Core collections in the animal genebank for livestock species in the Netherlands

Current state and future strategies to complete the core collections of the Dutch livestock breeds in the genebank for animal genetic resources in the Netherlands

CGN report 57 English

Mira A. Schoon, Malou van der Sluis

WAGENINGEN UNIVERSITY & RESEARCH

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Current state and future strategies to complete the core collections of the Dutch livestock breeds in the genebank for animal genetic resources in the Netherlands.

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The Centre for Genetic Resources the Netherlands (CGN) of Wageningen University & Research (WUR) manages genebank collections of livestock species: cattle, pigs, horses, sheep, goats, dogs, ducks, geese, rabbits and chickens (no material from pigeons and bees has been stored as of July 2022). The objective of the animal genebank is to safeguard the genetic diversity between and within livestock species and breeds. The emphasis is on the conservation of the endangered Dutch native and locally adapted breeds. The main goal is to create a core collection for all relevant breeds. A core collection is defined as the set of sufficient genetic material, in terms of number of minimal doses and unique donors, stored in the genebank, that is required to recover a breed lost by extinction. For most breeds, there is limited material of sufficient quality present in the genebank and insufficient genetic diversity is stored to recover an entire population of healthy animals after extinction. In this report, first the required size of the core collections - to recover an completely extinct breed - was calculated for three different risk scenarios. Secondly an overview of the completeness of the core collections was provided (reference date July 2022). In the discussion, the opportunities and constraints per species are discussed. Based on the information gathered, priorities are set for each breed and animal species to focus on in the coming years .

Dutch summary: Het Centrum voor Genetische Bronnen Nederland (CGN) van Wageningen University & Research (WUR) beheert genenbankcollecties van landbouwhuisdieren: runderen, varkens, paarden, schapen, geiten, honden, eenden, ganzen, konijnen en kippen (er was in juli 2022 nog geen materiaal opgeslagen van duiven en bijen). Het doel van de dierlijke genenbank is om de genetische diversiteit van landbouwhuisdierrassen in Nederland veilig te stellen voor de toekomst. Hierbij ligt de nadruk op het behoud van de (zeldzame) Nederlandse rassen, waarbij een kerncollectie per ras wordt ontwikkeld. Een kerncollectie dient een zodanige omvang te hebben dat het mogelijk is om een ras terug te fokken wanneer het zou uitsterven. Voor de meeste rassen geldt dat er beperkt materiaal van voldoende kwaliteit is opgeslagen en dat er onvoldoende genetische diversiteit opgeslagen is om, in het meest extreme geval, een gehele populatie gezonde dieren terug te kunnen fokken. In dit rapport wordt een overzicht gegeven van de kerncollecties van Nederlandse landbouwhuisdierrassen in de Nederlandse genenbank, peildatum juli 2022, en wordt de benodigde grootte van de collecties berekend om een ras terug te kunnen fokken indien nodig. In de discussie worden de kansen en beperkingen per diersoort besproken. Op basis van de verzamelde informatie wordt per ras en diersoort een prioriteit vastgesteld om in de komende jaren al dan niet aandacht aan te besteden.

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Contents

Preface

The Centre for Genetic Resources, the Netherlands (**CGN**) of Wageningen University & Research (**WUR**) conducts, on behalf of the Dutch government, statutory research tasks associated with the conservation of genetic diversity between and within species and breeds that are important for agriculture and forestry (livestock, plant, forest and aquatic species).

Within the domain of livestock species, CGN is responsible for safeguarding genetic diversity between and within the Dutch livestock breeds in genebank collections. CGN evaluates on a regular basis to what extent the goals of the genebank are realised. An important goal is to ensure that for each (endangered) Dutch livestock breed there is sufficient, high-quality genetic material stored in the genebank to, in the most extreme case, recover an entire population of healthy animals by a back-cross breeding programme. The complete collection of genetic material per breed is referred to as the core collection[1].

This report provides the results of the evaluation and contains conclusions and priorities for further development of the CGN animal genebank collections.

We would like to express our gratitude to the experts who have helped us with advice on the technical parameters for the calculations and evaluation. And to our colleagues at Wageningen Livestock Research (**WLR**) and from the Animal Breeding and Genomics (**ABG**) group of WUR who assisted in translating the Dutch report.

This report is a translation of the original Dutch report: https://www.wur.nl/nl/show/kerncollecties-van-nederlandse-landbouwhuisdierrassen-in-de-genenbank.htm

[1] The term 'core collection' can be used differently in other domains, like for plant or forestry genebanks it is defined by the FAO as: "*A subset selected to contain the maximum available variation in a small number of accessions*" (https://www.fao.org/wiews/glossary/en/)). In this report we are referring to the minimal number of doses and unique donors needed per breed to recover an extinct population by backcrossing with genetic material from the genebank for livestock species.

Summary

In the animal genebank for livestock species in the Netherlands, managed by Centre for Genetic Resources, the Netherlands (**CGN**) of Wageningen University & Research (**WUR**), genetic material from Dutch livestock breeds is stored. The objective of the genebank is to safeguard the genetic diversity within and between breeds and to, in the most extreme case, be able to recover an entire population of healthy animals with genetic material stored in the genebank.

This report first provides an overview of the current genebank collections of Dutch livestock breeds (reference date July 19, 2022). Secondly it shows the minimal number of doses and unique donors required to be able to recover a breed after extinction (core collection). The calculations of the required core collections per species were performed with Conservation Planner (CGN/SZH, 2005), once for a situation with only semen samples and once for a situation with semen and embryo's. Reproduction parameters of different animal species used (for example the average number of offspring per birth and average insemination success rate), were based on literature and consultation of experts. The required core collections were calculated for three scenarios, based on rate of inbreeding per generation (**ΔF**) and the effective population size ($\mathbf{N_e}$):

1) Safe (**S**)	**ΔF** = 0,5% and	$\mathbf{N_e}$ = 100 animals,
2) Compromised (**C**)	**ΔF** = 0,67% and	$\mathbf{N_e}$ = 74,
3) Risky (**R**)	**ΔF** = 1% and	$\mathbf{N_e}$ = 50 animals.

Comparison of the current collections with the desired core collections shows that, although there are breeds that have enough material available for the Safe (**S**) scenario, for many of the breeds in the genebank there is insufficient material available for a full reconstruction, even for the Risky (**R**) scenario.

Based on a set number of male donors, 50% of the desired effective population size, there is sufficient material (at least for the Risky scenario) for a reconstruction of 9 out of 11 cattle breeds, 10 out of 14 pig breeds, 1 out of 5 goat breeds, 7 out of 14 sheep breeds, 2 out of 11 horse breeds, 1 out of 31 chicken breeds, 1 out of 5 duck breeds, and none of the 9 dog breeds, 10 rabbit breeds or few goose breeds. The Dutch pigeon breeds and the one native honey bee were not included in the analyses and the results, given that at the reference date (July 19, 2022) there was no material stored in the genebank yet.

The results of the analyses show that over the coming years it is necessary to collect additional material for all the breeds for which the core collection is currently incomplete. In this way we can ensure that the genetic diversity between and within the Dutch livestock breeds can be safeguarded for the future. A priority level for the next five years has been advised per breed (no priority, low priority, and high priority). For this we looked at the f necessity to expand the core collection as well as actual possibilities. In order to expand the core collections, CGN is dependent of external factors (protocols, laws and regulations, cooperation of breeding organisations) and internal factors (expertise, capacity and budget).

Parallel to expanding the genebank collections by means of constructing the core collections, CGN also offers advice to the breeding organisations of the Dutch livestock breeds about sustainable breeding programmes. The ex-situ genebank and the in-situ activities and advice are complementary towards the common goal of preserving genetic diversity within the Dutch native livestock species and breeds in the Netherlands.

1 Introduction

In the Dutch genebank for livestock species, managed by the Centre for Genetic Resources, the Netherlands (**CGN**), of Wageningen University & Research (**WUR**), genetic material from Dutch livestock breeds is stored. This genebank is one of the largest livestock genebanks in Europe, with a collection of over 350,000 samples (mostly sperm) of almost 9,000 different donor animals and more than 135 different livestock breeds. The objective of the animal genebank is to safeguard the genetic diversity of livestock breeds for the future. The genetic material can, for example, be used to limit inbreeding in current populations or to recover lost populations of extinct breeds. However, whether this is possible depends on the amount and quality of the material that is stored in the genebank.

A core collection is defined as the minimal genebank collection that, in case a breed goes extinct, is required to recover the breed. The required size of the core collection depends (among other things) on the reproductive characteristics of the animal species and the type of genetic material that is stored. Most of the genetic material that is stored in the Dutch genebank consists of sperm cells. However, oocytes, embryos, primordial germ cells (PGCs) or somatic cells can also be stored through means of cryoconservation and, if necessary, be used, to produce new individuals (FAO, 2023).

How a potential reconstruction of a breed will look strongly depends on the type of genetic material that is used and the requirements that need to be met, for example the breed purity of the animal, the success rate of the reconstruction and the maximum expected inbreeding rate per generation. Breed purity signifies the purity of a specific breed in an animal. A 100% ‘purebred’ animal is an animal that consists of 100% genetics of one specific breed. Due to the development of (new) breeds and exchange between populations, there is a margin within the breed purity. The CGN advises to uphold a 87.5% breed purity (as a minimum) in order to classify an individual animal as purebred (this is equivalent to a three generations backcross). For individual animals this is considered acceptable: if a few animals within the breeding population have a breed purity of 87.5%, the average influence of the other breed on the total breeding population is not very high. However, if an entirely ‘new’ breeding population would be 87.5% purebred for the breed that is to be recovered, the average proportion of the other breed would be a lot higher. 100% purebred back breeding is not feasible, which is why the reconstructions featured in this report are based on a breed purity of 97.5% or higher (**Figure 1**).

In order to recover a breed that has gone extinct with only sperm requires multiple generations. The sperm of a breed in the genebank from a 100% purebred animal is first used to inseminate female animals from a different breed (given that female animals of the corresponding breed are no longer available). This first generation offspring is also referred to as F1 (50% breed A – 50% breed B). Subsequently, the animals are backcrossed, which means that the female F1 animals (50% breed A – 50% breed B) are inseminated with the sperm from the genebank of the breed that is to be recovered (100% breed A). The animals born are the F2 generation (75% breed A – 25% breed B), and the female animals from this generation are in turn inseminated with sperm from the genebank of the breed that is to be recovered. Throughout this process the percentage of breed purity rises with each new generation, and with each generation the animals resemble the breed that is to be recovered more. This results in six generations backcross to achieve the desired minimal breed purity of 97.5% for the entire population (**Figure 1**).

When embryos are used, purebred embryos are implanted in female animals of a different breed, which means the resulting offspring are already purebred for the breed that is to be recovered. Subsequently, the female offspring can be inseminated with purebred animal sperm from the genebank, or mate with other purebred animals that were born out of the embryos. This method allows for a breed to be recovered in a much shorter amount of time. A breed is considered to be ‘recovered’ when there are sufficient purebred animals to maintain a healthy population.

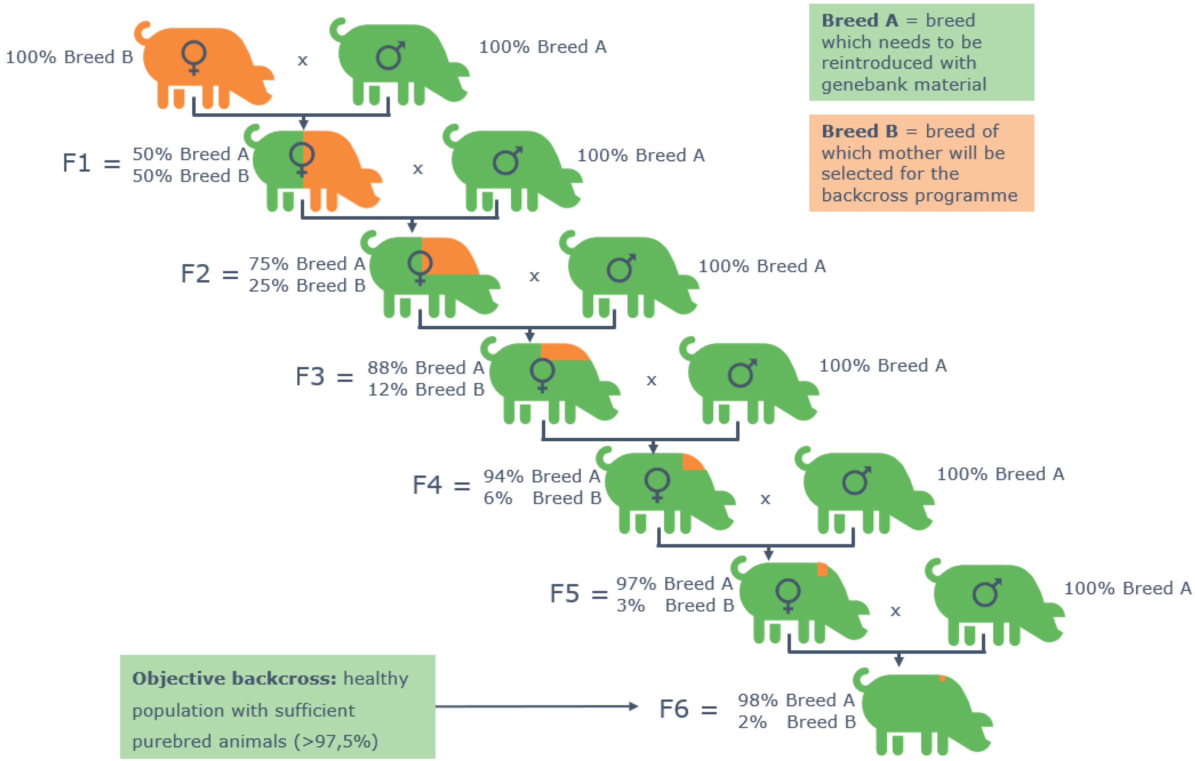


Figure 1 *Schematic visualisation of how to recover a(n extinct) breed with a backcross programme with stored sperm from the genebank to reach the desired breed purity in the sixth generation.*

Apart from the purity of a population, it is also important that the recovered population will not immediately perish due to an accumulation of hereditary defects. The increase in inbreeding per generation provides a good indication for the health of the population after six generations. As the inbreeding per generation rises, the risk of hereditary defects being expressed also rises (**Figure 2**). The absolute maximum is 1% rate of inbreeding per generation, as higher percentages are linked to the risk of extinction due to an accumulation of hereditary defects. In general, a rate of inbreeding per generation of less than 0.5% is considered acceptable for small populations and rare breeds.

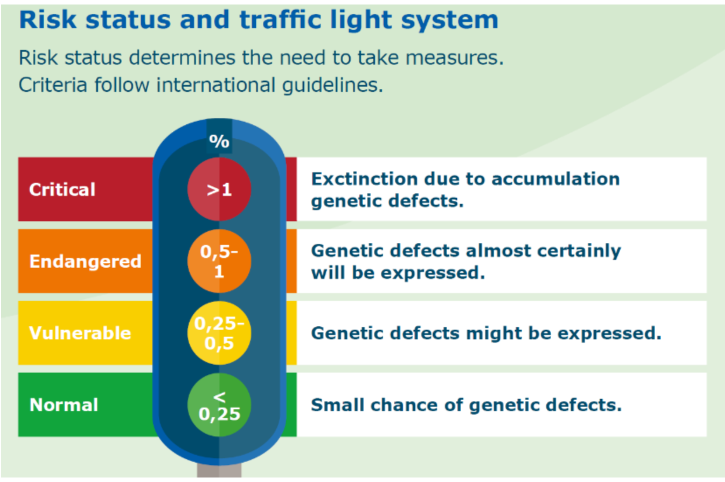


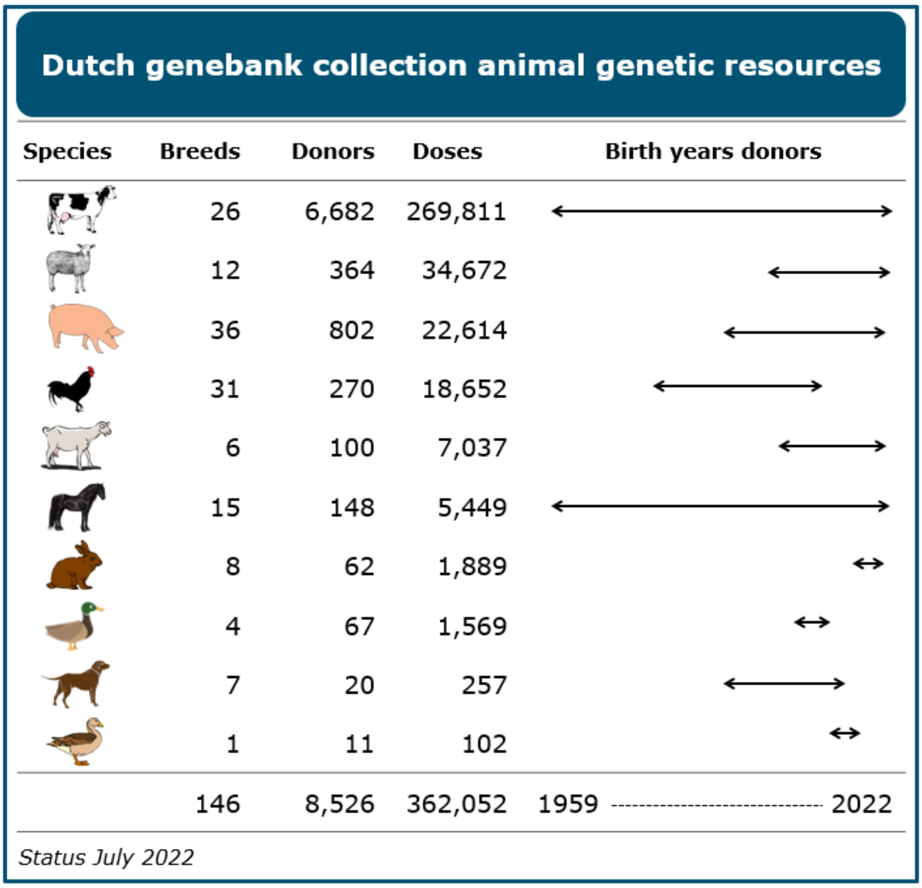
Figure 2 *Rate of inbreeding per generation and corresponding risks (source: www.fokkenmetverstand.nl).*

To get a clear and complete view of the size of the current genebank collection and identify where additions are required to reach the desired core collections, this report provides an overview of the current genebank collection. In addition, we provide indications for the required number of donors and doses of the core collection per animal breed. Lastly, we provide several focus points and priorities for the coming years to strengthen the genebank collection.

