

# Constitution of the first French patrimonial genetic stock for *ex situ* management in the species *Gallus gallus*

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This project was planned to deal with the reduction in genetic variability of domestic bird livestock and the increasing risk of line extinction for health and safety reasons (e.g.: bird influenza epidemic). The aim of the present study was to examine the feasibility of the creation of genetic stock to preserve and improve the management of rare chicken strains and lines. Embryo cryopreservation is not routinely feasible in birds. As a consequence, the collection includes semen and blood samples frozen in optimal safety and traceable conditions. This project is taking place in the construction of the French National Cryobank of Domestic Animals (CNAD).

## Characterisation of the strain and lines stored, safety status, cleaning up

A limited number of lines and one strain were chosen to examine the feasibility of the project. Three INRA experimental lines were chosen for their value in the research fields of nutritional and growth metabolism and pathology: i.e. lines R+, Y33 and B4/B4 (Gabarrou et al, 1997; Le bihan et al, 1998). A traditional strain, the “Gauloise dorée” (coq gaulois) was also chosen for its patrimonial interest.

The three INRA lines were more or less infertile due to the selected character (deficit in energy metabolism for R+ line, growth pressure for Y33) or to the succession of many inbred generations. The reproductive performance of the gauloise dorée is not good (high seasonality and short reproductive period; low production of semen and eggs).

The INRA experimental lines are raised in controlled safety status (CSS) for the R+ and Y33 lines, or in specific free status (SSF) for the B4 line.

The strain “Gauloise dorée” is dispersed in different breeding centres throughout France. Its health safety status was unknown at the start of the project. As a consequence, we established a clean up system to eliminate potential carrying of contagious infectious diseases, especially salmonella and mycoplasma. For this strain, animals originating from 10 different breeding centres were taken in the form of eggs for brooding (EFB) after treatment of the parents of the eggs against infectious diseases. Eggs were then incubated and also treated by injection of tiamuline during the incubation period. The newly hatched chickens were grown on in isolated rooms up to 1 to 2 months of age and treated with tiamutine to the point of eradication of salmonella and mycoplasma contamination. The young males were grown further for semen collection and freezing in controlled health safety conditions and submitted to a classical vaccination programme as performed for INRA lines.

## Construction of a general infrastructure for the cleaning up, collection and storage of frozen cells

We established two complementary structures

- a) *A structure for cleaning up, animal breeding, semen collection and freezing of samples*. The site of this structure is the INRA centre of Tours with experimental units specializing in avian pathology (UE PAP), avian breeding (UE SRA), and the research unit specializing in Avian Biology (UR SRA).

- b) *Site of storage of frozen avian samples that is also the secondary avian site of the CNAD*. This site is located at the EFS (French Blood Institute) of Tours.

These two structures are part of the Biological Centre of Touraine (CRBT) created for that purpose. The aim of this centre is to manage the collection, freezing and storage of biological samples from the Tours city area in the most highly traceable quality conditions.

## Breeder male management, sample collection, freezing, storage and fertility

After the raising period, the adult males were housed in individual battery cages under a 14L/10D photoperiod and fed a standard diet of 12.5 MJ/day. Semen was routinely collected twice a week by the method of

Burrows and Quinn (1937) and semen concentration and quality regularly assessed (number of spermatozoa and motility). The best ejaculates were frozen in 0.5 ml straws with the cryoprotectant glycerol, as described by Seigneurin and Blesbois (1995).

The results presented in the Table show that it was possible to prepare and store 491 straws for the gauloise dorée strain, more that 900 for Y33 and R+ lines, and 474 for the B4/B4 line. For each male giving frozen semen, 15 straws of blood were also frozen for further analysis.

For security reasons, half of the straws were stored at the EFS, and the other half at the primary storage site of the CNAD, on the site at Maison Alfort (France).

**Table Stocks of frozen semen**

Line or strain	Number of males	Minimum number of Straws per male	Maximum number of straws per male	Total number of straws per line
Y 33	22	21	61	982
B 4	20	7	41	474
R +	22	30	55	994
Gauloise Dorée	20	8	40	491

To examine the feasibility of restoration of the lines or strains with the frozen ejaculates, a fertility test was organised with the frozen ejaculates of Y33, R+ and Gauloises dorées males and commercial laying females (ISA Brown). Fertility varied significantly between the lines and strain (Y33>Gauloise>R+: 52>37>25% fertility rates). These fertility rates are sufficient to ensure the restoration of the lines (with the exception of genes shared by the W chromosome).

## Conclusion

This project permitted the construction of a complete infrastructure of an avian germ cell cryobank. It also led to the cryopreservation of semen and blood samples of 1 strain and 3 lines of the species *Gallus-gallus*.

The frozen sperm were also proven to be effective in yielding offspring after thawing, even when the lines were subfertile.

Finally, the feasibility of an avian germcell cryobank for patrimonial preservation and management of genetic resources is now proven.

## References

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