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

GÁSPÁRDY, András[1*] – KALTENEKKER, Endre[2] – BORICS, Imre [2]

[1]Institute of Animal Breeding, Nutrition and Laboratory Animal Science, University of Veterinary Medicine Budapest, István utca 2, 1078 Budapest, Hungary;
[2]Association of the Hungarian Grey Cattle Breeders, Lőportár utca 16, 1134 Budapest, Hungary
*corresponding author: gaspardy.andras@univet.hu

Abstract

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

Keywords: cryopreservation, artificial insemination, rare breed conservancy

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Introduction

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

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

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

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Material and methods

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

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

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

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Results and discussion

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

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

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

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

[a,b] different superscript letters differ significantly

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

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

Conclusion and recommendation

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

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