2 Overview current CGN genebank collections

Figure 3 shows, for the reference date July 2022, the size of the overall genebank collection (at species level), with the sizes of the different breeds[2], donors and doses per animal species, as well as the distribution in year of birth of the donors. This chapter will focus on the amount and quality of the material that is available per breed in the current CGN genebank collections. To create this overview, we used data from Cryoweb[3] from July 19, 2022. The focus is on Dutch breeds (see **Appendix 1: Dutch – English breed names** for an overview of the breed names in English, where available); although the collection also contains material from non-native Dutch livestock breeds, CGN does not often receive this type of material. Only for cattle are embryos available in the genebank, not for the other animal species, which is why only for cattle information about the embryo collections is provided.

Apart from the animal species that were represented in the genebank up until July 2022, CGN now also supports the Dutch pigeon breeds and the native *Zwarte bij* (European Dark bee). These two animal species were not included in the analyses and are not discussed in the results.


Dutch genebank collection animal genetic resources

Species	Breeds	Donors	Doses	Birth years donors
	26	6,682	269,811	
	12	364	34,672	
	36	802	22,614	
	31	270	18,652	
	6	100	7,037	
	15	148	5,449	
	8	62	1,889	
	4	67	1,569	
	7	20	257	
	1	11	102	
	146	8,526	362,052	1959 – 2022

Status July 2022

Figure 3 *Overview per animal species with the number of breeds (not just Dutch breeds), the number of individual donors, total number of doses and the distribution in years of birth. Reference date July 2022.*

[2] Number of breeds: the total number of breeds for which material is stored. Not limited to Dutch livestock breeds. Other chapters and calculations only include Dutch livestock breeds (native & locally adapted).
[3] Cryoweb: online database used to register and maintain the genebank collection (locations, quality and animal records). As of 2024 Cryoweb is no longer used and the CGN uses the new database Biolomics.

2.1 Cattle

The amount of genetic material (sperm) in the genebank for different cattle breeds is presented in **Table 1**. For two of the cattle breeds in the Dutch genebank, embryos are also available. For *Brandrood rund* there are in total 15 embryos stored of 3 parent combinations, with respectively 4, 5 and 6 embryos per unique parent combination. For the subpopulation *Roodbont Fries vee* there are in total 42 embryos stored of 7 parent combinations, with respectively 1, 4, 4, 6, 7, 10 and 10 embryos per unique parent combination.

Table 1 *Amount of material in the genebank for cattle breeds.*

Breed	Number of unique sperm donors	Total number of straws	Average number of straws per donor (min-max)
Brandrood rund	28	8,870	317 (84-600)
Fries-Hollands vee (zwartbont)[1]	209	28,144	135 (1-887)
Roodbont Fries vee[2]	70	25,037	358 (17-1,685)
Groninger Blaarkop	103	20,960	203 (4-1,464)
Heiderund	6	588	98 (94-100)
Holstein zwartbont	4,558	119,215	26 (10-1,164)
Holstein roodbont[2]	1,247	32,115	26 (8-314)
Lakenvelder	43	2,639	61 (9-428)
MRIJ (Maas-Rijn-IJssel)	369	26,569	72 (3-1,034)
Verbeterd Roodbont	37	2,279	62 (20-400)
Witrik, Vaal en Baggerbont	12	3,366	281 (24-670)

[1]Fries-Hollands zwartbont and fundamental breeding line combined; [2]Animals of specific colour varieties within the breed.

2.2 Pigs

The amount of genetic material in the genebank for different pig breeds is presented in **Table 2**. For cases where the exact number of straws per dose was unknown, 10 straws per dose was assumed. Over the course of time, some breeds were conjoined, changed name or were terminated all together. In **Appendix 2: pig breeds classification** the current classification of the pig breeds are presented. The report "*Varkensrassen in de genenbank: beschrijving van de rassen en de ontwikkelingen in de varkensfokkerij*" contains more information about the history of the different pig breeds (https://edepot.wur.nl/423162).

Table 2 *Amount of material and quality of material in the genebank for pig breeds.*

Breed	Number of unique sperm donors	Total number of straws	Total number of doses[1]	Average number of doses per donor (min-max)
Bonte Bentheimer landvarken	1	345	69	69 (69-69)
Hypor Landras	0	0	0	0 (0-0)
Hypor Large White	0	0	0	0 (0-0)
Meishan*	29	7,051	1,540	53 (8-81)
Nederlands Landvarken	79	15,506	3,232	41 (6-263)
Topigs Norsvin A-lijn* (Large White)	48	9,084	1,301	27 (3-102)
Topigs Norsvin B-lijn*	30	6,046	1,122	37 (2-103)
Topigs Norsvin D-lijn (TN Talent, Duroc)	76	10,880	1,979	26 (1-63)
Topigs Norsvin E-lijn (TN Tempo, Large White)	85	18,502	2,557	30 (3-132)
Topigs Norsvin N-lijn (Nederlands landras)	124	17,319	2,963	24 (4-90)
Topigs Norsvin P-lijn (TN Select, TN Top Select, Piétrain)	50	8,203	881	18 (1-42)
Topigs Norsvin T-lijn	43	9,127	1,548	36 (8-86)
Topigs Norsvin Y-lijn*	8	4,406	865	108 (16-175)
Topigs Norsvin Z-lijn (Large White)	158	24,524	3,819	24 (0-127)

[1]Rounded down; * Lines or breeds no longer available.

2.3 Goats

The amount of genetic material stored in the genebank for goat breeds is presented in **Table 3**.

Table 3 *Amount of material in the genebank for goat breeds.*

Breed	Number of unique sperm donors	Total number of straws	Average number of straws per donor (min-max)
Nederlandse Melkgeit	2	89	45 (32-57)
Nederlandse Bonte geit	21	926	44 (2-118)
Nederlandse Landgeit	36	4,713	131 (32-364)
Nederlandse Witte geit	21	937	45 (2-179)
Nederlandse Toggenburger geit	15	302	20 (5-35)

2.4 Sheep

The amount of genetic material for sheep breeds is presented in **Table 4**.

Table 4 *Amount of material in the genebank for sheep breeds.*

Breed	Number of unique sperm donors	Total number of straws	Average number of straws per donor (min-max)
Bonte schaap	0	0	0 (0-0)
Drents Heideschaap	71	5,694	80 (4-219)
Flevolander	17	2,555	150 (3-393)
Groot Heideschaap	0	0	0 (0-0)
Fries melkschaap[1]	69	3,285	48 (1-249)
Kempisch Heideschaap	32	5,028	157 (23-263)
Mergelland schaap	38	4,144	109 (22-195)
Noord Hollander	5	347	69 (44-103)
Schoonebeeker	33	3,918	119 (2-228)
Swifter	0	0	0 (0-0)
Texelaar	0	0	0 (0-0)
Blauwe Texelaar[2]	13	1,306	100 (45-203)
Dassenkop Texelaar[2]	0	0	0 (0-0)
Veluws Heideschaap	49	5,223	107 (25-427)
Zwartbles	32	2,970	93 (5-615)

[1]Before *Fries and Zeeuws Melkschaap*, there are currently no more living *Zeeuwse melkschapen*; [2]Animals of specific colour varieties within the breed.

2.5 Dogs

The amount of genetic material in the genebank for dog breeds is presented in **Table 5**.

Table 5 *Amount of material and quality of the material in the genebank for dog breeds.*

Breed	Number of unique sperm donors	Total number of straws	Total number of doses[1]	Average number of doses per donor (min-max)
Drentse Patrijshond	0	0	0	0 (0-0)
Hollandse herder	0	0	0	0 (0-0)
Hollandse smoushond	0	0	0	0 (0-0)
Kooikerhondje	0	0	0	0 (0-0)
Markiesje	0	0	0	0 (0-0)
Nederlandse schapendoes	0	0	0	0 (0-0)
Saarloos Wolfhond	1	6	2	2 (2-2)
Stabyhoun	8	140	103	13 (1-27)
Wetterhoun	4	76	76	19 (11-39)

[1]Rounded down.

2.6 Horses

The amount of genetic material in the genebank for horse breeds is presented in **Table 6**. For some registrations only mixed samples are available (multiple donors in 1 straw/insemination dose), these registrations are not included in the table.

Table 6 *Amount of material and quality of the material in the genebank for horse breeds.*

Breed	Number of unique sperm donors	Total number of straws	Total number of doses[1]	Average number of doses per donor (min-max)
Fries paard	178	9,932	1,510	8 (0-140)
KWPN Gelders paard	18	10,033	523	29 (1-76)
Groninger paard	51	9,784	1,522	30 (0-118)
Klassiek Gelderlander paard	2	562	59	30 (26-34)
KWPN Rijpaard[2]	8	1,605	193	24 (5-55)
NRPS Rijpaard	0	0	0	0 (0-0)
NRPS Rijpony	0	0	0	0 (0-0)
Trekpaard	50	8,392	1,037	21 (1-90)
KWPN Tuigpaard	17	3,680	559	33 (3-83)
Zwaar Warmbloed paard[3]	1	141	8	8 (8-8)

[1]Rounded down; [2]*KWPN Rijpaard* or *KWPN Springpaard* in the genebank; [3]*Nederlands Warmbloed* in the genebank.

2.7 Chickens

The amount of genetic material in the genebank for chicken breeds is presented in **Table 7**.

Table 7 *Amount of material in the genebank for chicken breeds.*

Breed	Number of unique sperm donors	Total number of straws	Average number of straws per donor (min-max)
Assendelfts Hoen[1]	12	642	54 (3-93)
Baardkuifhoen[1]	11	851	77 (21-153)
Barnevelder[1]	14	1,124	80 (5-229)
Brabanter[1]	10	1,203	120 (46-186)
Chaams Hoen	10	991	99 (11-186)
Drentse Hoen	13	672	52 (26-99)
Drentse Hoen bolstaart	0	0	0 (0-0)
Drentse kriel	0	0	0 (0-0)
Drentse kriel bolstaart	0	0	0 (0-0)
Eikenburger kriel[2]	7	201	29 (2-50)
Fries Hoen[1]	17	1,142	67 (32-99)
Groninger Meeuw[1]	10	851	85 (18-138)
Hollands Hoen[1]	31	1,182	38 (1-107)
Hollandse kriel	11	455	41 (30-50)
Hollandse kuifhoenders[1]	11	827	75 (2-239)
Kraaikop[1]	10	980	98 (48-185)
Lakenvelder hoen[1]	9	879	98 (14-170)
Leghorn (Nederlands type)[1]	0	0	0 (0-0)
Nederlandse sabelpootkriel	20	564	28 (1-47)
Noord-Hollandse Blauwe[1]	17	1,571	92 (2-240)
Schijndelaar	5	496	99 (52-143)
Twents hoen[1]	14	990	71 (28-145)
Uilebaard[1]	12	848	71 (27-101)
Welsumer[1]	13	910	70 (12-111)
ISA/HG Rhode Island Red	0	0	0 (0-0)
ISA/HG Rhode Island White	0	0	0 (0-0)
ISA/HG barred Plymouth Rock	0	0	0 (0-0)
ISA/HG Australorp	0	0	0 (0-0)
ISA/HG New Hampshire	0	0	0 (0-0)
ISA/HG White Leghorn	0	0	0 (0-0)
ISA/HG Sussex	0	0	0 (0-0)

[1]In the genebank (at the moment) no difference between *kriel* (bantam) or not, so including *kriel*; [2]White and black combined.

2.8 Geese

The amount of genetic material in the genebank for geese breeds is presented in **Table 8**.

Table 8 *Amount of material in the genebank for geese breeds.*

Breed	Number of unique sperm donors	Total number of straws	Average number of straws per donor (min-max)
Twentse landgans	11	102	9 (2-25)

2.9 Ducks

The amount of genetic material in the genebank for duck breeds is presented in **Table 9**.

Table 9 *Amount of material in the genebank for duck breeds.*

Breed	Number of unique sperm donors	Total number of straws	Average number of straws per donor (min-max)
Hollandse kuifeend (incl. dwarf)	0	0	0 (0-0)
Hollandse Kwaker	14	218	16 (2-46)
Noord Hollandse Krombekeend	34	815	24 (1-104)
Noord Hollandse witborsteend	18	499	28 (3-56)
Overbergse eend	1	37	37 (37-37)

2.10 Rabbits

The amount of genetic material in the genebank for rabbit breeds is presented in **Table 10**.

Table 10 *Amount of material and quality of the material in the genebank for rabbit breeds.*

Breed	Number of unique sperm donors	Total number of straws	Total number of doses[1]	Average number of doses per donor(min-max)
Beige	8	297	297	37 (12-56)
Deilenaar	3	209	209	70 (63-73)
Gouwenaar	12	367	353	29 (4-73)
Havana	11	233	213	19 (1-35)
Hulstlander	8	180	180	23 (4-61)
Klein lotharinger	0	0	0	0 (0-0)
Nederlandse hangoordwerg	0	0	0	0 (0-0)
Nederlandse kleurdwerg	0	0	0	0 (0-0)
Sallander	3	95	95	32 (23-48)
Thrianta	9	327	327	36 (20-62)

[1] Rounded down.

3 Method for calculating required core collections

3.1 Conservation Planner

To calculate the minimum size required for core collections, the Conservation Planner tool (CGN/SZH, 2005) was used. This Excel tool provides the user with the possibility to calculate, depending on different input parameters, the required number of donors, sperm doses, or embryos for reconstruction of a breed. This tool was developed by the CGN and Stichting Zeldzame Huisdierrassen (SZH) and can be accessed through https://www.wur.nl/nl/onderzoek-resultaten/kennisonline-onderzoeksprojecten-lvvn/centrum-voor-genetische-bronnen-nederland-1/dier/expertise-en-advies.htm.

3.2 Representative reproduction values

There are differences between animal species regarding the required size of the core collection in order to reconstruct a breed. This depends, for example, on the average litter size per birth. In addition, the type of genetic material which is stored is also significant, and for this certain factors affect species differently. For example, the average success rate of insemination using sperm can differ between species. In addition, there can be differences between species in terms of the percentage of viable embryos after thawing, the average success rate after implantation, and the number of embryos per implantation.

Table 11 shows the average litter size per species, the number of living offspring to be expected from one dose of frozen sperm, the number of living offspring to be expected from one frozen embryo, and the (usual) number of embryos that are implanted at a time. These values are based on consultations of experts and are explained in more detail below. If additional literature was consulted, this has been indicated. Given that the numbers in **Table 11** indirectly include the success rate of insemination and the viability of the offspring after birth/hatching, the calculations that follow include the numbers from **Table 11** for the pregnancy rate (sperm, embryos), whereas the survival rate up until fertile age (sperm, embryos) and the percentage of viable embryos after thawing (embryos) are set to one.

Table 11 *Representative average reproduction values for cattle, pigs, goats, sheep, dogs, horses, chickens, ducks and geese, and rabbits.*

Species	Cattle	Pig	Goat	Sheep	Dog	Horse	Chicken	Goose, duck	Rabbit
Average litter size	1	12[a]	2	2	5[b]	1	3-6[c]	10[d]	7[e]
Average number of offspring per dose of frozen sperm (or insemination success for multiple offspring per insemination)	0.50	0.50	0.20	0.20	0.65	0.40	0.5[f]	0.50[g]	0.8
Average number of offspring per frozen embryo	0.35	0.14	0.30	0.30	N.a.	0.52	N.a.	N.a.	0.23[h]
Number of embryos implanted at a time	1	30	3	3	N.a.	1	N.a.	N.a.	10[h]

[a] AHDB (2021); [b] Borge et al. (2011); [c] Bakst (2011); [d] de Vos (2014); [e] Blasco et al. (2017); [f] Donoghue & Wishart (2000); [g] Váradi et al., 2019; [h] Marco-Jiménez et al. (2018).; N.a. = Not applicable.

3.2.1 Cattle

For cattle it is expected that, on average, two doses of sperm will result in one calf being born. For this reproduction value it has been taken into account that some individuals may have a lower sperm quality and that, consequently, a single dose may consist of a larger number of straws (see also the calculations of the current core collections). For embryos an average calving percentage of 35-50% is to be expected. To be on the safe side, we assumed an calving percentage of 35%. The average litter size for cattle is 1.

3.2.2 Pigs

In Europe the average number of weaned piglets per birth is 12.8 (AHDB, 2021). To stay on the safe side, we assumed an average of 12 piglets per birth. It is expected that, on average, two doses of sperm result in one piglet being born. For *in vivo* produced or collected embryos that were thawed after vitrification, a realistic estimate to work with is that seven previously thawed embryos result in one living piglet, which corresponds to 0.14 offspring per frozen embryo. On average 30 embryos are implanted simultaneously.

3.2.3 Goats and sheep

The average expected litter size for goats and sheep is two. In addition, it is expected that, on average, five doses of sperm result in one lamb being born. For embryos it is expected that the average number of offspring per frozen embryo is somewhat lower than it is for cattle, which is why the estimated value for goats and sheep is 0.30.

3.2.4 Dogs

For dogs the litter size differs significantly between breeds. However, for simplification purposes we assumed an average of 5 offspring per litter, based on a study of 224 purebred dogs (Borge et al., 2011). The average success rate of insemination ranges from 65% to 80% in case of intrauterine endoscopic inseminations with frozen sperm during the optimum breeding period of the female dog. To be on the safe size, we assumed an insemination success rate of 65%. Embryo techniques for dogs have not been developed because it is prohibited by law to surgically harvest embryos or flush these from the ovaries or uterus of dogs. There are also practical limitations for using embryo techniques, for example the complex synchronisation of the oestrous cycle of the female dog and the fact that the oocytes still need to mature and divide in the oviduct after ovulation.

3.2.5 Horses

For horses a pregnancy rate of 40% (with a range between 35% to 50%) is expected for thawed sperm. For embryos it depends on the type of embryo that has been frozen. For fresh embryos (obtained from a mare by embryo collection after artificial insemination) it is expected that approximately 80% will be viable after freezing. Consequently, a pregnancy rate of 65-70% is expected. For ICSI embryos (produced through oocyte recovery and in vitro production through intracytoplasmic sperm injection and culturing up to the blastocyst stage) pregnancy rates of approximately 70% are expected after implantation. Hence, we work with an average number of offspring per frozen embryo of 0.52 (0.8 * 0.65) to stay on the safe side in the calculations.

3.2.6 Chicken

How many offspring a chicken will produce after one insemination strongly depends on the breed, given that breeds can differ in the number of eggs they produce per time unit. Chickens can store sperm internally from a few days up to several weeks (Bakst, 2011). To stay on the safe side, we assumed one insemination for a laying period of one week. Commercial poultry breeds produce, on average, 300 eggs per year, while local breeds produce approximately 150 eggs per year. Per week, this corresponds to approximately 6 eggs for commercial breeds and 3 eggs for local breeds. For chickens, the fertilisation success of artificial inseminations with frozen sperm is approximately 0.5 (Donoghue & Wishart, 2000).

