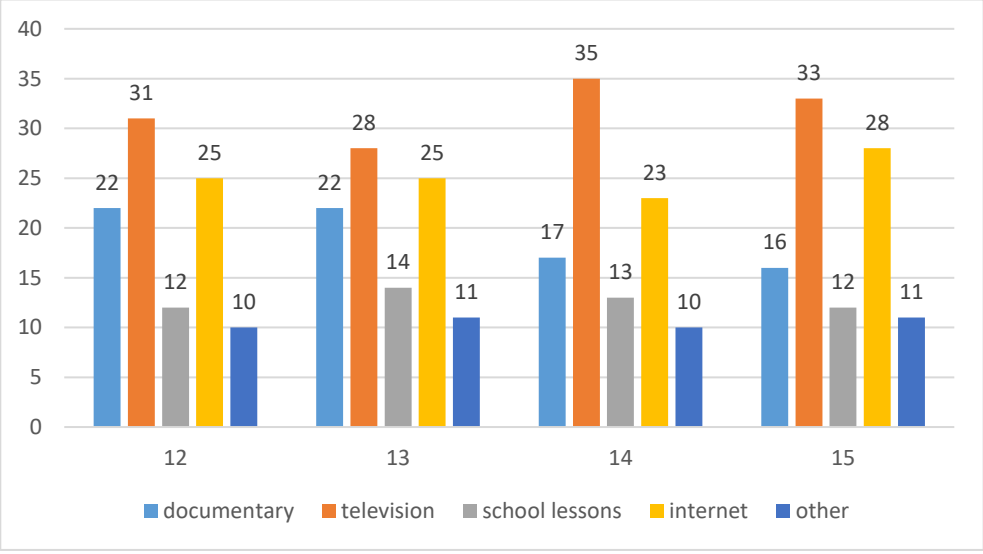

Figure 5: Where did you get your knowledge about Slovenian autochthonous breeds?

Conclusion and recommendation

The results of the study lead to the following conclusions. The first one is that the recognition of written livestock breeds as Slovenian native breeds among learners is not the best. The second one is that most of the learners had already heard the term native breed. The learners know best that the Lipizzan horse and the Carniolan bee are Slovenian native breeds (45 % correct answers). Almost the same result (44 % correct answers) was obtained for the Styrian hen. However, the learners do not know that the Bosnian mountain horse is a native breed on Slovenian territory as

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well. Few learners recognised the Drežnica goat and the Cika cattle as Slovenian native breeds. Television and Internet are the most important sources of information about autochthonous breeds, while school education obviously plays no a role.
The findings of this study may help agricultural education coordinators and agricultural teacher trainers in planning training courses on the integration of farm animal biodiversity into the curriculum.

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Gene bank for Animal Genetic Resources in the Republic of Croatia

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Abstract

The Gene bank for domestic animals in the Republic of Croatia is provided by the National Program for the conservation of local and endangered breeds of domestic animals from 2010. For this purpose, the National Gene bank Network was established, which consists of (i) the Gene bank for domestic animals as a central point under the jurisdiction of the Ministry of Agriculture, and (ii) Recognized Gene banks (authorized by the Ministry of Agriculture and operated by organizations, associations and/or authorized institutions). Until 2023, more than 32,000 samples from six types of domestic animals are stored in the Gene bank as follows: cattle (57.7%), horses (13.2%), sheep (12.1%), pigs (10%), donkeys (5%), and goats (1.9%). Tissue samples (29.2%), hair (28%), semen (42.2%) and blood (0.6%) are mainly stored in the Gene bank. The implementation of the National Program is supported by the Reference Expert Network for the Conservation of Animal Genetic Resources, which promotes greater collaboration with stakeholders. Future plans include further characterization of breeds with emphasis on adaptive traits, and the necessary monitoring and implementation of new and improved procedures in the conservation of local breeds.

