Cryoconservation of cattle breeds in The Netherlands
Introduction
Various European indigenous regional cattle breeds have become endangered. The EU-funded project EURECA (Towards (self)sustainability of EUropean REgional CAttle breeds) aims to analyse the factors that are important for success of a breed in in situ use and development, as well as in ex situ (gene banking) conservation. The project started in May 2007 and involves partners from 10 European countries (see: www.regionalcattlebreeds.eu).

In this brochure, an overview is given of ex situ conservation of regional breeds in The Netherlands, i.e. Brandrode Rund, Fries Hollands, Fries Roodbont Vee, Groninger Blaarkop, Lakenvelder, Maas-Rijn-IJssel, Verbeterd Roodbont Vleesras, and Witrik.

History of AI and use of frozen semen
The function of spermatozoa, first described by Van Leeuwenhoek in 1677, was not understood until the 19th century. Despite this, Lazaro Spallanzani, performed successful artificial insemination (AI) of a dog as early as 1783. In the early 20th century, AI was developed further, but the first ‘routine’ application in cattle did not arise before the 1930s, at least in western Europe. In the Netherlands, the first calf conceived by AI was born in Elsloo, Holland, in 1935. The use of cattle AI and the number of AI stations grew rapidly after the Second World War. Currently, the majority of cows in the Netherlands are serviced by AI.

In 1949, Chris Polge and co-workers reported the first successful cryopreservation of semen of a vertebrate species (fowl), soon to be followed by successful cryopreservation of bull semen. As it was demonstrated that frozen semen stored in liquid nitrogen (-196°C) maintained its fertility during long-time storage, the use of frozen cattle semen spread around the world. The use of frozen semen was one of the factors that led to the decline in the number of independent AI stations in the Netherlands. Presently, there are five cattle AI organisations in the Netherlands, CRV, Alta Genetics, KI-Kampen, KI-Samen and KI-de Toekomst.

Breed organisations
All Dutch cattle breeds are registered in officially recognised herd books. NRS (currently known as CRV holding) and the Fries Hollands Cattle Syndicate (FHRS) are recognised for herd book registration of a large number of cattle breeds. Some breeds opted for recognition of their breed-specific herd book (e.g. Brandrode rund).

Besides the official herd book registration, some breeds have one or several breed specific interest groups. E.g. for the Groninger Blaarkop cattle there are regional breeders networks, one national ‘breed foundation’ and a ‘syndicate’ that promotes the use of quality bulls and semen of this breed.
Freezing of semen enabled the global use of Holstein Friesian. At the same time, frozen semen is an instrument in conservation of breeds and of genetic diversity within breeds.

The breeds are visualised top down according to Table 1.

Table 1. Dutch regional cattle breeds

<table>
<thead>
<tr>
<th>Name of the breed</th>
<th>English name</th>
<th>Breed code</th>
<th>Breed status*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brandrode Rund</td>
<td>Deep Red Cattle</td>
<td>BRR</td>
<td>rare; endangered; growing</td>
</tr>
<tr>
<td>Fries Hollands</td>
<td>Dutch Friesian</td>
<td>FH</td>
<td>rare; vulnerable; declining</td>
</tr>
<tr>
<td>Fries Roodbont Vee</td>
<td>Friesian Red and White</td>
<td>FR</td>
<td>rare; endangered; growing</td>
</tr>
<tr>
<td>Groninger Blaarkop</td>
<td>Groningen White-Headed</td>
<td>G</td>
<td>rare; vulnerable; stable</td>
</tr>
<tr>
<td>Lakenvelder</td>
<td>Dutch Belted</td>
<td>LV</td>
<td>rare; vulnerable; stable</td>
</tr>
<tr>
<td>Maas-Rijn-Ussel</td>
<td>Meuse-Rhine-Yssel</td>
<td>MRU</td>
<td>not rare; declining</td>
</tr>
<tr>
<td>Verbeterd Roodbont Vleesras</td>
<td>Improved Red and White</td>
<td>VRB</td>
<td>rare; vulnerable; declining</td>
</tr>
<tr>
<td>Wiltrik</td>
<td>Coloursided White Back</td>
<td>WR</td>
<td>rare; vulnerable; stable</td>
</tr>
</tbody>
</table>

* Breed status: 1) rare or not rare; 2) If rare: vulnerable or endangered or critical; 3) declining or stable or growing
Developments towards conservation of rare breeds

In 1976, the Stichting Zeldzame Huisdierrassen (SZH, Foundation for rare farm animal breeds) was founded. In the early 1990’s, SZH, NRS, and the Research institute IVO-DLO, initiated the Gene Bank Foundation for Farm Animals (Stichting Genenbank Landbouwhuisdieren, SGL).