3.2.7 Geese

For the number of offspring per clutch for geese we specifically looked at the *Twentse landgans*, the only Dutch geese breed. The *Twentse landgans* usually lays 10 to 12 eggs per clutch (de Vos, 2014). To stay on the safe side, we assumed a clutch of 10 eggs. Research has shown that for geese, a fertility percentage of 58.5% can be achieved with frozen sperm (Váradi et al., 2019). To stay on the safe side, we assumed an insemination success rate of 50% for geese.

3.2.8 Ducks

The reproductive success of ducks through artificial insemination is not entirely clear yet, so for ducks we used the same values as for geese.

3.2.9 Rabbits

For rabbits the litter size differs between litters, but the average litter size is slightly above 7 lampreys per birth (Blasco et al., 2017). The average insemination success for frozen sperm is approximately 80%. A recent study has shown that there is a success rate of 23% for offspring by using frozen embryos (Marco-Jiménez et al., 2018). On average, 10 embryos were implanted per implantation (Marco-Jiménez et al., 2018).

3.3 Scenarios for calculating required core collections

To calculate the required core collections to restore breeds, three different scenarios were assumed: Safe (**S**), Compromised (**C**), and Risky (**R**; **Table 12**). The scenarios differ in terms of effective population size and, consequently, also in the expected rate of inbreeding per generation. The scenarios also differ in their respective chance of success under specific conditions. For the Risky scenario there is only a chance of success if the scenario is applied meticulously, in which case every male animal is replaced by one son, and every female animal by one daughter. This can be difficult to apply in conventional livestock breeding. For such a scenario, selection is almost impossible: there is only a choice between full blood brothers or full blood sisters if multiple animals of the same sex are born. Consequently, it might happen that less suitable animals have to be used in the breeding programme. Within the Safe scenario, selection is possible, and this scenario is well executable in conventional livestock breeding. For the Compromised scenario, some chance selection is possible, for example if one animal has a larger litter size than another animal.

For all scenarios we focused on a core collection that is large enough to reconstruct a breed (as a one-off, not multiple times) to the desired breed purity of at least 97.5%, using only sperm (not embryos). When using only sperm, multiple generations are required to cross back a breed, as the sperm of a breed is used to inseminate female animals of a different breed, and the female offspring are in turn inseminated with the sperm of the breed that is to be crossed back. Through this process, each generation of animals will start to resemble the breed that is to be crossed back more (**Figure 1**).

In addition to these three scenarios for using only sperm, a calculation was made for the combination of sperm and embryo availability. When embryos are used, these are implanted in female animals of a different breed, and the offspring resulting from this insemination are directly purebred. Consequently, the female offspring are inseminated with the sperm (from the genebank) of the breed that is to be crossed back. Thus, using embryos significantly speeds up the process.

Table 12 *Scenarios for calculating the required core collections.*

Scenario	**Safe**	**Compromised**	**Risky**
Effective population size	100	74	50
Rate of inbreeding per generation	0.5%	0.67%	1%
Number of male donors[1]	50	37	25
Calculated minimum number of female animals required per generation (see 3.4.3)	53	40	28
Calculated inbreeding rate after full programme of 6 generations with sperm only (see 3.4.6)	2.9%	3.9%	5.8%

[1] For these scenarios the number of male donors is fixed at 50% of the effective population size.

3.4 Calculations of scenarios with sperm only

The three abovementioned scenarios were calculated with the Conservation Planner (CGN/SZH Conservation Planner, 2005) and additional manual calculations based on the Conservation Planner. The calculations are explained below, with scenario Risky (**R**) for cattle as a calculation example.

3.4.1 Input data

Required input data are:

- the number of male animals (**M**) per generation in the reconstruction programme
- the desired effective population size (**N_e**)
- the pregnancy rate for sperm insemination (**PR_s**)
- the average litter size (**LS**)
- the survival rate until fertile age (**S**)
- the desired breed purity (**BP**; 0.975 for current calculations)
- the success rate of the reconstruction (**SR**; 0.95 for current calculations)
- the number of reconstructions or cycles (**C**; 1 for current calculations)

3.4.2 Calculation of the required length of the reconstruction programme (in generations)

With each generation of the reconstruction programme, the percentage of genetic material originating from the breed that was used for the reconstruction of the animals (that is, the breed from which the mothers are recruited to reconstruct the desired breed) decreases by half (**Figure 1**). The required length of the reconstruction programme in generations (**L**) can be calculated as followed:

$$L = \frac{\log_{10}(1 - BP)}{\log_{10} 0.5}$$

For a desired breed purity (BP) of 0.975 this results in a programme length of 6 generations (rounded up).

3.4.3 Calculation of the minimum number of female animals required per generation

The minimum number of female animals required per generation (**min F for N_e**) is calculated as followed:

$$\min F\ voor\ N_e = \frac{1 + \left(1 + \frac{1}{M}\right)/M}{\frac{4}{N_e} - \left(1 + \frac{1}{M}\right)/M}$$

For 25 male animals (**M**) and an effective population size (**N_e**) of 50 animals, this results in a total minimum number of 28 required female animals per generation (rounded up).

3.4.4 Calculation of the number of successful inseminations required to produce sufficient fertile female offspring

The calculation for the number of successful inseminations (**SI**) that is required to produce sufficient female offspring, consists of multiple steps.

First, the chance of at least one fertile female offspring per pregnancy is calculated. The overall chance of a female offspring at the birth of one animal is 0.5. If the survival rate until fertile age is equal to **S**, that means that the chance of no female fertile offspring for a litter size of **LS** is equal to $(1-0.5*S)^{LS}$. Consequently, the chance of at least one fertile female offspring per pregnancy ($\boldsymbol{P(1fF/Pr)}$) equals:

$$P(1fF/Pr) = 1-(1-0.5*S)^{LS}$$

With the chance of at least one fertile female offspring per pregnancy ($= P(1fF/Pr)$) we can calculate the chance of at least one fertile female animal per insemination ($\boldsymbol{P(1fF/i)}$). Here, the pregnancy rate (**PR$_s$**) is important, and the chance of at least one fertile female animal per pregnancy is multiplied with the pregnancy rate after insemination:

$$P(1fF/i) = P(1fF/Pr)*PR_s$$

Once we know if the insemination will result in at least one fertile female offspring, we can also calculate how many successful inseminations are required to reach the minimum number of female animals for a reconstruction programme. You can never be 100% certain, because random coincidences can result in fewer female animals being born than expected. However, a probability distribution can calculate how many successful inseminations are required to succeed in 95% of the cases. Here, an inverse normal distribution was used, with a mean of 0 and a standard deviation of 1 (NORMINV(1-succes rate, 0, 1)). The final calculation of the number of successful inseminations (**SI**) needed to obtain sufficient fertile females, was calculated using:

$$SI = \left(\frac{\left(a*\sqrt{P(1fF/i)*(1-P(1fF/i))}+\sqrt{a^2*(P(1fF/i)*(1-P(1fF/i)))+P(1fF/i)*min\,F\,voor\,N_e*4}\right)}{2*P(1fF/i)}\right)^2$$

Here a equals NORMINV(1-0.95, 0, 1) = 1.644.

Concluding, for a pregnancy rate of 0.5, a litter size of 1 and a survival rate of 1, as well as a success rate of 0.95, this calculation results in 147 required successful inseminations (rounded up).

3.4.5 Calculation of the required number of sperm doses

The required number of sperm doses is found by multiplying the number of inseminations by the number of generations of the reconstruction cycle and the number of reconstruction cycles::

$$doses\ sperm = SI*L*C$$

Using the previously calculated or input data (**SI** = 147, **L** = 6, **C** = 1) this results in 890 required sperm doses (rounded up to the nearest ten).

3.4.6 Calculation of the inbreeding percentage per generation

The calculation used to determine the final inbreeding percentage (**I**), assuming a situation where the animals used at the start were not related and were not inbred at the start of the reconstruction, consists of two steps.

First, the increase in inbreeding ($\Delta \boldsymbol{F}$) per generation was calculated with the following equation:

$$\Delta F = 0.125 * \left(\frac{\left(1 + \frac{1}{M}\right)}{M} + \frac{1 + \frac{1}{M}}{\min F\ voor\ N_e} \right)$$

In case of 25 male animals (**M**) and at least 28 female animals (**min F for N_e**) per generation, this results in an increase in inbreeding of 1% per generation (rounded up).

Subsequently, the final inbreeding percentage ($\boldsymbol{I}$) can be calculated, taking the length of the reconstruction programme (number of generations) into account:

$$I = 1 - (1 - \Delta F)^L$$

In case of 25 male animals and the calculated 28 female animals per generation, this results in a total increase in inbreeding over the entire length of the reconstruction programme of 5.8%.

3.5 Calculations of the scenarios with both sperm and embryos available

All three scenarios were also calculated for the use of both sperm and embryos, with the Conservation Planner. The calculations are explained below, again with scenario Risky (**R**) for cattle as a calculation example.

3.5.1 Input data

Required input data are:

- the number of male animals (**M**) per generation in the reconstruction programme
- the desired effective population size (**N_e**)
- the pregnancy rate for sperm insemination (**PR_S**)
- the average litter size (**LS**)
- the survival rate until fertile age (**S**)
- the success rate of the reconstruction (**SR**; 0.95 for current calculations)
- the number of reconstructions or cycles (**C**; 1 for current calculations)
- the percentage of viable embryos after thawing (**V**)
- the pregnancy rate when embryos are used (**PR_E**)
- the number of embryos per implantation (**E**)
- whether the sex of the embryos is known (sex is assumed to be known in the current calculations)

3.5.2 Calculation of the required number of embryos

The required number of embryos can be calculated via a series of steps. First, the required number of female animals for the effective population size ($\boldsymbol{number\ F\ for\ N_e}$) is calculated:

$$number\ F\ for\ N_e = 1/\left(\frac{4}{N_e} - \frac{1}{M}\right)$$

In addition, the odds of a female offspring per pregnancy ($\boldsymbol{P(1fF/Pr)}$) is determined. In this calculation the odds of the embryo being female ($\boldsymbol{P(embryo_s = F)}$) is determined first. For embryos where the sex is determined beforehand (as is the case in this analysis), these odds are equal to one. The calculation of the odds of a female offspring per pregnancy are as followed:

$$P(1fF/Pr) = 1 - (1 - P(embryo_s = F) * S * V)^{LS}$$

With a survival rate of 1, a percentage of viable embryos after thawing of 1, and an average litter size of 1, this will result in the odds of a female offspring from the birth also being 1.

Consequently, with the odds of a female offspring per birth, the odds of a female offspring per embryo transfer can be calculated ($\boldsymbol{P(1fF/t)}$). These odds are calculated based on the odds of a female offspring per birth and the pregnancy rate when embryos are used:

$$P(1fF/t) = P(1fF/Pr) * PR_E$$

The next step is to calculate the maximum number of implantations (**I_{max}**) by using the following formula:

$$I_{max} = \left(\frac{\left(\alpha_2 * \sqrt{P(1fF/t) * (1 - P(1fF/t))} + \sqrt{\alpha_2{}^2 * (P(1fF/t) * (1 - P(1fF/t))) + 4 \ * P(1fF/t) * number\ F\ for\ N_e}\right)}{2 * P(1fF/t)}\right)^2$$

Where $\boldsymbol{\alpha_2}$ = NORMINV(error level, 0, 1) = 1.644 (see paragraph **3.4.4**) and error level = 1 – the success rate.

Finally, the required number of embryos can be calculated as followed:

$$required\ number\ of\ embryos = E * I_{max}$$

Summarising, using the previously mentioned example values (**S** = 1, **V** = 1, **LS** = 1) and one embryo per implantation, a pregnancy rate when embryos are used of 0.35 and an effective population size of 50 animals, of which 25 are male, this results in 94 required embryos.

3.5.3 Calculation of the number of sperm doses per available male animal for an embryo and sperm scenario

The required number of sperm doses per available male animal are also calculated via a series of steps. First, the odds of at least one fertile female offspring per pregnancy ($\boldsymbol{P(1fF/Pr)}$) is calculated by using the following formula:

$$P(1fF/Pr) = 1 - (1 - 0.5 * S)^{LS}$$

Consequently, the odds of at least one fertile female offspring per insemination ($\boldsymbol{P(1fF/i)}$) can be calculated:

$$P(1fF/i) = P(1fF/Pr) * PR_s$$

This data can be used to calculate the maximum number of inseminations ($\boldsymbol{max\ \#\ insem}$):

$$max\ \#\ insem = \left(\frac{\left(a * \sqrt{P(1fF/i) * (1 - P(1fF/i))} + \sqrt{a^2 * (P(1fF/i) * (1 - P(1fF/i))) + 4 * P(1fF/i) * min\ F\ for\ N_e}\right)}{2 * P(1fF/i)}\right)^2$$

Where $\boldsymbol{a}$ = NORMINV(error level, 0, 1) = 1.644 (see paragraph **3.4.4**) and error level = 1 – the success rate.

Consequently, the required number of sperm doses can be calculated by multiplying the maximum number of inseminations by the number of reconstruction cycles:

$$doses = C * max\ \#\ insem$$

Current calculations are based on one reconstruction cycle (C = 1).

Lastly, the number of sperm doses per available male animal, in addition to the embryos, can be calculated by using the following formula:

$$sperm\ doses\ per\ male\ animal = doses/M$$

Where **M** is the number of available male animals (maximum 25, even if there are more male animals available, 25 is used as a maximum).

If the pregnancy rate is 0.5, the litter size is 1, the survival rate is 1, and the success rate is 0.95, this results in 133 doses in total and 10 doses per male animal (rounded up to the nearest ten).

3.5.4 Calculation of the total required number of sperm doses

The total required number of sperm doses is calculated as followed:

$$required\ number\ of\ sperm\ doses = doses * M$$

For the previously calculated 10 doses per male animal and 25 male animals, this results in 250 sperm doses.

3.5.5 Calculation of the expected number of female animals

The expected number of female animals in a scenario with embryos and sperm is calculated as followed:

$$expected\ F = \frac{max\ \#\ embryos\ per\ cycle}{E} * PR_E * \ P(1fF/Pr)$$

Where $max\ \#\ embryos\ per\ cycle$ equals the number of embryos per implantation (**E**) multiplied by $\mathbf{I_{max}}$. For $\mathbf{I_{max}}$ = 94, **E** = 1, $\mathbf{PR_E}$ = 0.35 en $\boldsymbol{P(1fF/Pr)}$ = 1, this results in 32 female animals (rounded to an integer).

3.5.6 Calculation of the expected number of male animals

The expected number of male animals in a scenario with embryos and sperm can be calculated in two ways. For scenarios where the sex of the embryos is known, the expected number of male animals equals the pre-assigned number of male animals. If the sex of the embryos is not known, the expected number of male animals equals the expected number of female animals (see paragraph **3.5.5**).

3.5.7 Calculation of the realised effective population size

The realised effective population size is calculated as followed:

$$realised\ N_e = 4 * \frac{1}{\frac{1}{expected\ F} + \frac{1}{expected\ M}}$$

For 32 expected female animals and 25 expected male animals, this results in a realised effective population size of 56.

3.5.8 Calculation of the rate of inbreeding per generation

The rate of inbreeding per generation is calculated as follows:

$$\Delta F = \frac{1}{2 * realised\ N_e}$$

For a realised N_e of 56, this results in a rate of inbreeding of 0.9% per generation.

4 Calculated required numbers for different species

The calculations explained in chapter 3 provide the required numbers for the core collections for the different species. These numbers are provided in **Table 13** and **Figure 4** for sperm-only scenarios and **Table 14** and **Figure 5** for sperm and embryo scenarios. Both show the most optimal situation for both scenarios, assuming the most favourable ratios in the sex of the animals that are born and the most favourable distribution in the number of doses per donor.

Table 13 *Required numbers of sperm doses for **sperm-only** scenarios. ΔF = rate of inbreeding per generation, N_e = effective population size, N_M = required number of male animals. In every case, six generations are required in the restoration programme to reach the desired breed purity (>97.5%).*

		Safe ΔF = 0.5% N_e = 100 N_M = 50	**Compromised** ΔF = 0.67% N_e = 74 N_M = 37	**Risky** ΔF = 1% N_e = 50 N_M = 25
Cattle	Required number of successful inseminations per generation for the required number of adult female offspring	258	201	147
	Required number of doses (total)	1,550	1,210	890
Pigs	Required number of successful inseminations per generation for the required number of adult female offspring	125	97	70
	Required number of doses (total)	750	590	420
Goats	Required number of successful inseminations per generation for the required number of adult female offspring	435	339	249
	Required number of doses (total)	2,610	2,040	1,500
Sheep	Required number of successful inseminations per generation for the required number of adult female offspring	435	339	249
	Required number of doses (total)	2,610	2,040	1,500
Dogs	Required number of successful inseminations per generation for the required number of adult female offspring	97	75	54
	Required number of doses (total)	590	450	330
Horses	Required number of successful inseminations per generation for the required number of adult female offspring	325	253	185
	Required number of doses (total)	1,950	1,520	1,110
Chickens (commercial/ native)	Required number of successful inseminations per generation for the required number of adult female offspring	127/144	98/112	71/81
	Required number of doses (total)	770/870	590/680	430/490
Geese, ducks	Required number of successful inseminations per generation for the required number of adult female offspring	125	97	70
	Required number of doses (total)	750	590	420
Rabbits	Required number of successful inseminations per generation for the required number of adult female offspring	74	57	41
	Required number of doses (total)	450	350	250