Keywords: Gene Bank, Republic of Croatia, local breed, conservation

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Introduction

The original domestic breeds were bred over thousands of years in specific environmental and economic niches that outlined the unique nature of the breed, the unique records in its genome. Their value is reflected in their adaptability, specificity of genotype and phenotype, and a large preserved genetic variability for future requirements that we do not know what they will be. From a global perspective, local breeds contribute to the conservation of biodiversity. The adoption of the Convention on Biological Diversity (CBD 1992) laid the foundations for the conservation and sustainable use of biological diversity, with emphasis on the equitable sharing of benefits arising from the use of genetic resources. The Republic of Croatia is one of approximately 200 signatory countries to the Convention. Recognizing the importance of biodiversity conservation and protection, the Republic of Croatia has prepared several legal and key documents, such as the Strategy and Action Plan for the Protection of Biological and Landscape Diversity (Official Gazette 81/99, 143/08), the National Program for the Conservation and of Local and Endangered Breeds of Domestic Animals in Croatia (2010, 2023) and others.

History and Organisation

The legal basis, the National Program for the Conservation of Local and Endangered Breeds of Domestic Animals in Croatia (2010) and the Operational Program for the Establishment of the Gene Bank of Domestic Animals in the Republic of Croatia (2012) led to the establishment of the Gene bank for Domestic Animals in the Republic of Croatia within the Croatian Agricultural Agency in early 2013. The Gene bank represents a collection of animal genetic material for ex-situ conservation and is a permanent support for in-situ conservation programs for domestic animals. In 2017, the Gene bank became an integral part of the Genetic Evaluation Service for Domestic Animals, which provides support for the evaluation of breeding value for various species in Croatia. Within the Gene bank there is also a laboratory equipped to perform molecular analyses and cryopreservation techniques and is involved in the implementation of commercial breeding programmes in animal breeding (e.g. pigs, bees).
As of January 1, 2019, the Gene bank organizationally belongs to the Ministry of Agriculture, which becomes the umbrella institution, as shown in the schematic Figure 1.

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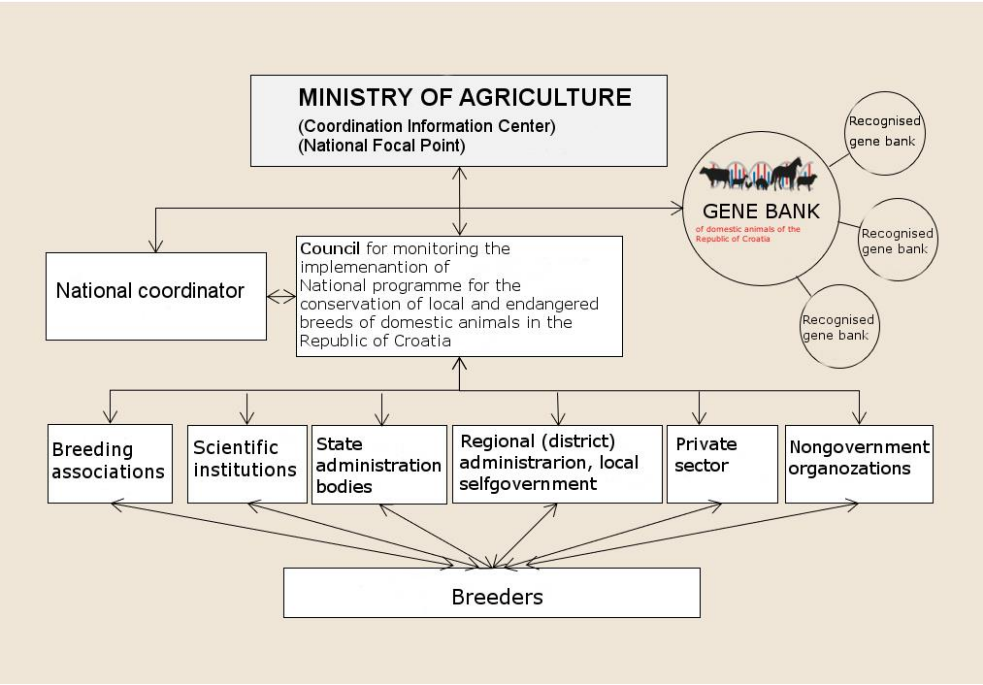