In 2002, the Ministry of Agriculture, Nature and Food Quality decided to fund and promote conservation and sustainable use of animal genetic resources (AnGR), through a year programme for the Centre for Genetic Resources, the Netherlands (CGN). CGN is an independent organisation within Wageningen University and Research Centre (WUR). CGN-AnGR is located in the Animal Sciences Group of Wageningen UR at Lelystad. Its main tasks are to organise and perform ex situ conservation, to support in situ conservation, and to supply policy support to the Dutch government and international organisations. Since 2001 CGN-AnGR manages an animal gene bank, which includes the original SGL stocks since 2006.

Dutch regional cattle breeds

In the EURECA project, a number of Dutch regional breeds, with varying levels of being endangered, were taken into account (Table 1). The ranking of the breeds according to size is: Maas-Rijn-IJssel (MRIJ), Fries Hollands (FH), Groninger Blaarkop, other breeds. The importance of breed improvement as a breeding goal, as well as the level of coordination of a breeding programme, seems to decline with breed size. For the MRIJ breed

Conservation strategies

The CGN gene bank stocks are kept for two purposes or conservation strategies. One is long-term storage of germplasm with the aim to preserve genetic diversity of the present for future generations. The second is short-term storage: semen may be distributed to farmers provided that it is necessary to use gene bank material to maintain the breed. Also, CGN will first consult the breed interest group. In addition, whenever the number of doses available in the CGN gene bank drops below 50, CGN will issue the semen only in exceptional cases.

Table 2. Numbers of bulls and doses of stored semen of Dutch regional cattle breeds

<table>
<thead>
<tr>
<th>Name of the breed</th>
<th># bulls</th>
<th># doses</th>
<th>Number of bulls with doses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;200</td>
</tr>
<tr>
<td>Brandrode rund</td>
<td>13</td>
<td>4,804</td>
<td>3</td>
</tr>
<tr>
<td>Fries Hollands</td>
<td>203</td>
<td>&gt;42,726</td>
<td>126</td>
</tr>
<tr>
<td>Fries Roodbont Vee</td>
<td>38</td>
<td>14,925</td>
<td>7</td>
</tr>
<tr>
<td>Groninger Blaarkop</td>
<td>69</td>
<td>&gt;44,664</td>
<td>38</td>
</tr>
<tr>
<td>Lakenvelder</td>
<td>34</td>
<td>&gt;19,567</td>
<td>15</td>
</tr>
<tr>
<td>Maas-Rijn-IJssel</td>
<td>246</td>
<td>&gt;925,970</td>
<td>111</td>
</tr>
<tr>
<td>Verbeterd Roodbont Vleesras</td>
<td>52</td>
<td>&gt;41,877</td>
<td>38</td>
</tr>
<tr>
<td>Witrik</td>
<td>4</td>
<td>629</td>
<td>2</td>
</tr>
</tbody>
</table>

Figure 1. Stocks of frozen semen per breed and per owner/storage site.

still 8-10 new bulls are tested every year by CRV. In the FH breed, the members of the FH breed interest group apply ‘fundament breeding’ i.e. farmers breed with FH bulls born on their own farms, thus maintaining many separate lines on farm.

Number of sires and doses in storage per breed

Stocks of semen of the regional breeds are available at CGN, at the AI stations and at private farms, but number of bulls and number of doses per bull vary widely between breeds (see Table 2). Figure 1 shows that there are several semen owners and...
storage sites. Bulls of breeds which still show a good market perspective are well represented at the AI organisations. For the rare local breeds a large percentage of all semen available for the breed is stored in the gene bank of CGN or at private farms.

Figure 2 shows the availability of bull semen by year of birth of the bull. For breeds with a short history (Verbeterd Roodbont, Brandrood) there is no semen stored before the 1980s. The breeds Verbeterd Roodbont and Brandrood belonged to the MRIJ breed in the past and specific herdbooks were established only recently.