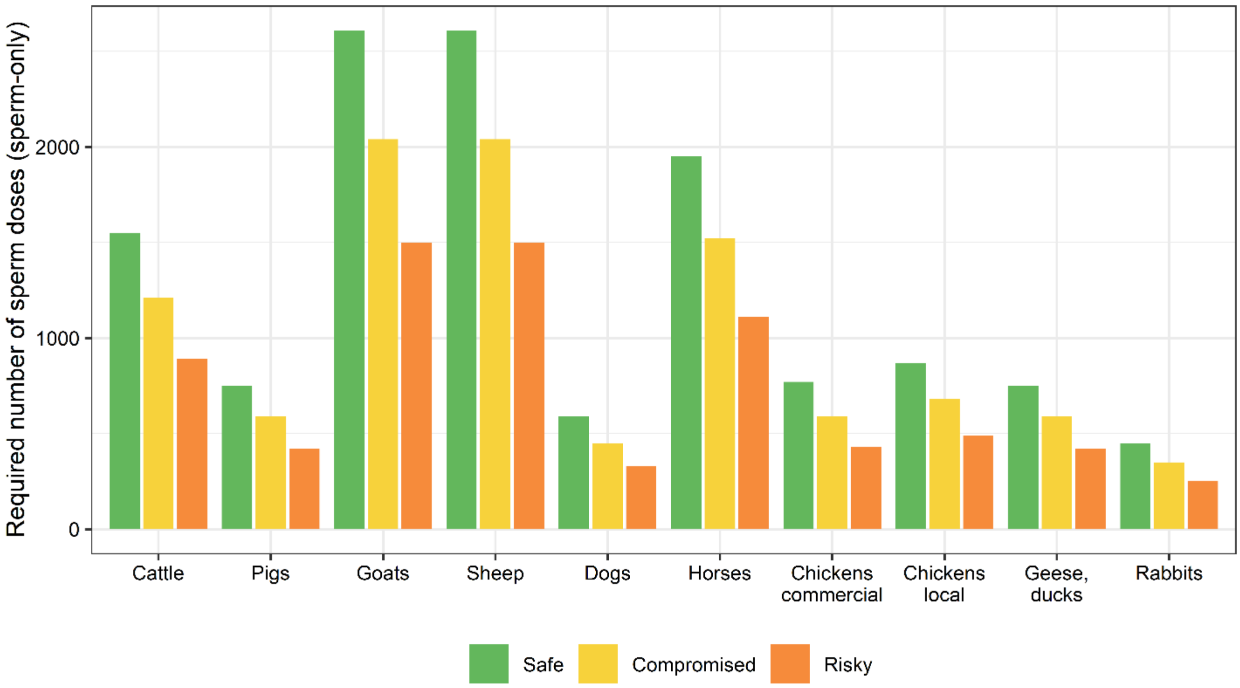


Figure 4 *Required numbers of sperm doses for sperm-only scenarios for different scenarios and animal species.*

Table 14 *Required numbers of embryos and sperm doses for **sperm and embryo** scenarios. ΔF = rate of inbreeding per generation, N_e = effective population size, N_M = required number of male breeding animals. In every case, six generations are required in the restoration programme to reach the desired breed purity (>97.5%).*

		Safe ΔF = 0.5% N_e = 100 N_M = 50	**Compromised** ΔF = 0.67% N_e = 74 N_M = 37	**Risky** ΔF = 1% N_e = 50 N_M = 25
Cattle	Required number of embryos	173	132	94
	Required number of sperm doses (total)	500	370	250
Pigs	Required number of embryos	13,290	10,200	7,260
	Required number of sperm doses (total)	500	370	250
Goats	Required number of embryos	609	465	330
	Required number of sperm doses (total)	1,000	740	250
Sheep	Required number of embryos	609	465	330
	Required number of sperm doses (total)	1,000	740	250
Dogs	Required number of embryos	N.a.	N.a.	N.a.
	Required number of sperm doses (total)	N.a.	N.a.	N.a.
Horses	Required number of embryos	113	86	61
	Required number of sperm doses (total)	1,000	370	250
Chickens	Required number of embryos	N.a.	N.a.	N.a.
	Required number of sperm doses (total)	N.a.	N.a.	N.a.
Geese,	Required number of embryos	N.a.	N.a.	N.a.
ducks	Required number of sperm doses (total)	N.a.	N.a.	N.a.
Rabbits	Required number of embryos	2,670	2,040	1,450
	Required number of sperm doses (total)	500	370	250

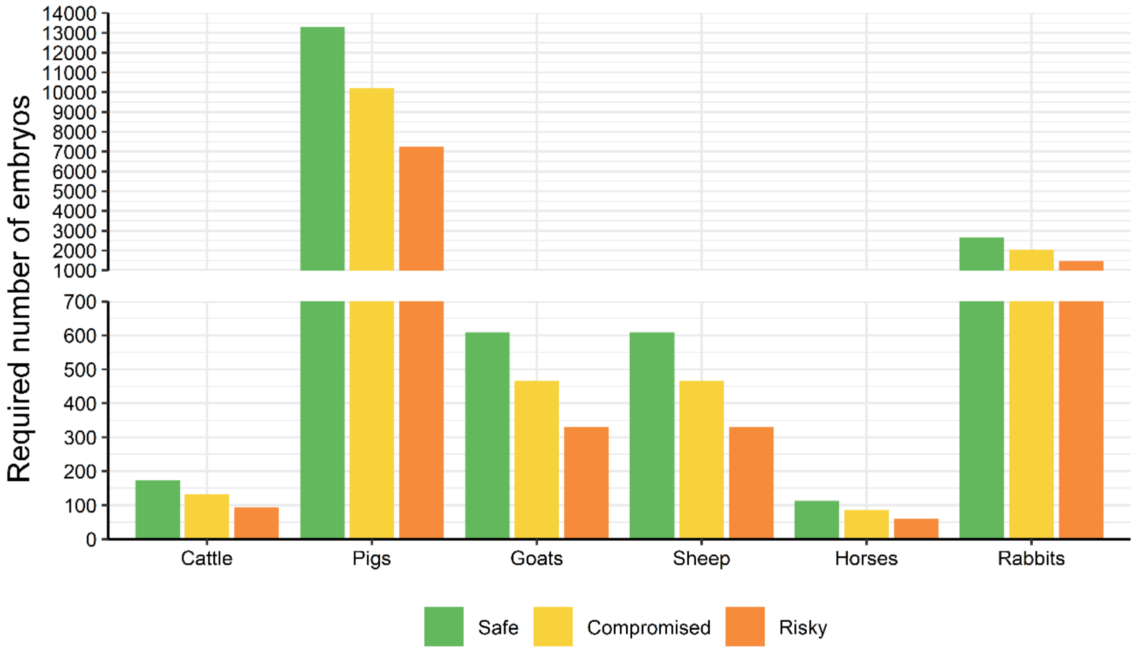


Figure 5 *Required number of embryos for sperm and embryo scenarios for different scenarios and animal species.*

4.1 Number of male donors

In the calculations of the abovementioned scenarios we assumed a set number of male donors to examine specific effective population sizes and inbreeding rates (50% of the effective population size, that is 50, 37 and 25 male donors for the Safe (**S**), Comprised (C) and Risky (**R**) scenarios; see **Table 12**). It is also possible to perform a reconstruction with fewer male donors. However, these should be paired with more females to reach the desired effective population size. This will require significantly more doses. The required number of doses for different numbers of donors has been calculated per animal species. As an example, **Figure 6** shows for cattle the correlation between the number of male donors and the total number of doses per scenario. It is important to keep in mind that, in this example, there is an equal number of doses available per male animal, or rather that the total amount of doses is divided proportionally over the number of male donors. In reality, however, this is not often the case. In addition, **Figure 6** also shows that there is a minimum amount of donors that is needed to reach the required effective population size, where, for example, the Risky (**R**) scenario requires at least 14 male donors, regardless of the number of doses.

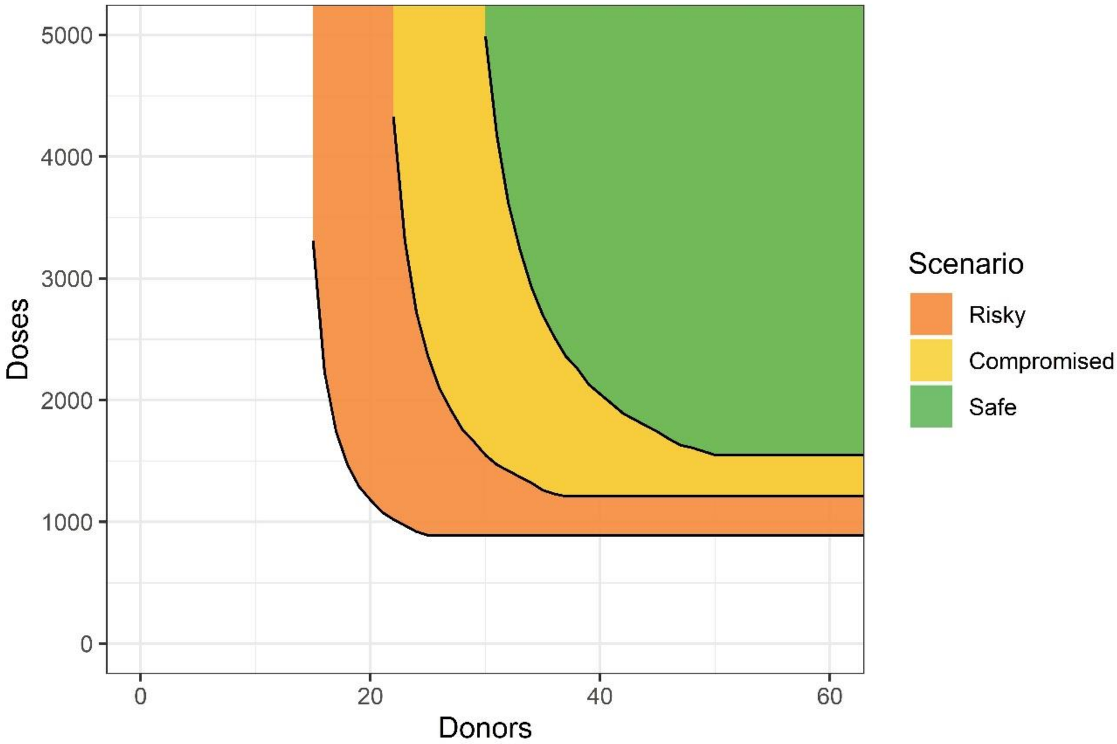


Figure 6 *Correlation between the number of male donors and the required amount of doses per scenario for cattle.*

5 Evaluation required versus actual core collections

This chapter contains a comparison between the current core collections of the Dutch livestock breeds (see chapter 2) and the calculated required core collections (see chapter 4). The results for each animal species – and the different breeds within the species – are explained in more detail in the paragraphs below. Only for cattle the scenario for embryos has been included, as this is the only animal species for which embryos are stored in the genebank.

5.1 Cattle

For the Safe scenario, 1,550 sperm doses are required of at least 50 male donors. As shown in **Table 15**, these numbers are met by the *Fries-Hollands vee* (black and white; and also specifically for the red-and-white subpopulation *Roodbont Fries vee), Groninger Blaarkop, Holstein Friesian* (also specifically for the *Holstein red-and-white* subpopulation) and *Maas-Rijn-IJsselvee*. There is insufficient material available for the *Brandrode rund*, *Heiderund, Lakenvelder, Verbeterd Roodbont* and colour variations *Witrik, Vaal and Baggerbont*. For the Compromised scenario, 1,210 sperm doses are required of at least 37 male animals. These numbers, in addition to the breeds that already had sufficient for the Safe scenario, are met by *Lakenvelder* and *Verbeterd Roodbont*. For the Risky scenario, 890 sperm doses are required of at least 25 male animals. These numbers, in addition to the breeds that already had sufficient for the Compromised scenario, are met by *Brandrode rund*. For both the *Heiderund* and the colour variations *Witrik, Vaal and Baggerbont* there is insufficient material available for each of the scenarios. Regarding the use of embryos: the Risky scenario already requires 94 embryos per breed, and this number is met by none of the cattle breeds (**Table 15**).

Table 15 *Overview per cattle breed whether or not there is sufficient material (doses and donors) available for the different scenarios, including sperm and embryos scenarios.*

Breed	**Scenario Safe (sperm)**	**Scenario Compromised (sperm)**	**Scenario Risky (sperm)**	**All embryo-scenarios**
Brandrood rund	✗	✗	✓	✗
Fries-Hollands vee (zwartbont)[1]	✓	✓	✓	✗
Roodbont Fries vee[2]	✓	✓	✓	✗
Groninger Blaarkop	✓	✓	✓	✗
Heiderund	✗	✗	✗	✗
Holstein zwartbont	✓	✓	✓	✗
Holstein roodbont[2]	✓	✓	✓	✗
Lakenvelder	✗+	✓	✓	✗
MRIJ (Maas-Rijn-IJssel)	✓	✓	✓	✗
Verbeterd Roodbont	✗	✓	✓	✗
Witrik, Vaal en Baggerbont	✗	✗	✗	✗

[1]Fries-Hollands zwartbont and fundamental breeding line combined; [2]Animals of specific colour varieties within the breed; [+]Sufficient if fewer male donors are used.

As previously indicated, it is also possible to achieve a scenario with fewer male donors, but with more doses. **Figure 7** shows the current state of affairs for the different cattle breeds if different numbers of male donors are also taken into consideration. It shows that the required number of doses per donor is higher when there are fewer donors. In **Table 15** a plus sign has been added to indicate for which breeds there is sufficient material available if fewer male donors are used.

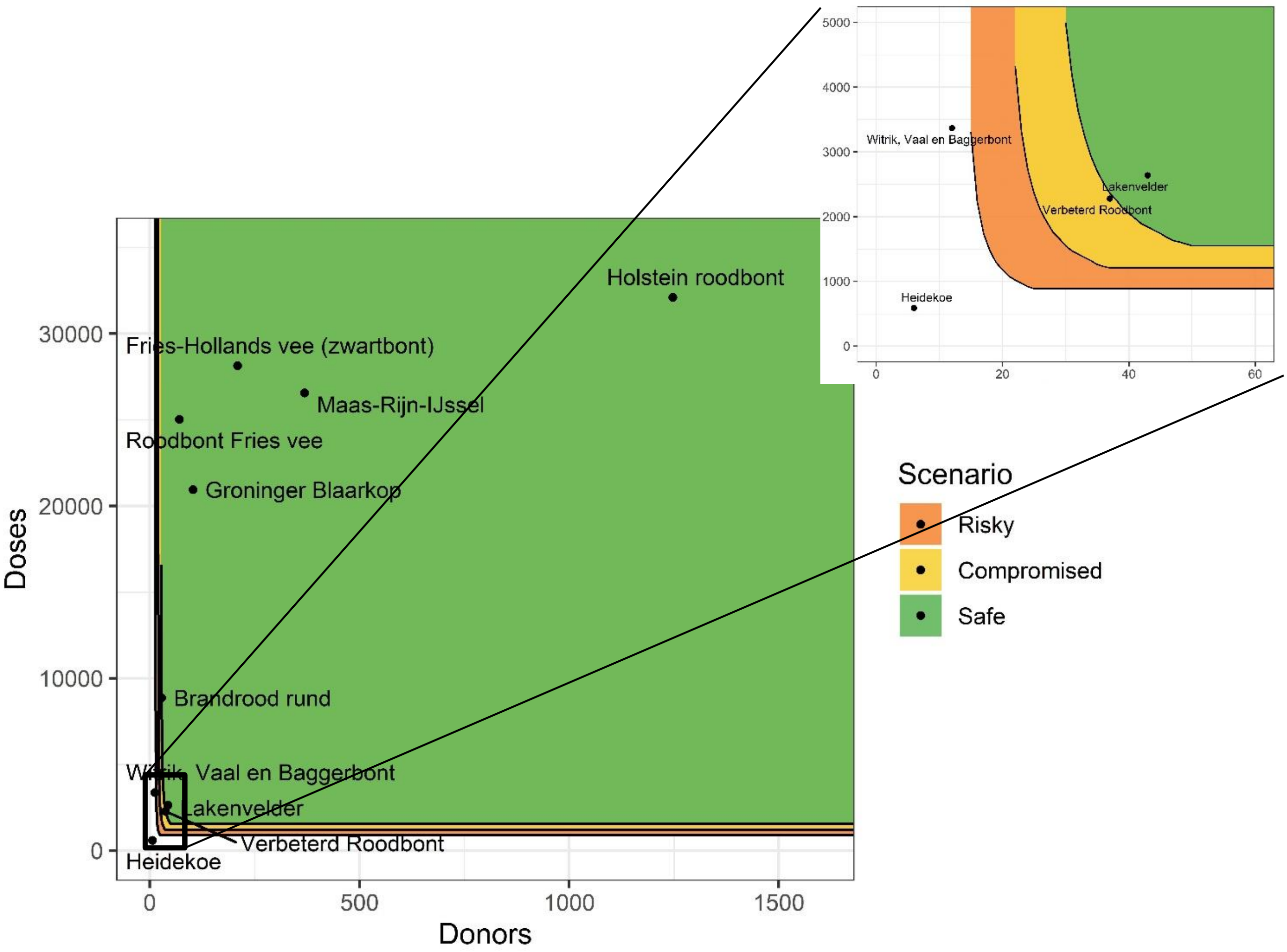


Figure 7 Overview of doses and donors in relation to the calculated scenarios with differing numbers of male donors, provided per cattle breed *(Holstein Friesian black and white is out of range with 120,000 doses).*

5.2 Pigs

For the Safe scenario, 750 sperm doses are required of at least 50 male donors. As shown in **Table 16**, these numbers are met by the *Nederlands Landvarken* and *Topigs Norsvin-lines D, E, P, N and Z*. For the Compromised scenario, 590 sperm doses are required of at least 37 male donors. These numbers, in addition to the breeds that already had sufficient for the Safe scenario, are met by the *Topigs Norsvin A-line* and *T-line*. For the Risky scenario, 420 sperm doses are required of at least 25 male donors. These numbers, in addition to the breeds that already had sufficient for the Safe and Compromised scenario, are met by Meishan and the Topigs Norsvin B-line. For the *Bonte Bentheimer landvarken*, *Hypor Landras, Hypor Large White* and *Topigs Norsvin Y-line* there is insufficient material available for each of the scenarios.

Table 16 *Overview per pig breed whether or not there is sufficient material (doses and donors) available for the different scenarios.*

Breed	Scenario Safe (sperm)	Scenario Compromised (sperm)	Scenario Risky (sperm)
Bonte Bentheimer landvarken	✗	✗	✗
Hypor Landras	✗	✗	✗
Hypor Large White	✗	✗	✗
Meishan*	✗	✗+	✓
Nederlands Landvarken	✓	✓	✓
Topigs Norsvin A-lijn* (Large White)	✗+	✓	✓
Topigs Norsvin B-lijn*	✗	✗+	✓
Topigs Norsvin D-lijn (TN Talent, Duroc)	✓	✓	✓
Topigs Norsvin E-lijn (TN Tempo, Large White)	✓	✓	✓
Topigs Norsvin N-lijn (Nederlands landras)	✓	✓	✓
Topigs Norsvin P-lijn (TN Select, TN Top Select, Piétrain)	✓	✓	✓
Topigs Norsvin T-lijn	✗+	✓	✓
Topigs Norsvin Y-lijn*	✗	✗	✗
Topigs Norsvin Z-lijn (Large White)	✓	✓	✓

[*] Lines or breeds no longer available; [+] Sufficient if fewer male donors are used.

It is also possible to achieve a scenario with fewer male donors, but with more doses. **Figure 8** shows the current state of affairs for the different pig breeds if different numbers of male donors are also taken into consideration. In **Table 16** a plus sign has been added to indicate for which breeds there is sufficient material available if fewer male donors are used.