Figure 1. Organisation of a National Network of Gene banks for domestic animals in the Republic of Croatia (https://bag.mps.hr/nacionalna-mreza-banke-gena/)

The National network of the Gene bank for domestic animals of the Republic of Croatia is composed of: i) the Gene bank for domestic animals as the National Gene bank and ii) Recognized Gene banks. The National Gene bank is an integral part of the Ministry of Agriculture and is the national contact point and the coordination and information centre while Recognized Gene banks are established according to the Law on Domestic Animal Breeding (Official Gazette, No. 115/18 and 52/21). Currently, there are four Recognized Gene banks: Agency for Rural Development Istria, Central Association of Croatian Cold Blooded Horse Breeders, Central Association of Croatian Posavina Horse Breeders and Croatian Agency for Agriculture and Food. The Council for monitoring the implementation of the National Program and the National coordinator work closely with the Ministry of Agriculture and the stakeholders in the breeding process, taking into account the opinions of breeders, breeding organizations, scientific institutions, state and local bodies and non-governmental organizations, etc.

One of the documents currently in force is the National Program for the Conservation of Local and Endangered Breeds of Domestic Animals in the Republic of Croatia 2021-2025, adopted by the Ministry of Agriculture. The Program defines two key points: i) strategic guidelines for the development of the national policy for the

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conservation of local and endangered domestic animal breeds and ii) the National Network of Gene banks in the Republic of Croatia. In addition, the National program also establishes strategic guidelines for regional and international cooperation and carries out conservation of animal genetic resources at the global level.

The Reference Expert Network for the conservation of animal genetic resources, which consists of scientific and professional stakeholders, participates in the work of the Gene bank. In Croatia, the Reference Expert Network consists of seven main areas, whose activities range from breeding, valorisation of breeding programs and action plans, conservation of local breeds through methods of in-situ and ex-situ protection, economic use and promotion of breeds, health and legislation, with the main goal of preservation of animal genetic resources.

Collections

From 2013 to 2022, more than 31,300 samples from 43 breeds were stored in the Gene bank of the Republic of Croatia, and the dynamics of the collection is shown in Figure 2.