Figure 2 also shows that there has not been any addition of FH semen to the gene bank during the last decade. The FH breed society has its own breeding structure including storage of semen on farm, so CGN didn’t have to take care for short term storage. Also, no semen was supplied to CGN for long term storage during the last decade.

**Stocks of semen at the CGN gene bank**

The CGN gene bank contains stocks of semen (and some embryos) of the Dutch regional cattle breeds, as well as the commercial breeds. The CGN gene bank has an agreement with Alta Genetics and CRV that CGN obtains 25 doses from each of the young bulls in their breeding programmes. With regard to the rare breeds, the objective of CGN is to cryopreserve 400 sperm doses per bull, and 25 bulls per breed. The semen is obtained from AI stations or from on farm collections of bulls that are chosen in collaboration with the breed interest group/herd book of the breed. For on farm collection, the animals are serologically tested for all relevant diseases.

**Numbers of bulls available vary widely between breeds**
The main CGN storage location is on the premises of the Animal Sciences Group (ASG) in Lelystad, and consists of one EU-certified storage site and one separate site for not EU certified material. For security reasons, there is a duplicate site for not EU-certified material at the Faculty for Veterinary Sciences in Utrecht.

Stocks of semen at AI organisations
In addition to the CGN gene bank, the Dutch AI organisations CR-Delta, KI-Kampen, KI-Samen, and KI-de Toekomst, have stocks of semen of regional Dutch breeds. However, they will not guarantee long term storage.

Moreover, the AI stations will only have semen of bulls as long as it is commercially interesting. For the smaller breeds (Fries Hollands, Fries Roodbont, Groninger Blaarkop, Lakenvelder, Verbeterd Roodbont Vleesras), there is not a structural breeding and testing programme, and the number of bulls available from those breeds is limited.

Stocks of semen at private farmers
Lastly, some stocks of semen are stored by private farmers. Data have become available for Groninger Blaarkop and Verbeterd Roodbont Vleesras, but also for other breeds there may be significant stocks of privately owned semen.

CGN wants to ensure the availability of semen from a sufficient number of bulls for each rare breed.
SWOT analysis: cryoconservation

Using the information obtained, a SWOT-analysis (strengths, weaknesses, opportunities, threats) was performed for cryoconservation of cattle breeds in the Netherlands.

Strengths and weaknesses

An important strength of the present situation with regard to (cryo)conservation of Dutch regional breeds is that there is a national (government funded) organisation for conservation of farm animal genetic resources (CGN), embedded in Wageningen-UR. Another strength is the co-operation of breeding organisations, e.g. by donating sperm from young unproven bulls to CGN. There is a good and regular contact of CGN with breed societies, AI organisations, and LNV, and collaboration between these stakeholders could be further strengthened.

An important weakness is that for several (rare) breeds, the availability of good quality bulls is limited. Also, especially the smaller breeds lack structured breeding programmes.

Opportunities and threats

Opportunities may lie in a new generation of farmers, with possibly increased interest in some breeds for specific characteristics or purposes, e.g. organic farming, robust animals, low input systems, regionalization of the breed; connection between breed and farming area of origin, etc.

Another opportunity/challenge is to further develop and implement the interest of large and commercial breeding organisations in taking co-responsibility for long term conservation of rare breeds. A third opportunity would be to redefine breeding goals and breeding structures for local breeds, including the role of AI, rotational mating, natural mating, etc. and to strengthen the collaboration between different stakeholders.

A threat is the possibility that the national government could cut funding for conservation efforts. Government involvement is seen as necessary in the light of the short term perspective of breeding industry and farmers, and the costs of collection and storage of semen and embryos. Another threat is the possibility that AI organisations would close their breeding infrastructure, to only focus on a small number of commercial breeds.
This overview was compiled by Henri Woelders, with help of Ina Hulsegge, Henk Sulkers, Rita Hoving, and Sipke-Joost Hiemstra. Information was kindly provided by breed interest groups and AI organisations. More information about the EURECA-project can be found on the website: www.regionalcattlebreeds.eu. Veeteelt and SZH (Hinke Fiona Cnossen) are acknowledged for the cattle photos.

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Cover photography: View into a liquid nitrogen storage tank.
This tank contains the oldest semen stocks in the CGN gene bank.

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