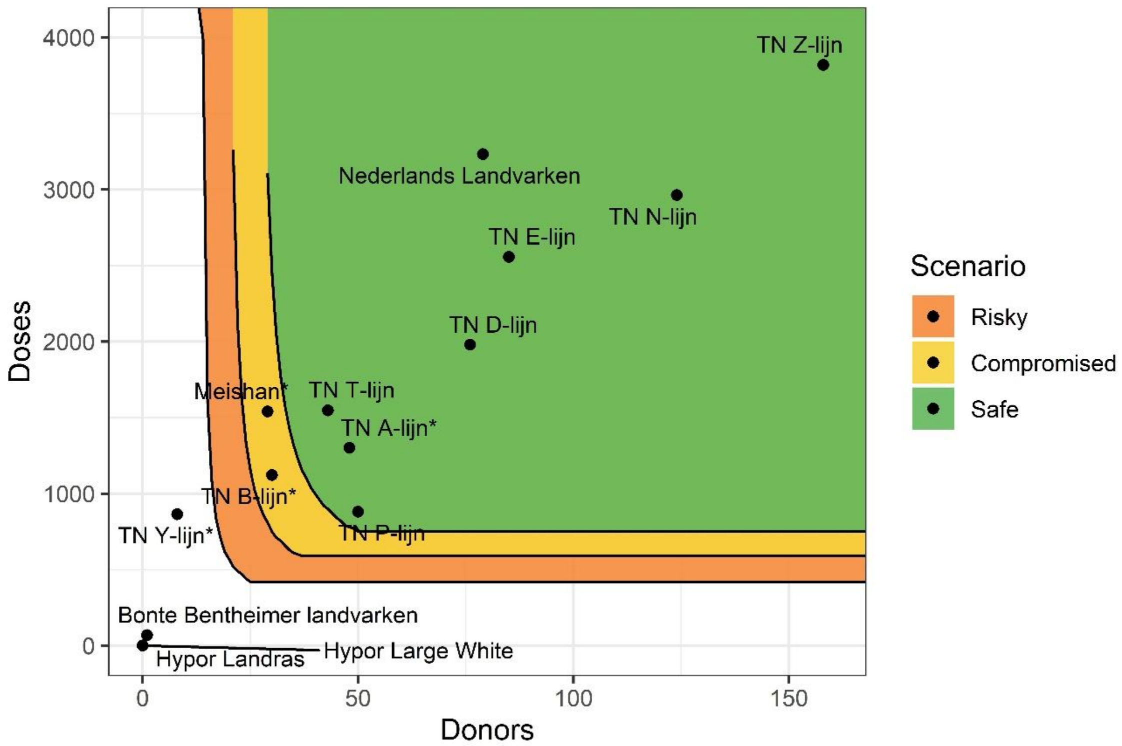


Figure 8 *Overview of doses and donors in relation to the calculated scenarios with differing numbers of male donors, provided per pig breed.*

5.3 Goats

For the Safe scenario, 2,610 sperm doses are required of at least 50 male donors. As shown in **Table 17**, these numbers are met by none of the goat breeds. For the Compromised scenario, 2,040 sperm doses are required of at least 37 male donors. These numbers are met by none of the goat breeds. For the Risky scenario, 1,500 sperm doses are required of at least 25 male donors. These numbers are met only by the *Nederlandse landgeit*.

Table 17 *Overview per goat breed whether or not there is sufficient material (doses and donors) available for the different scenarios.*

Ras	Scenario Safe (sperm)	Scenario Compromised (sperm)	Scenario Risky (sperm)
Nederlandse Melkgeit	✗	✗	✗
Nederlandse Bonte geit	✗	✗	✗
Nederlandse Landgeit	✗+	✗+	✓
Nederlandse Witte geit	✗	✗	✗
Nederlandse Toggenburger geit	✗	✗	✗

+ Sufficient if fewer male donors and more doses are used.

It is also possible to achieve a scenario with fewer male donors, but with more doses. **Figure 9** shows the current state of affairs for the different goat breeds if different numbers of male donors are also taken into consideration. In **Table 17** a plus sign has been added to indicate for which breeds there is sufficient material available if fewer male donors are used.

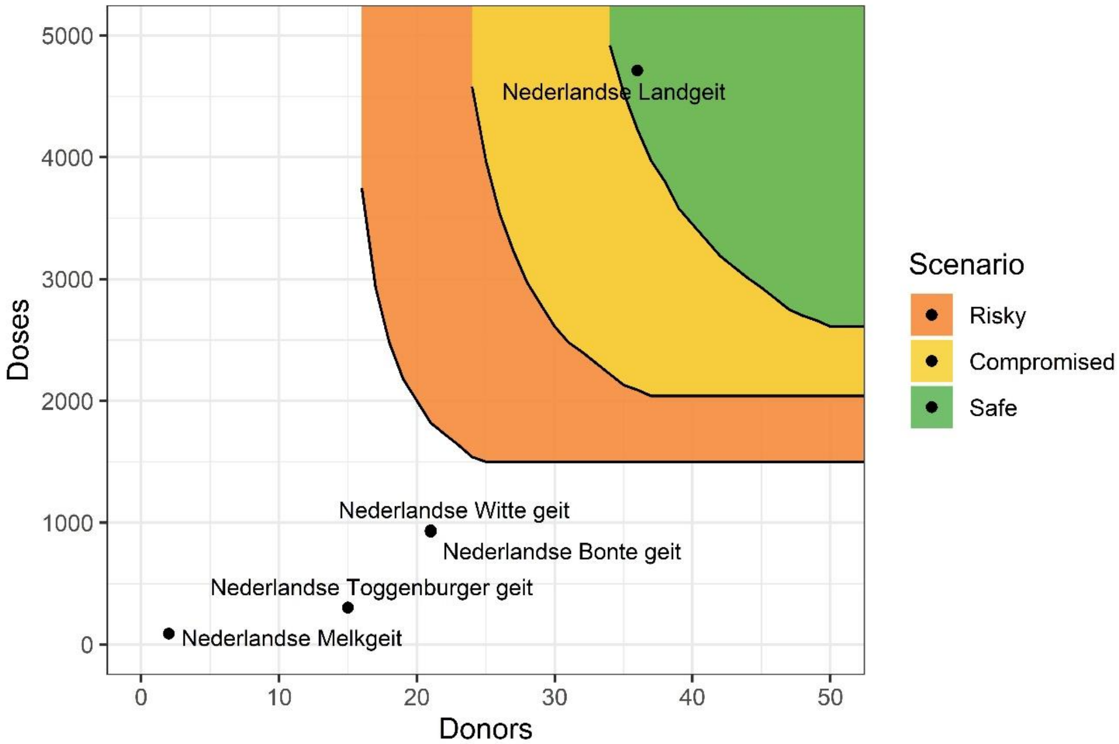


Figure 9 *Overview of doses and donors in relation to the calculated scenarios with differing numbers of male donors, provided per goat breed.*

5.4 Sheep

For the Safe scenario, 2,610 sperm doses are required of at least 50 male donors. As shown in **Table 18**, these numbers are met by the *Drents Heideschaap* and the *Fries melkschaap*. For the Compromised scenario, 2,040 sperm doses are required of at least 37 male donors.

These numbers, in addition to the breeds that already had sufficient for the Safe scenario, are met by the *Mergelland* schaap and the *Veluws Heideschaap*. For the Risky scenario, 1,500 doses are required of at least 25 male donors. These numbers are met by the *Kempisch Heideschaap*, the *Schoonebeeker* and the *Zwartbles*.

Table 18 *Overview per sheep breed whether or not there is sufficient material (doses and donors) available for the different scenarios.*

Breed	Scenario Safe (sperm)	Scenario Compromised (sperm)	Scenario Risky (sperm)
Bonte schaap	✗	✗	✗
Drents Heideschaap	✓	✓	✓
Flevolander	✗	✗	✗
Groot Heideschaap	✗	✗	✗
Fries melkschaap[1]	✓	✓	✓
Kempisch Heideschaap	✗	✗+	✓
Mergelland schaap	✗+	✓	✓
Noordhollander	✗	✗	✗
Schoonebeeker	✗	✗+	✓
Swifter	✗	✗	✗
Texelaar	✗	✗	✗
Blauwe Texelaar[2]	✗	✗	✗
Dassenkop Texelaar[2]	✗	✗	✗
Veluws Heideschaap	✗+	✓	✓
Zwartbles	✗	✗+	✓

[1]Before *Fries and Zeeuw Melkschaap*, there are currently no more living *Zeeuwse melkschapen*; [2]Animals of specific colour varieties within the breed; [+]Sufficient if fewer male donors are used.

It is also possible to achieve a scenario with fewer male donors, but with more doses. **Figure 10** shows the current state of affairs for the different sheep breeds if different numbers of male donors are also taken into consideration. In **Table 18** a plus sign has been added to indicate for which breeds there is sufficient material available if fewer male donors are used.

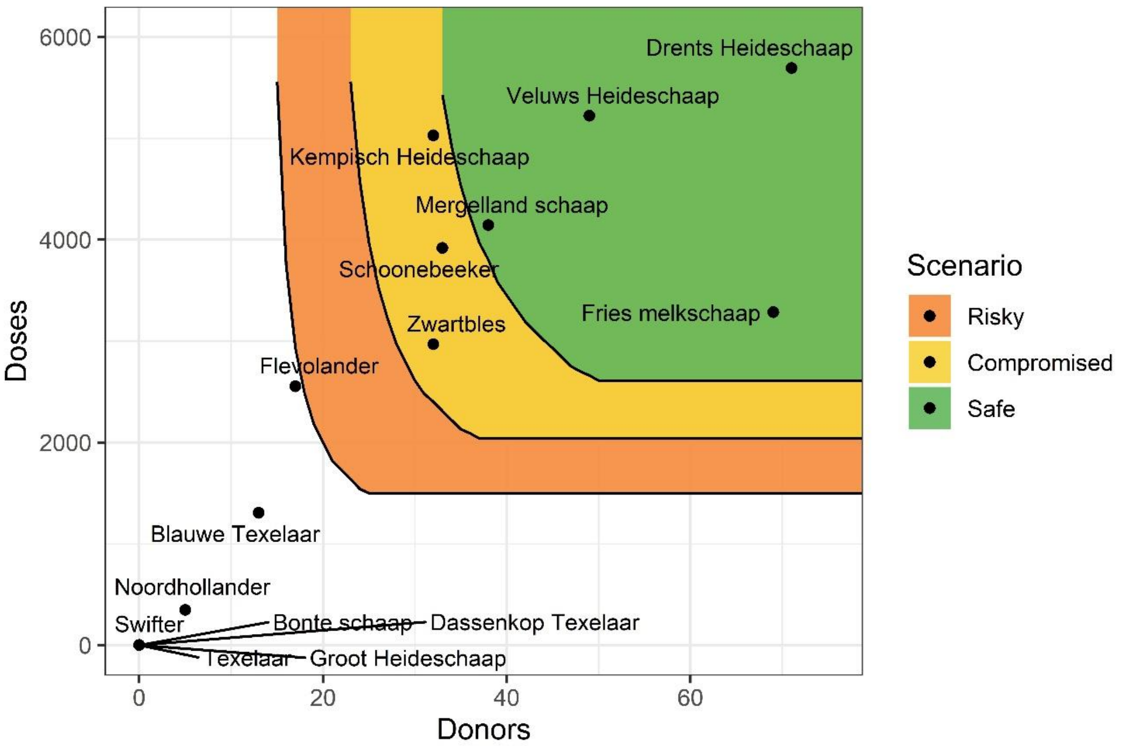


Figure 10 *Overview of doses and donors in relation to the calculated scenarios with differing numbers of male donors, provided per sheep breed.*

5.5 Dogs

For the Safe scenario, 590 sperm doses are required of at least 50 male donors. For the Compromised scenario, 450 sperm doses are required of at least 37 male donors, and for the Risky scenario, 330 sperm doses are required of at least 25 male donors. These numbers are met by none of the breeds (**Table 19**).

Table 19 *Overview per dog breed whether or not there is sufficient material (doses and donors) available for the different scenarios.*

Breed	Scenario Safe (sperm)	Scenario Compromised (sperm)	Scenario Risky (sperm)
Drentse Patrijshond	✗	✗	✗
Hollandse herder	✗	✗	✗
Hollandse smoushond	✗	✗	✗
Kooikerhondje	✗	✗	✗
Markiesje	✗	✗	✗
Nederlandse schapendoes	✗	✗	✗
Saarloos Wolfhond	✗	✗	✗
Stabyhoun	✗	✗	✗
Wetterhoun	✗	✗	✗

It is also possible to achieve a scenario with fewer male donors, but with more doses. However, as **Figure 11** shows, also for this option there are insufficient donors and doses for each of the scenarios.

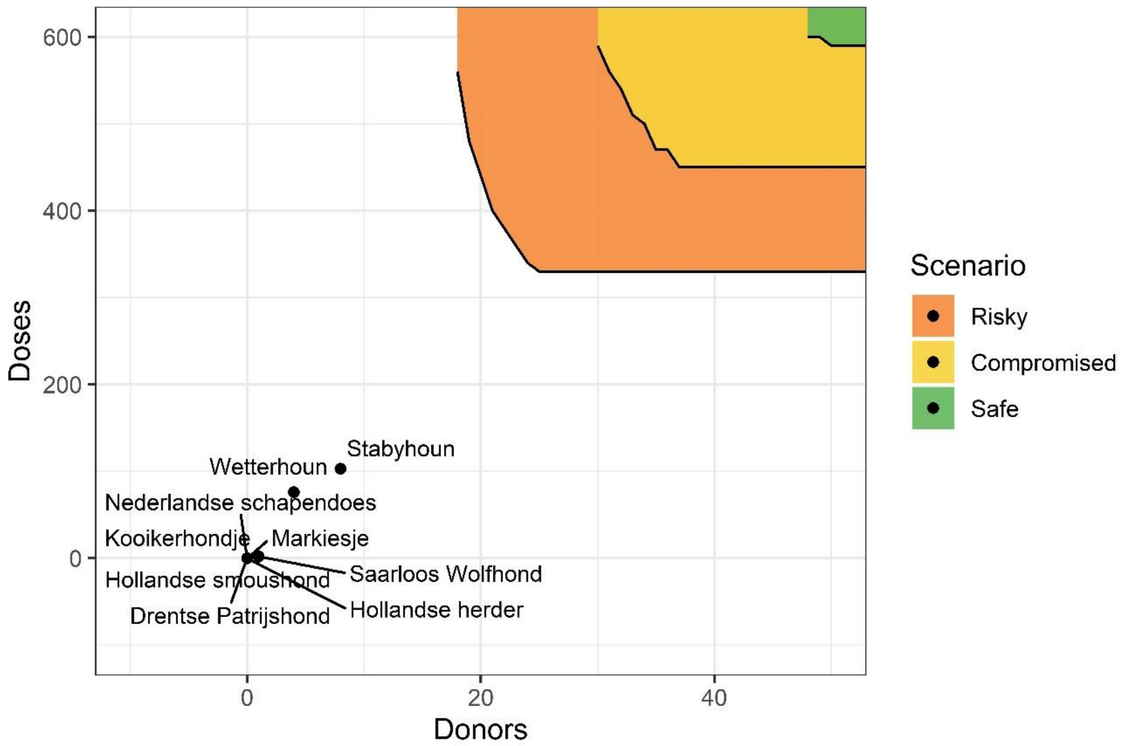


Figure 11 *Overview of doses and donors in relation to the calculated scenarios with differing numbers of male donors, provided per dog breed.*

5.6 Horses

For the Safe scenario, 1,950 sperm doses are required of at least 50 male donors. As shown in **Table 20**, these numbers are met by none of the horse breeds. For the Compromised scenario, 1,520 sperm doses are required of at least 37 male donors. This number is met by the *Groninger paard*. For the Risky scenario, 1,110 sperm doses are required of at least 25 male donors. Apart from the *Groninger paard*, this number is met only by the *Friesian* horse.

Table 20 *Overview per horse breed whether or not there is sufficient material (doses and donors) available for the different scenarios.*

Breed	Scenario Safe (sperm)	Scenario Compromised (sperm)	Scenario Risky (sperm)
Fries paard	✗	✗	✓
KWPN Gelders paard	✗	✗	✗
Groninger paard	✗	✓	✓
Klassiek Gelderlander paard	✗	✗	✗
KWPN Rijpaard[1]	✗	✗	✗
NRPS Rijpaard	✗	✗	✗
NRPS Rijpony	✗	✗	✗
Nederlandse Shetland Pony	✗	✗	✗
Trekpaard	✗	✗	✗
KWPN Tuigpaard	✗	✗	✗
Zwaar Warmbloed paard[2]	✗	✗	✗

[1] *KWPN Rijpaard or KWPN Springpaard* in the genebank; [2] *Nederlands Warmbloed* in the genebank.

It is also possible to achieve a scenario with fewer male donors, but with more doses. **Figure 12** shows the current state of affairs for the different horse breeds if different numbers of male donors are also taken into consideration. However, this image does not differ from the results presented in **Table 20**.