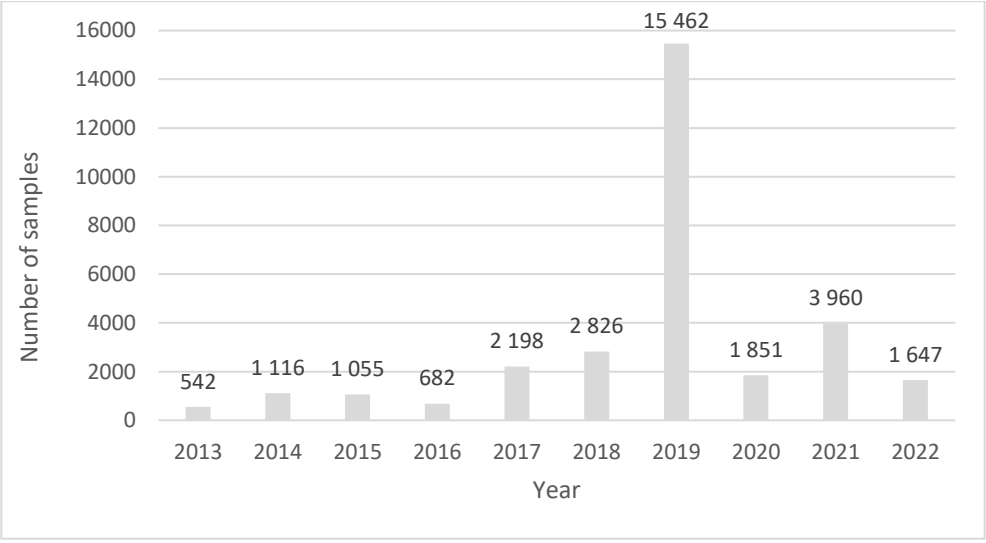

Figure 2. Dynamics and quantity of domestic animal samples collected in the Gene bank of the Republic of Croatia from 2013 to 2022 (HAPIH, personal communication).

It should be noted that 25 local breeds of domestic animals and 22,477 samples are included in the genome collection of the Gene bank of domestic animals of the Republic of Croatia, which is 76% of all collected samples (https://stocarstvo.mps.hr/app/uploads/2021/12/brosura-banka-gena.pdf). Genetic

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material of breeds with local, regional and global importance is stored in the Gene bank, and the stored material represents a public good. Cattle (57.7%) account for the largest proportion of stored samples by domestic animal species, followed by horses (13.2%), sheep (12.1%), pigs (10%), donkeys (5%) and goats (1.9%), as shown in Figure 3a. Within the project OPTI-SHEEP (HRZZ IP -2019-04-3559), more than 1,400 samples of Istrian sheep and Pag sheep were collected, which will be stored in one of the Recognised Gene banks after the completion of the project. Such a synergetic action of several institutions and activities is a good basis for a more comprehensive implementation of additional analyzes contributing to the ex-situ and in-situ conservation of the local breeds. The majority of biological samples stored at the National Gene bank are semen (42.2%), followed by tissue (29.2%), hair (28%), and blood (0.6%; Figure 3b).

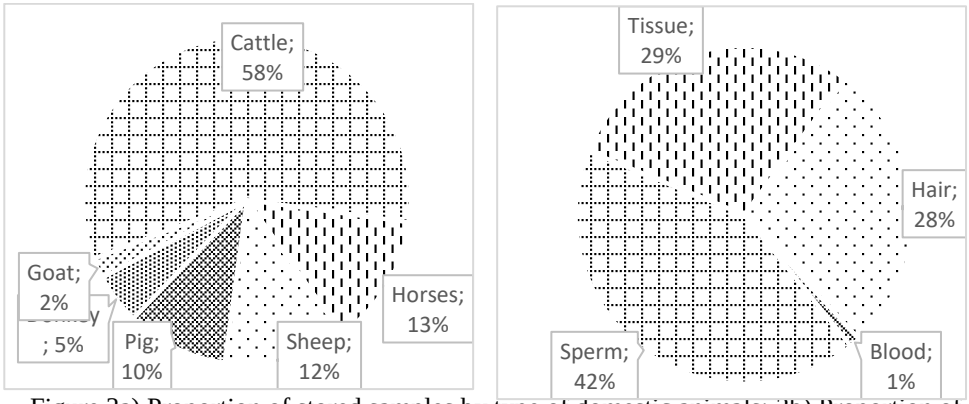

Figure 3a) Proportion of stored samples by type of domestic animals; 3b) Proportion of stored biological samples by sample type in Gene bank.

The introduction should briefly place the study in a broad context and highlight why it is important.

Material and methods

Materials and Methods should be described with sufficient details to allow others to replicate and build on published results.

Results and discussion

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation. Authors should discuss the results and how they can be interpreted in perspective of previous studies and of the working hypotheses.

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Conclusion and recommendation

The work of Gene banks (National and Recognised) is of great importance for the conservation and improvement of all domestic breeds, especially local and endangered ones. Therefore, special attention is focused to the future work plans of the Gene banks, which include continuation of necessary monitoring and collection of samples and further characterization of breeds with emphasis on adaptive traits. Special attention must be directed to introduction of new and improved procedures for conservation of local breeds.

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