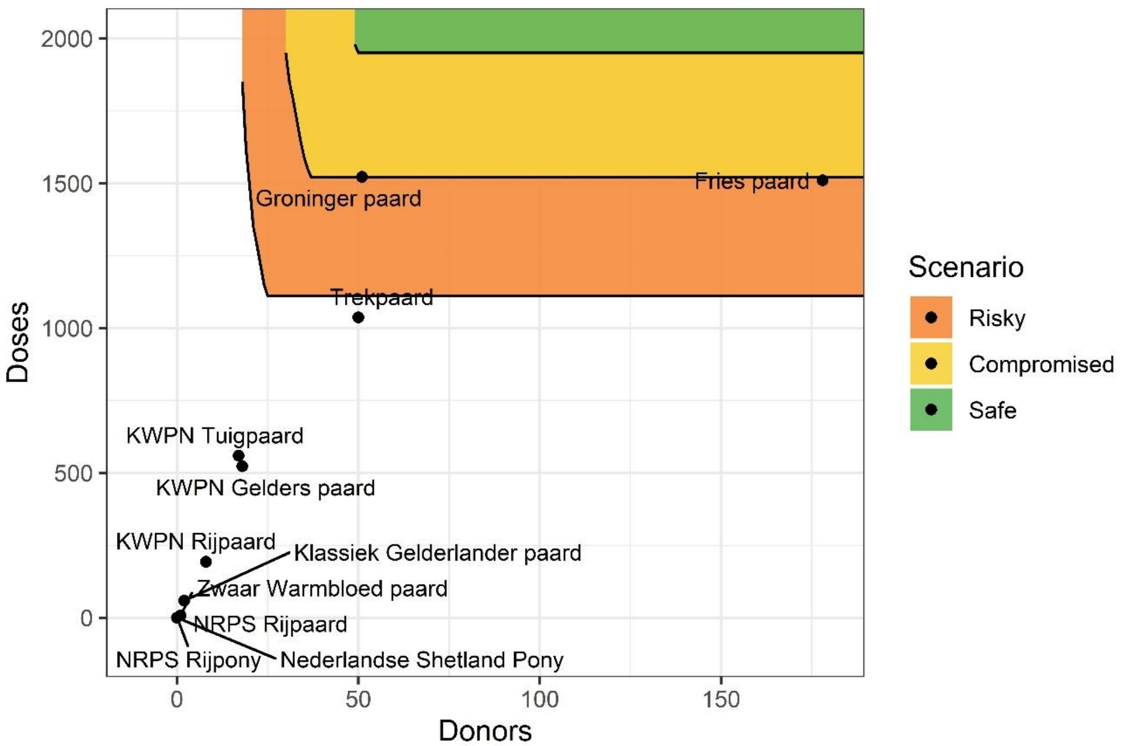


Figure 12 *Overview of doses and donors in relation to the calculated scenarios with differing numbers of male donors, provided per horse breed.*

5.7 Chickens

For the Safe scenario, 770 or 870 (commercial vs local breeds) sperm doses are required of at least 50 male donors. This number is met by none of the chicken breeds (**Table 21**). For the Compromised scenario, 590 or 680 sperm doses are required of at least 37 male donors. For the Risky scenario, 430 or 490 sperm doses are required of at least 25 male donors. The numbers of the Risky scenario are met only by the *Hollandse Hoen*.

Table 21 *Overview per chicken breed whether or not there is sufficient material (doses and donors) available for the different scenarios.*

Breed	**Local or commercial**	**Scenario Safe (sperm)**	**Scenario Compromised (sperm)**	**Scenario Risky (sperm)**
Assendelfts Hoen[1]	Local	✗	✗	✗
Baardkuifhoen[1]	Local	✗	✗	✗
Barnevelder[1]	Local	✗	✗	✗
Brabanter[1]	Local	✗	✗	✗
Chaams Hoen	Local	✗	✗	✗
Drentse Hoen	Local	✗	✗	✗
Drentse Hoen bolstaart	Local	✗	✗	✗
Drentse kriel	Local	✗	✗	✗
Drentse kriel bolstaart	Local	✗	✗	✗
Eikenburger kriel[2]	Local	✗	✗	✗
Fries Hoen[1]	Local	✗	✗	✗+
Groninger Meeuw[1]	Local	✗	✗	✗
Hollands Hoen[1]	Local	✗	✗+	✓
Hollandse kriel	Local	✗	✗	✗
Hollandse kuifhoenders[1]	Local	✗	✗	✗
Kraaikop[1]	Local	✗	✗	✗
Lakenvelder hoen[1]	Local	✗	✗	✗
Leghorn (Nederlands type)[1]	Commercial	✗	✗	✗
Nederlandse sabelpootkriel	Local	✗	✗	✗
Noord-Hollandse Blauwe[1]	Local	✗	✗	✗
Schijndelaar	Local	✗	✗	✗
Twents hoen[1]	Local	✗	✗	✗
Uilebaard[1]	Local	✗	✗	✗
Welsumer[1]	Local	✗	✗	✗
ISA/HG Rhode Island Red	Commercial	✗	✗	✗
ISA/HG Rhode Island White	Commercial	✗	✗	✗
ISA/HG barred Plymouth Rock	Commercial	✗	✗	✗
ISA/HG Australorp	Commercial	✗	✗	✗
ISA/HG New Hampshire	Commercial	✗	✗	✗
ISA/HG White Leghorn	Commercial	✗	✗	✗
ISA/HG Sussex	Commercial	✗	✗	✗

[1] In the genebank (at the moment) no difference between *kriel* (bantam) or not, so including *kriel*; [2] White and black combined; [+] Sufficient if fewer male donors are used.

It is also possible to achieve a scenario with fewer male donors, but with more doses. **Figure 13** shows the current state of affairs for the different local chicken breeds if different numbers of male donors are also taken into consideration. In **Table 21** a plus sign has been added to indicate for which breeds there is sufficient material available if fewer male donors are used. For commercial breeds (*Leghorn* and the *ISA/HG lines*) no material is available.

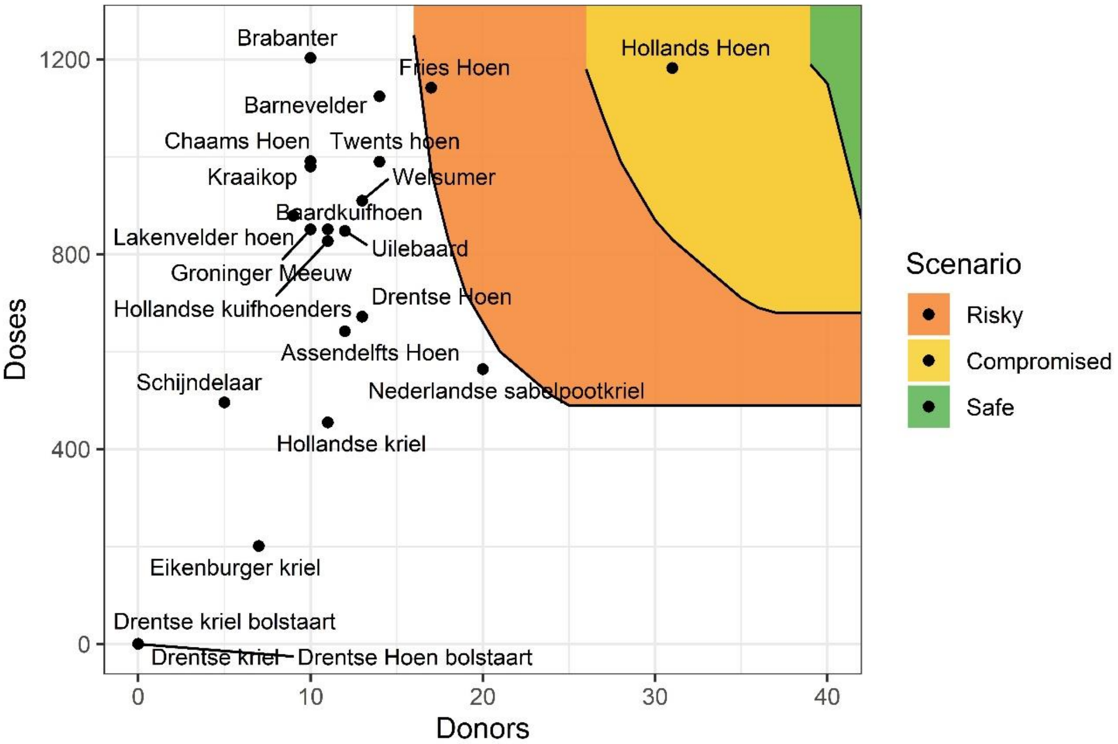


Figure 13 *Overview of doses and donors in relation to the calculated scenarios with differing numbers of male donors, provided per chicken breed.*

5.8 Geese

For the Safe scenario, 750 sperm doses are required of at least 50 male donors. This number is not met by the *Twente landgans* (**Table 22**). Also, the requirements for the Compromised scenario (590 doses of 37 animals) and the Risky scenario (420 doses of 25 animals) are not met.

Table 22 *Overview per geese breed whether or not there is sufficient material (doses and donors) available for the different scenarios.*

Breed	Scenario Safe (sperm)	Scenario Compromised (sperm)	Scenario Risky (sperm)
Twentse landgans	✗	✗	✗

It is also possible to achieve a scenario with fewer male donors, but with more doses. However, **Figure 14** shows that this is currently not feasible for the *Twentse landgans*.

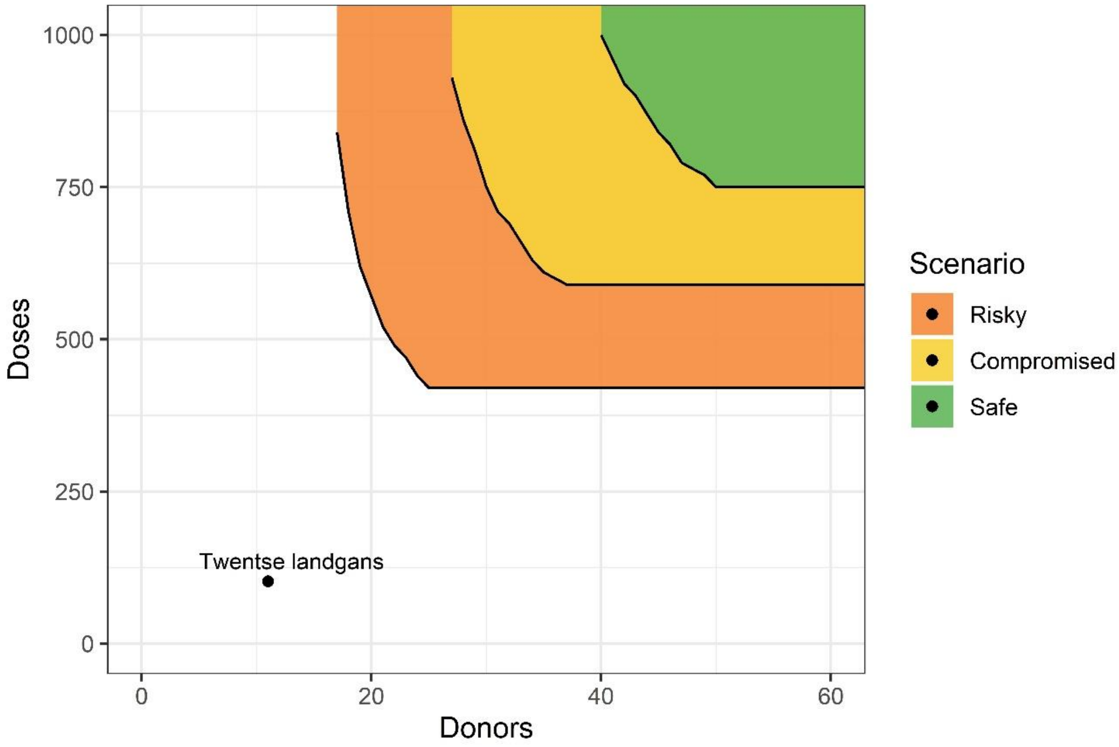


Figure 14 *Overview of doses and donors in relation to the calculated scenarios with differing numbers of male donors, provided for the Twentse landgans.*

5.9 Ducks

For the Safe scenario, 750 sperm doses are required of at least 50 male donors. This number is met by none of the duck breeds (**Table 23**). For the Compromised scenario, 590 sperm doses are required of 37 male donors. This number is also not met by any of the duck breeds. For the Risky scenario, 420 sperm doses are required of at least 25 male donors. These numbers are only met by the *Noord Hollandse Krombekeend*, but not by any of the other duck breeds.

Table 23 *Overview per duck breed whether or not there is sufficient material (doses and donors) available for the different scenarios.*

Breed	Scenario Safe (sperm)	Scenario Compromised (sperm)	Scenario Risky (sperm)
Hollandse kuifeend (incl. dwerg)	✗	✗	✗
Hollandse Kwaker	✗	✗	✗
Noord Hollandse Krombekeend	✗	✗+	✓
Noord Hollandse witborsteend	✗	✗	✗
Overbergse eend	✗	✗	✗

+ Sufficient if fewer male donors are used.

It is also possible to achieve a scenario with fewer male donors, but with more doses. **Figure 15** shows the current state of affairs for the different duck breeds if different numbers of male donors are also taken into consideration. In **Table 23** a plus sign has been added to indicate for which breeds there is sufficient material available if fewer male donors are used.

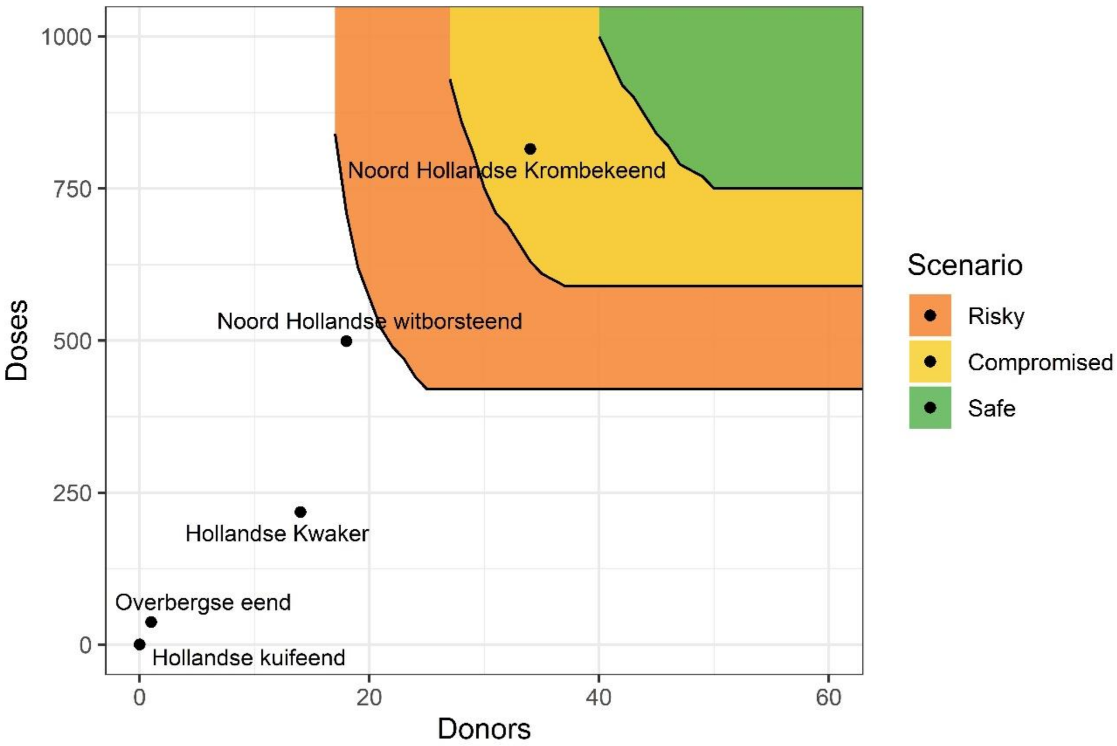


Figure 15 *Overview of doses and donors in relation to the calculated scenarios with differing numbers of male donors, provided per duck breed.*

5.10 Rabbits

For the Safe scenario, 450 sperm doses are required of at least 50 male donors. These numbers are met by none of the rabbit breeds in the genebank (**Table 24**). For the Compromised scenario, 350 sperm doses are required of at least 37 male donors. These numbers are met by none of the rabbit breeds in the genebank. For the Risky scenario, 250 sperm doses are required of at least 25 male donors. These numbers are also not met by any of the rabbit breeds in the genebank.

Table 24 *Overview per rabbit breed whether or not there is sufficient material (doses and donors) available for the different scenarios.*

Breed	**Scenario Safe (sperm)**	**Scenario Compromised (sperm)**	**Scenario Risky (sperm)**
Beige	✗	✗	✗
Deilenaar	✗	✗	✗
Gouwenaar	✗	✗	✗
Havana	✗	✗	✗
Hulstlander	✗	✗	✗
Klein lotharinger	✗	✗	✗
Nederlandse hangoordwerg	✗	✗	✗
Nederlandse kleurdwerg	✗	✗	✗
Sallander	✗	✗	✗
Thrianta	✗	✗	✗

It is also possible to achieve a scenario with fewer male donors, but with more doses. However, as **Figure 16** shows, there is still insufficient material for a successful reconstruction of any of the rabbit breeds.

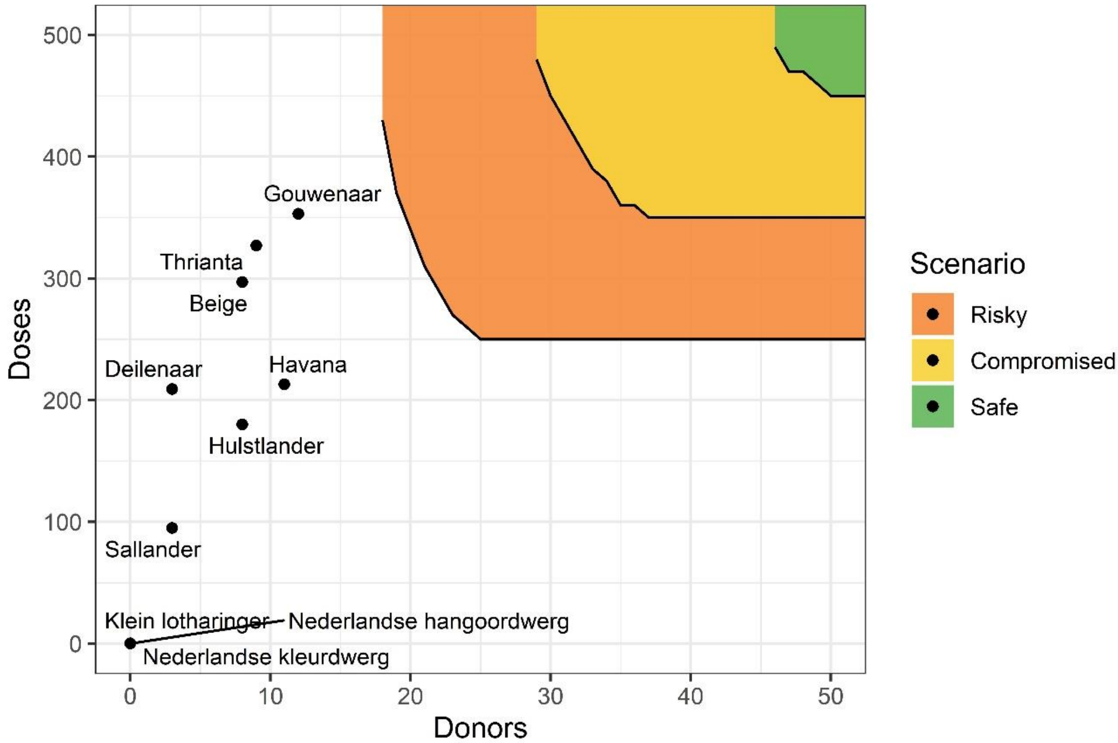


Figure 16 *Overview of doses and donors in relation to the calculated scenarios with differing numbers of male donors, provided per rabbit breed.*

5.11 Summary: status core collections Dutch breeds per species

Within all animal species there are several or multiple breeds for which there is currently insufficient sperm stored in the genebank for the purpose of reconstructing a breed (sperm-only scenarios). **Figure 17** shows the percentage of the breeds (per animal species) for which there is insufficient material available.

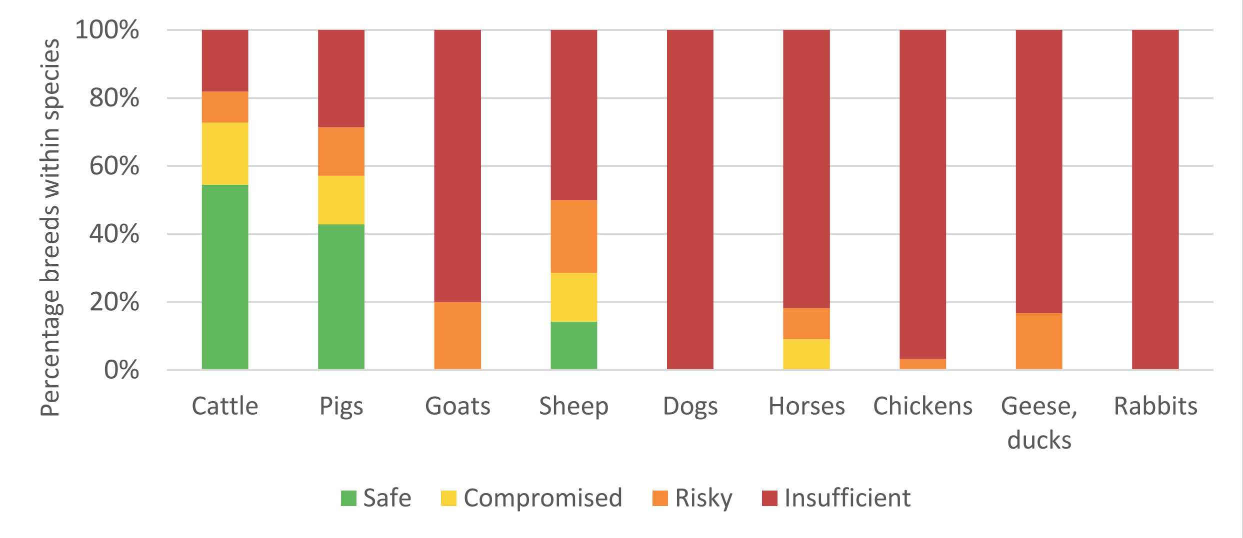


Figure 17 *Overview of the percentages Dutch breeds within an animal species with insufficient or sufficient material available for the three reconstruction scenarios (Safe, Compromised, Risky or Insufficient for all scenarios).*

Important factors that need to be taken into account when collecting sperm are the available protocols per animal species, the use of frozen sperm for insemination, the availability of male animals, the enthusiasm of the animal owners and the (limitations resulting from) laws and regulations that apply. Furthermore, perhaps the most important part is the contact between CGN and breeding organisations. Without knowledge about the importance of the genebank and the continuous collection of material, breeding organisations are less likely to collaborate with CGN. The CGN is very much aware of the need to create more visibility and is investing more time and effort in the relationships with the breeding organisations to establish and continue long-term collaborations.

6 Discussion: priorities for expanding the genebank collections

This research has provided an overview of the current genebank collection of Dutch livestock breeds and the size of the current collection per animal species and breed (chapter 2), compared to the required numbers of doses and donors to, in the event of a breed going extinct, reconstruct a breed; the core collection (chapter 4). The required numbers of doses to reconstruct a breed were calculated based on specific assumptions per animal species (chapter 3). These assumptions were determined based on literature and advice from experts. The practical situation per animal species or breed can, of course, differ from the average values established by literature and experts. To ensure that a breed can in fact be reconstructed and possesses sufficient variation, it is advised to, aside from the minimal size of the core collection presented in this report, continue to keep collecting and storing material to ensure that the changing diversity from living populations is also represented in the genebank collection. This applies to both the rare breeds and larger populations of commercial breeds.

However, expansion of the genebank collection and the realisation of core collections for different animal species should be prioritised. This chapter contains discussions about the start and development of the current core collection per animal species, as well as the technical limitations and possibilities. Future opportunities are based on the information that is available about methods and protocols about freezing of sperm, embryos and other material that is suitable for reproduction as well as collaborations with the sector and (other) priorities arising from the CGN work programme (chapter 7).

6.1 Cattle

Cattle is the animal species for which, compared to other animal species, a significant amount of genetic material is stored in the genebank. For almost all the Dutch cattle breeds a large number of unique donors (bulls) is represented in the genebank, and the total number of sperm doses for many cattle breeds is sufficient to reconstruct a breed. Moreover, for a number of breeds there are also (some) embryos stored. Six out of eleven cattle breeds have sufficient material in the genebank for the Safe scenario (nearly 60%). For some breeds different subpopulations have been taken into account as separate breeds. One example is the *Roodbont Fries vee,* red and white subpopulation which is officially part of the breeding programme of the *Fries-Hollands* breed (which mainly consists of black and white). Given the history and importance of the *Roodbont Friese* subpopulation, this population is considered a 'separate' breed in this discussion.

It is not surprising that there is a lot of genetic material available in the genebank already. The first animals for which sperm was stored in the genebank were bulls that were born around 1960 (**Figure 3**). A second reason that explains why this collection is so vast, is because of the continuous flow of so-called 'snapshots' from commercial AI-organisations. A 'snapshot' is the material that is stored from each bull that becomes commercially available via one of the sperm-collection centres in the Netherlands. For each bull, 25-400 doses are stored in the genebank. The number of doses varies, with a smaller snapshot for large breeds such as the Holstein Friesian, and a larger number of doses per breed for rare but original breeds such as the *Fries-Hollands* cattle and the *Brandrode rund* which are historically significant in the development of the cattle farming business in the Netherlands. For these breeds the genebank is also important for current breeding practices. In addition, the CGN can (because of an exceptional position regarding the veterinary regulations) visit cattle farmers on location in order to collect sperm and add the material to the genebank collection. Because there are often fewer AI-bulls available for rare breeds, this material is not just relevant for the long-term, but it is also used more frequently in current breeding practices. However, in order to find the right bulls and owners who are interested in cooperating, as well as for making good agreements, it is essential to maintain a good relationship with the breed organisations and studbook keepers that are involved.

For cattle, the addition of ‘snapshots’ of sperm doses of new AI-bulls will continue via good agreements with the AI-organisations. For several of the original Dutch breeds and colour varieties, particularly the *Brandrode rund*, the *Lakenvelder* and the colour varieties *Witrik, Vaal* and *Baggerbont*, the CGN will also take on an active role regarding the potential genebank candidates and, if necessary, collect sperm on corporate level. The CGN will also emphasise this in the contact and during consultations with the breeding organisations that are concerned with these matters. This approach is in keeping with the results of the core-collection analysis that shows that there is still insufficient material available for these breeds.

For cattle, more information and experience is becoming available in the field of cryoconservation (freezing) of embryos and oocytes. To better secure the genetic diversity of the female side of the species, the CGN will give high priority to this topic and, whenever possible, contact breeding organisations of the original Dutch rare breeds to take action. Embryos of other cattle breeds in the Netherlands will, when offered and assigned to the CGN, also be stored in the genebank.

6.2 Pigs

In relation to the other animal species in the genebank, pigs are the species with the second-highest percentage of breeds for which sufficient material is stored. In the Risky scenario, this is the case for 9 out of 13 breeds (70%). For 5 out of 13 Dutch pig breeds (40%) sufficient material is stored for the Safe scenario. The genebank collection for pigs contains mostly sperm of the (now no longer available) breeds or lines of commercial pig breeding organisations in the Netherlands. The exceptions are the *Bonte Bentheimer* and the *Nederlandse landvarken*, which are currently the rarest breeds with only a few breeders who are represented by relevant breed organisations.

The *Bonte Bentheimer landvarken* and the *Nederlandse landvarken* are on the brink of extinction. For both breeds there are currently less than 100 breeding sows registered in the studbook (reference date 2023). Whereas for the *Nederlandse landvarken* there is sufficient material stored for all three scenarios, the *Bonte Bentheimer* only has sperm available of one boar. Given the high veterinary requirements in pig breeding, it is nearly impossible to exchange boars between companies, or to use boars of small-scale farms on an AI-station to collect sperm for the genebank or for ‘regular’ AI. In addition, the high costs of pig-AI are not in proportion to the number of doses that are actually used for such a small breed.

Although the *Meishan* has already disappeared in practice, there is still a small amount of sperm available in the genebank. This material is used for a restoration breeding plan. For the long term it is necessary to store the genetic material from the animals that are born out of the restoration plan to ensure that this breed can still be used in the future.

The commercial pig breeding industry has made a significant contribution to the current genebank collection of the commercial breeds and lines. The CGN will continue to store the ‘snapshot’ lines of Topigs Norsvin when these are offered to the genebank. This is relevant to secure the unique genetic diversity of the lines that may be terminated or combined in the future.

There is a high priority to secure the genetic material of the two rare Dutch pig breeds (*Bonte Bentheimer* and the *Nederlands landvarken* pig). The most important step right now is to collect and freeze boar sperm, but before this can be done, a plan of action will need to be formulated and an assessment will need to be made regarding the possibilities to collect sperm. In addition, the focus will be on research in ways to add embryos and oocytes of these breeds to the genebank.

6.3 Goats

Only for the *Nederlandse Landgeit* there is sufficient genetic material available for the Risky scenario. For the other scenarios and the other four goat breeds (*Nederlandse Melkgeit, Nederlandse Bonte Geit, Nederlandse Witte geit* and *Nederlandse Toggenburger*) there is insufficient sperm available to recover the breeds, should this be necessary.

AI is now used more often for goats which makes it possible to use male goats on more locations (throughout the Netherlands and in different companies). The downside is that the number of available male goats that is actively being used is limited. Hence, it is important to secure the full genetic diversity in current populations for the future, should variation be lost over time. The CGN will collaborate with the goat AI-organisations to store 'snapshots' of male goats and, where possible, deliver male goats of other breeds than the ones currently available to the commercial AI-organisations.

The results clearly indicate that actions are required regarding the four Dutch goat breeds. However, the *Nederlandse Landgeit* should not be overlooked, as the periodical supplementation is also important to secure the genetic diversity over the years. For all the goat breeds the focus will be on the collection of sperm. An additional assessment of requirements should result in a plan of action and collaboration with relevant AI-organisations and breed organisations.

6.4 Sheep

There are 15 Dutch sheep breeds, two of which are, officially, a subpopulation of the *Texelaar* based on their colour varieties. For the Safe scenario, there is only sufficient material available for two breeds: the *Fries melkschaap* and the *Drents heideschaap*. For 7 out of 15 Dutch breeds there is sufficient material stored for the Risky scenario (approximately 50%). This means that for the other half of the Dutch breeds there is still insufficient material (or no material at all) stored in the genebank to recover a breed.

For approximately half of the Dutch sheep breeds, a start has been made with the storage of a small core collection. Apart from a continuation of the sporadic additions and (smaller) breed-specific actions to collect material, several breeds require more priority, and the CGN should assume a more proactive attitude. Rare breeds such as the *Flevolander, Noord Hollander, Swifter* and the *Nederlandse Bonte schaap* need to be prioritised, as little or no material has been collected of these breeds. In addition, to further develop and optimise the currently available insemination techniques, a good collaboration with sheep farmers is a very valuable addition to the work of the CGN and the value of the genebank collection. This is because, in contrast to several other animal species, successful artificial insemination with (frozen) sperm is more difficult with sheep. One reason for this is due to the anatomy of the cervix and the quality of the sperm post-thawing. There are also indications that these causes may differ between breeds, which means that methods that have been proven to work well for one breed, might not be as effective for other breeds. In the future, attention will also be paid to the creation of protocols to store embryos and female reproduction material. However, this research is currently still in its infancy.

6.5 Dogs

For 3 out of 9 Dutch dog breeds the CGN has a minimal amount of sperm stored in the genebank. For this, there are several explanations. First, of all the animal species the CGN represents, the dog is the one with the least priority and focus. In addition, the costs for collecting material in relation to the number of doses obtained is relatively high compared to other animal species for which the CGN collects genetic material. Particularly the number of doses per semen collection is very low, and it requires a lot of specific expertise, materials and equipment. Hence, the CGN itself does not freeze sperm but collaborates with Cryolab Eersel, who are experts in the field of sperm collection and insemination for dogs. Through the Cryolab there are several options to – as a breeding organisation or owner of a male dog – supply (frozen) material to the CGN to store in the genebank.

One important issue encountered by dog owners and breeding organisations is the ownership of the material. A possible explanation is that dogs are generally viewed and kept as companion animals rather than production animals. In addition, AI for dogs is still quite new and rarely used. In the Netherlands, AI for dogs is even prohibited by law. Despite the exception to use AI for the purpose of maintaining a breed, vets are not always open to the possibilities. Also, for many dog breeds there are strict mating quotas and breed standards that the male dog has to adhere to in order to breed, which means that owners are less inclined to donate material to the genebank.

A positive development is that several breeding organisations are setting up a genebank themselves. The importance of maintaining genetic variation is thereby enhanced among dog owners, which may also have a positive effect on their inclination to donate material to the genebank. The CGN will assume a proactive attitude in 'promoting' the possibilities through Cryolab Eersel in future meetings with breeding organisations and the periodical dog platform meeting.

6.6 Horses

Out of all the 11 Dutch horse breeds, there is sufficient material for the Compromised scenario of the *Groninger paard* and sufficient material for the Risky scenario for the *Friese paard*. For the other 9 breeds there is insufficient material for each of the scenarios.

An important factor for horses are the high costs per (artificial) insemination in practice. The costs of a mating with an approved KWPN stallion are generally higher than € 1,000.- (in comparison to the price of a straw of Holstein cattle sperm which is less than € 50.-). This means that the owner misses out on a substantial sum when they donate material to the CGN. Consequently, many stallion keepers refrain from cooperating.

For several years now, the CGN has had a valuable agreement with the *Friese paarden* studbook. Each year when the stallions go to the horse clinic in Wolvega for a veterinary and sperm-quality check, the CGN can freeze the leftover sperm. This agreement is a very valuable way of storing sperm from a large and varied group of stallions. This is also the reason why for the *Friese paarden* there is a large number of donors, each with several doses of sperm. Investigations into whether this agreement can also be made with other breeding organisations are currently ongoing.

At this moment there are several options for collecting sperm from horses for the genebank. There are 'standard' methods such as sperm winning at corporate level or via an official sperm collection centre, but besides that there is also epididymal sperm collecting. With the epididymal method, the testicles of a recently castrated stallion are brought to the CGN. The CGN can extract sperm from the epididymis and freeze it. Also, for horses there are many developments in the field of oocytes, embryos and different insemination techniques.

Several Dutch horse breeds are under pressure, with very small populations and indications of problems regarding inbreeding and limited stores of material in the genebank. This applies to, among others, the *Nederlandse Trekpaard, KWPN Tuigpaard, KWPN Gelders paard*, *Klassiek Gelderlander paard*, *Zwaar warmbloed paard* and *Groninger paard*. The CGN will contact the individual breeding organisations per breed to discuss possibilities for cooperation.

6.7 Chickens

For only one chicken breed (*Hollands Hoen*) there is sufficient material available in the genebank for the Risky scenario. For the other 30 breeds there is insufficient material available for each of the scenarios. Existing protocols for freezing and successful use of rooster sperm in commercial chicken breeds proved not ideal for (smaller) Dutch chicken breeds in the past. Within the CGN several research projects were initiated to examine the development of these protocols for Dutch breeds.

In addition, a research project is initiated to examine the optimal use of PGCs (primordial germ cells). These are embryo stem cells that can develop into reproductive cells and can be stored in the genebank. In addition, the CGN is looking into setting up a campaign to collect material of Dutch chicken breeds that can be added to the genebank.

6.8 Ducks and geese

For only one duck breed (*Noord Hollandse Krombekeend*) there is sufficient material available in the genebank for the Risky scenario. For the *Hollandse kwaker, Noord Hollandse Witborsteend* and *Overbergse* duck there is insufficient material available for each of the scenarios, and for the *Kuifeend* there is no material available at all. The current collection largely stems from duck-specific genebank projects where sperm of the *Krombekeend* and *Witborsteend* (2011 and 2012) and the *Hollandse Kwaker* and *Overbergse* duck (2013) was collected.

The *Twentse landgans* is the only native Dutch geese breed. A limited amount of genetic material from this breed is stored in the genebank (sperm), but the amount is insufficient for each of the three scenarios.

In 2022 an insemination trial (with *Noord Hollandse Witborsteend*, *Krombekeend* and the *Twentse landgans*) was conducted at the request of the breeding organisation for domesticated waterbirds. Sperm collection and subsequent insemination for one of the duck breeds was successful, resulting in one chick. The male *Twentse landgans* in this trial no longer produced any sperm.

Neither ducks nor geese currently hold the highest priority for the CGN. However, the Dutch duck and geese breeds are considered as valuable historical and cultural heritage, and are therefore considered important by the CGN. Also, the rare status of these breeds plays a significant role in the urgency of any actions undertaken by the genebank. Before starting a new collection campaign, the CGN will first look into a possible insemination trial with sperm that is currently stored in the genebank.

6.9 Rabbits

In 2014 a rabbit-specific genebank project took place to collect sperm from 7 of the 10 Dutch rabbit breeds. However, there is currently insufficient material available to recover the breeds for all three scenarios. Rabbits currently do not hold a high priority for the CGN as these animals do not occupy an important position within the food production system. Over the past decades, worldwide there have been substantial developments in the field of cryoconservation for rabbits, both for sperm- and embryo collection. It is therefore important for the CGN to assess the current different possibilities. In collaboration with breeding organisations, the CGN can assess if there is sufficient interest to initiate a campaign.

6.10 Pigeons

Until now (reference date 2022) no pigeon sperm has been stored in the CGN genebank. The CGN prioritises a genebank project specifically aimed at pigeons. For this project, a temporary pigeon sperm collection station will be set up near Wageningen. Here, the CGN will initiate the collection and freezing of pigeon sperm (for five selected breeds). Further actions will be determined depending on the success of the project.

6.11 Bees

No genetic material of the only native honey bee, the *Zwarte bij* (Dark honeybee), is currently stored in the genebank. For the *Zwarte bij*, only two locations in the Netherlands are home to purebred populations: Texel, where the larger part of the Dutch *Zwarte bij* can be found, and Neeltje Jans, where there is a bee breeding station which is only accessible for purebred breeding.

Given the fact that bees live free and mate whilst flying high in the sky, they often crossbreed with other frequently occurring, non-native honey bee breeds. This situation makes the storage of genetic material of the purebred *Zwarte bij* very urgent. CGN has (yet) limited knowledge about bees and particularly about the successful freezing of genetic material of bees. Investigations into collaborations with experts to see whether and how the current genetic diversity of the *Zwarte bij* can be mapped are ongoing.

7 Conclusions and recommendations

This report contains an overview of the current collections in the genebank and the theoretically required sizes of the core collections to, if necessary, reconstruct a breed. For many animal species and breeds there is currently insufficient material available, as summarised in **Table 25**. We recommend to pay extra attention to several species and breeds in the future, to ensure the construction of adequate core collections (see **Table 25** and explanation for the priorities in chapter 6). This way we can ensure that the Dutch breeds will continue to exist in the future. Parallel to this support by means of constructing the core collections, the CGN also offers advice to the studbooks and breed organisations of the Dutch livestock breeds about sustainable breeding programmes. The strategy and priorities per animal species and breed may differ between both approaches, i.e., the ex-situ genebank and the in-situ advice.

The priority level indicates the necessity to expand the core collection. Here, we looked at the upcoming five years. In order to actually expand the core collections, the CGN is subject to external factors (protocols, laws and regulations, cooperation of breed organisations) and internal factors (expertise, capacity and budget). These have an effect on the feasibility of the desired activities based on the priority level.

Table 25 *Overview of the species and breeds in the genebank. It is indicated which sperm-based scenario is feasible with the current collection and what the current priority level is for additional collection of material in the upcoming years.*

Species/breed	**Feasible scenario** Based on sperm-only scenario with current collection and set number of male donors	**Priority level <5 years** ++ high priority + low priority - no priority
Cattle		
Brandrood rund	Risky	++
Fries-Hollands vee – incl. Roodbont Fries vee	Safe	-
Groninger Blaarkop	Safe	-
Heiderund	Insufficient	-
Holstein zwartbont – incl. Holstein roodbont	Safe	-
Lakenvelder	Compromised	+
Maas-Rijn-IJssel (MRIJ)	Safe	-
Verbeterd Roodbont	Compromised	+
Witrik, Vaal en Baggerbont (color varieties)	Insufficient	+
Pigs		
Bonte Bentheimer landvarken	Insufficient	++
Hypor Landras	Insufficient	-
Hypor Large White	Insufficient	-
Meishan*	Risky	-
Nederlands Landvarken	Safe	+
Topigs Norsvin A-lijn* (Large White)	Compromised	-
Topigs Norsvin B-lijn*	Risky	-
Topigs Norsvin D-lijn (TN Talent, Duroc)	Safe	-
Topigs Norsvin E-lijn (TN Tempo, Large White)	Safe	-
Topigs Norsvin N-lijn (Nederlands landras)	Safe	-
Topigs Norsvin P-lijn (TN Select, TN Top Select, Piétrain)	Safe	-
Topigs Norsvin T-lijn	Compromised	-
Topigs Norsvin Y-lijn*	Insufficient	-
Topigs Norsvin Z-lijn (Large White)	Safe	-

Species/breed	**Feasible scenario** Based on sperm-only scenario with current collection and set number of male donors	**Priority level <5 years** ++ high priority + low priority - no priority
Goats		
Nederlandse Melkgeit	Insufficient	+
Nederlandse Bonte geit	Insufficient	++
Nederlandse Landgeit	Risky	+
Nederlandse Witte geit	Insufficient	++
Nederlandse Toggenburger geit	Insufficient	++
Sheep		
Bonte schaap	Insufficient	++
Drents Heideschaap	Safe	+
Flevolander	Insufficient	++
Groot Heideschaap	Insufficient	++
Fries melkschaap	Safe	+
Kempisch Heideschaap	Risky	+
Mergelland schaap	Compromised	+
Noord Hollander	Insufficient	++
Schoonebeeker	Risky	++
Swifter	Insufficient	++
Texelaar – Blauwe Texelaar, Dassenkop Texelaar	Insufficient	++
Veluws Heideschaap	Compromised	+
Zwartbles	Risky	+
Dogs		
Drentse Patrijshond	Insufficient	-
Hollandse herder	Insufficient	-
Hollandse smoushond	Insufficient	-
Kooikerhondje	Insufficient	-
Markiesje	Insufficient	-
Nederlandse schapendoes	Insufficient	-
Saarloos Wolfhond	Insufficient	-
Stabijhoun	Insufficient	-
Wetterhoun	Insufficient	-
Horses		
Fries paard	Risky	+
KWPN Gelders paard	Insufficient	+
Groninger paard	Compromised	+
Klassiek Gelderlander paard	Insufficient	+
KWPN Rijpaard	Insufficient	-
NRPS Rijpaard	Insufficient	-
NRPS Rijpony	Insufficient	-
Nederlandse Shetland Pony	Insufficient	-
Trekpaard	Insufficient	++
KWPN Tuigpaard	Insufficient	+
Zwaar Warmbloed paard	Insufficient	+
Chickens		
Assendelfts Hoen	Insufficient	+
Baardkuifhoen	Insufficient	+
Barnevelder	Insufficient	+
Brabanter	Insufficient	+
Chaams Hoen	Insufficient	+
Drentse Hoen	Insufficient	+

Species/breed	**Feasible scenario** Based on sperm-only scenario with current collection and set number of male donors	**Priority level <5 years** ++ high priority + low priority - no priority
Drentse Hoen bolstaart	Insufficient	+
Drentse kriel	Insufficient	+
Drentse kriel bolstaart	Insufficient	+
Eikenburger kriel	Insufficient	+
Fries Hoen	Insufficient	+
Groninger Meeuw	Insufficient	+
Hollands Hoen	Risky	+
Hollandse kriel	Insufficient	+
Hollandse kuifhoenders	Insufficient	+
Kraaikop	Insufficient	+
Lakenvelder hoen	Insufficient	+
Leghorn (Dutch type)	Insufficient	+
Nederlandse sabelpootkriel	Insufficient	+
Noord-Hollandse Blauwe	Insufficient	+
Schijndelaar	Insufficient	+
Twents hoen	Insufficient	+
Uilebaard	Insufficient	+
Welsumer	Insufficient	+
ISA/HG Rhode Island Red	Insufficient	+
ISA/HG Rhode Island White	Insufficient	+
ISA/HG barred Plymouth Rock	Insufficient	+
ISA/HG Australorp	Insufficient	+
ISA/HG New Hampshire	Insufficient	+
ISA/HG White Leghorn	Insufficient	+
ISA/HG Sussex	Insufficient	+
Geese		
Twentse landgans	Insufficient	+
Ducks		
Hollandse kuifeend (incl. dwarf)	Insufficient	+
Hollandse Kwaker	Insufficient	+
Noord Hollandse Krombekeend	Risky	+
Noord Hollandse witborsteend	Insufficient	+
Overbergse eend	Insufficient	+
Rabbits		
Beige	Insufficient	-
Deilenaar	Insufficient	-
Gouwenaar	Insufficient	-
Havana	Insufficient	-
Hulstlander	Insufficient	-
Klein lotharinger	Insufficient	-
Nederlandse hangoordwerg	Insufficient	-
Nederlandse kleurdwerg	Insufficient	-
Sallander	Insufficient	-
Thrianta	Insufficient	-
Pigeons		
Amsterdamse baardtuimelaar	Insufficient	-
Amsterdamse Hoogvlieger	Insufficient	-
Amsterdamse tippler (pigeon)	Insufficient	-
Boerenmeeuw	Insufficient	-
Gelderse Slenk	Insufficient	-

Species/breed	**Feasible scenario** Based on sperm-only scenario with current collection and set number of male donors	**Priority level <5 years** ++ high priority + low priority - no priority
Groninger Slenk	Insufficient	-
Hagenaar	Insufficient	-
Hollandse kropper	Insufficient	-
Hyacinthduif	Insufficient	-
Nederlandse Helmduif	Insufficient	-
Nederlandse Hoogvlieger	Insufficient	-
Nederlandse Krulveerkropper	Insufficient	-
Nederlandse Schoonheidspostduif	Insufficient	-
Nonduif	Insufficient	-
Oud Hollandse Kapucijn	Insufficient	-
Oud Hollandse Tuimelaar	Insufficient	-
Oud Hollandse meeuw	Insufficient	-
Voorburgse schildkropper	Insufficient	-
Zeeuwse dwergkropper	Insufficient	-

* *Lines or breeds no longer available*

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Appendix 1 Dutch – English breed names

Table A1 gives an overview of all Dutch and English names for all Dutch native livestock breeds presented and discussed in this report. Breeds can be found in EFABIS DAD-IS via both names.

Table A2 **Overview of Dutch and English names for the Dutch native livestock breeds.Dutch breed name**		**English (transboundary) breed name(s)**
Cattle		
Brandrood rund		Deep Red
Fries-Hollands vee – incl. Roodbont Fries vee		Dutch Friesian - incl. Dutch Red and White Friesian
Groninger Blaarkop		Groningen White Headed
Heiderund		Jutland cattle
Holstein zwartbont – incl. Holstein roodbont		Holstein Friesian – incl. Red Holstein
Lakenvelder		Dutch Belted
Maas-Rijn-IJssel (MRIJ)		Meuse-Rhine-Yssel cattle (MRY)
Verbeterd Roodbont		Improved Red and White
Witrik, Vaal en Baggerbont (kleurslagen)		Coloursided white back
Pig		
Bonte Bentheimer landvarken		Bunte Bentheimer
Hypor Landras		Dutch Landrace
Hypor Large White		Large White
Meishan*		Meishan Line
Nederlands Landvarken		Dutch Landrace
Topigs Norsvin A-lijn* (Large White)		A-line AAAA Large White
Topigs Norsvin B-lijn*		B-line
Topigs Norsvin D-lijn (TN Talent, Duroc)		D-Line DDDD TN Talen Duroc, Duroc sireline
Topigs Norsvin E-lijn (TN Tempo, Large White)		Large White
Topigs Norsvin N-lijn (Nederlands landras)		Dutch Landrace
Topigs Norsvin P-lijn (TN Select, TN Top Select, Piétrain)		Pietrain
Topigs Norsvin T-lijn		T-Line TTTT
Topigs Norsvin Y-lijn*		Yorkshire
Topigs Norsvin Z-lijn (Large White)		Large White, Great Yorkshire damline (GY-z)
Goat		
Nederlandse Melkgeit		Dairy goat
Nederlandse Bonte geit		Dutch Pied
Nederlandse Landgeit		Dutch Landrace goat
Nederlandse Witte geit		Saanen, Dutch White goat
Nederlandse Toggenburger geit		Dutch Toggenburger
Sheep		
Bonte schaap		Dutch spotted sheep
Drents Heideschaap		Drenthe Heath sheep
Flevolander		Flevoland
Groot Heideschaap		Large Heath sheep
Fries melkschaap		Friesian Milk, Friesian dairy sheep
Kempisch Heideschaap		Kempen Heath
Mergelland schaap		Mergelland
Noord Hollander		North Holland
Schoonebeeker		Schoonebeek Heath sheep

Table A2 **Overview of Dutch and English names for the Dutch native livestock breeds.Dutch breed name**		**English (transboundary) breed name(s)**
Swifter		Swifter
Texelaar – Blauwe Texelaar, Dassenkop Texelaar		Texel – incl. Texel Blue and Badgerface
Veluws Heideschaap		Veluwe Heath
Zwartbles		Black Blazed sheep
Dog		
Drentse Patrijshond		Dutch Partridge dog
Hollandse herder		Dutch Shepherd Dog
Hollandse smoushond		Dutch Smoushond, Dutch Ratter
Kooikerhondje		Kooiker dog, Dutch Spaniel, Dutch Decoy Spaniel
Markiesje		Dutch Tulip Dog
Nederlandse schapendoes		Dutch Sheepdog
Saarloos Wolfhond		Saarloos Wolfdog
Stabijhoun		Stabyhoun
Wetterhoun		Wetterhoun
Horse		
Fries paard		Friesian Horse
KWPN Gelders paard		Gelder horse (KWPN)
Groninger paard		Groninger horse
Klassiek Gelderlander paard		Gelderlander horse
KWPN Rijpaard		Dutch Warmblood, KWPN riding horse
NRPS Rijpaard		Anglo-Arab, Dutch Riding horse
NRPS Rijpony		Anglo-Arab, Dutch Riding pony
Nederlandse Shetland Pony		Dutch Shetland pony
Trekpaard		Dutch Draught horse, Belgian Draught horse
KWPN Tuigpaard		Harness horse (KWPN)
Zwaar Warmbloed paard		Oldenburg, Heavy Warmblood
Chicken		
Assendelfts Hoen		Assendelft Fowl – incl. Bantam
Baardkuifhoen		Paduan, Polands (bearded) – incl. Bantam
Barnevelder		Barnevelder – incl. Bantam
Brabanter		Brabanter Fowl – incl. Bantam
Chaams Hoen		Chaam Fowl
Drentse Hoen		Drent Fowl
Drentse Hoen bolstaart		
Drentse kriel		Drent Bantam
Drentse kriel bolstaart		
Eikenburger kriel		Eikenburger Bantam
Fries Hoen		Friesland, Friesian Fowl
Groninger Meeuw		Groninger Mew incl. Bantam
Hollands Hoen		Hamburgh
Hollandse kriel		Dutch Bantam, Hamburgh Bantam
Hollandse kuifhoenders		Polands (non bearded) incl. Bantam
Kraaikop		Kraienkoppe incl Bantam, Breda Fowl incl. Bantam
Lakenvelder hoen		Lakenvelder Fowl incl. Bantam
Leghorn (Nederlands type)		Dutch Leghorn incl. Bantam
Nederlandse sabelpootkriel		Booted Bantam
Noord-Hollandse Blauwe		North Holland blue Fowl incl. Bantam
Schijndelaar		Schijndelaar Fowl
Twents hoen		Twente Fowl incl Bantam
Uilebaard		Owl-bearded Fowl incl. Bantam
Welsumer		Welsummer incl. Bantam
ISA/HG Rhode Island Red		Rhode Island Red, Hybro poultry

Table A2 **Overview of Dutch and English names for the Dutch native livestock breeds.Dutch breed name**		**English (transboundary) breed name(s)**
ISA/HG Rhode Island White		Rhode Island Red, Hybro poultry
ISA/HG barred Plymouth Rock		Plymouth Rock, Hybro poultry
ISA/HG Australorp		Australorp, Hybro poultry
ISA/HG New Hampshire		New Hampshire, Hybro poultry
ISA/HG White Leghorn		White Leghorn, Au
ISA/HG Sussex		Sussex, Hybro poultry, Australorp, Californian Grey Barren Plymouth Rock, Rhode Island Red, White Rhode Island Red, Poultry layerlines from White Leghorn
Goose		
Twentse landgans		Twentse landrace Geese
Duck (domesticated)		
Hollandse kuifeend (incl. dwerg)		Crested duck
Hollandse Kwaker		Call duck
Noord Hollandse Krombekeend		Hook Bill, North Holland Hook Bill
Noord Hollandse witborsteend		North Holland White Bibbed duck
Overbergse eend		Overberg duck
Rabbit		
Beige		
Deilenaar		Deilenaar
Gouwenaar		
Havana		Havanna
Hulstlander		
Klein lotharinger		
Nederlandse hangoordwerg		Holland Lop
Nederlandse kleurdwerg		Netherland dwarf
Sallander		
Thrianta		
Pigeon		
Amsterdamse baardtuimelaar		
Amsterdamse Hoogvlieger		
Amsterdamse tippler (duif)		Tippler
Boerenmeeuw		
Gelderse Slenk		
Groninger Slenk		
Hagenaar		
Hollandse kropper		
Hyacinthduif		
Nederlandse Helmduif		
Nederlandse Hoogvlieger		
Nederlandse Krulveerkropper		
Nederlandse Schoonheidspostduif		Dutch Show-Homer
Nonduif		
Oud Hollandse Kapucijn		
Oud Hollandse Tuimelaar		
Oud Hollandse meeuw		Boerenmeeuw, Old Dutch Turbit
Voorburgse schildkropper		
Zeeuwse dwergkropper		

Appendix 2 Pig breeds classification

Table A2 provides an overview of the classification of the pig breeds in the current analysis.

Table A2 *Overview of the classification of pig breeds in the current analysis.*

Original line	**Included in the analysis as**
Belgisch Landvarken	*Not included*
Bentheimer varken	Bonte Bentheimer varken
Cofok Norwegian and Finnish landrace	Topigs Norsvin N-lijn (Nederlands landras)
Cofok Yorkshire	Topigs Norsvin Z-lijn (Large White)
Dalland 20-line	Topigs Norsvin A-lijn (Large White)
Dalland 30-line	Topigs Norsvin B-lijn*
Dalland 80-line	Topigs Norvins E-lijn (TN Tempo, Large White)
Dalland meishan synthetic	Meishan*
Dumeco D-line	Topigs Norsvin D-lijn (TN Talent, Duroc)
Dumeco L-line	Nederlands landvarken
Dumeco N-line	Topigs Norsvin N-lijn (Nederlands landras)
Dumeco S-line	Topigs Norsvin T-lijn
Dumeco W-line	Topigs Norsvin Z-lijn (Large White)
Duroc	Topigs Norsvin D-lijn (TN Talent, Duroc)
Duroc s	Topigs Norsvin D-lijn (TN Talent, Duroc)
Duroc z	Topigs Norsvin D-lijn (TN Talent, Duroc)
Euribrid	Hypor Landras
Fins landras	Topigs Norsvin N-lijn (Nederlands landras)
Fomeva Z1-line	Nederlands landvarken
Groot Yorkshire	Hypor Large White
Hypor	Hypor Landras
Hypor Large White (line C)	Hypor Large White
Hypor Rock-Y (line G)	*Not included**
Meishan line	Meishan*
Nederlands Landras	Nederlands Landvarken
Pietrain	Topigs Norsvin P-lijn (TN Select, TN Top Select, Piétrain)
T lijn	Topigs Norsvin T-lijn
Topigs 080 - E-lijn - 8888	Topigs Norsvin E-lijn (TN Tempo, Large White)
Topigs A-lijn	Topigs Norsvin A-lijn (Large White)*
Topigs B-lijn	Topigs Norsvin B-lijn*
Topigs L-lijn	*Topigs Norsvin L-lijn (Noords landras; niet meegenomen)*
Topigs N-lijn	Topigs Norsvin N-lijn (Nederlands landras)
Topigs TN Talent	Topigs Norsvin D-lijn (TN Talent, Duroc)
Topigs TN Tempo	Topigs Norsvin E-lijn (TN Tempo, Large White)
Topigs Y-lijn	Topigs Norsvin Y-lijn*
Topigs Z line (Large White)	Topigs Norsvin Z-lijn (Large White)

* Lines or breeds no longer available.

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