# Discussion paper

# Gene banking strategies for livestock species involving germ cells or gonads

*State of the art and EU legislation - Results from the EU Horizon 2020 project IMAGE*

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*Statement:*
*Gene banking strategies involving both male and female contributions are needed. Recent gene banking technologies involving germ cells or gonads should be adopted"*

## Introduction

The FAO Global Plan of Action (GPA) for (farm) animal genetic resources (AnGR) involves both *in situ* as well as *ex situ* conservation strategies for livestock species. To this end, countries are establishing (national) *ex situ in vitro* gene banks for long term conservation purposes. Guidelines for the development and operation of national gene banks were developed by the FAO (2012).

The ERFP network has the objective to facilitate the implementation of the GPA for AnGR in Europe and supports the further development of *in situ* and *ex situ* conservation strategies. The ERFP Working Group on *ex situ* conservation specifically supports national gene bank development (in terms of efficiency and effectiveness) and the further development of the European Gene Bank Network for AnGR (EUGENA).

During the past 3 years, the EU Horizon 2020 research project 'IMAGE' carried out research on innovative reproduction and cryopreservation technologies. Published results are available on the IMAGE website[1]. At the ERFP WG Ex Situ meeting in Zagreb in 2018, Elisabeth Blesbois (IMAGE Work Package leader) presented the research carried out within IMAGE.IMAGE demonstrated the applicability of gene banking strategies involving cryopreserved primordial germ cells (PGCs) and gonads. Also, IMAGE addressed legal and ethical aspects of these reproductive technologies.

The aim of this discussion paper is to inform the ERFP WG Ex Situ specifically about this topic, in order to support discussions at national level on further steps towards implementation of novel reproductive technologies.

## Rationale for novel gene banking strategies

Currently, European national gene banks are cryobanking predominantly frozen semen (IMAGE survey). Cryopreserved semen can be readily used by farmers to sustain genetic diversity of local breeds in a number of (mammalian) livestock species, notably in cattle. However, in some species, semen is not a practical vehicle, especially for the aim of restoring a lost breed. For recovery of a lost breed, seven generations of back-crossing must be performed with cryopreserved semen, with sufficient numbers of male and female population sizes in each generation. This would require very large numbers of insemination doses. In various species, e.g. in poultry, freezability of semen can be low, and post-thaw

[1] http://www.imageh2020.eu/conteudo.php?idm=22&lang=en

fertility varies and is not guaranteed. Collection of sufficient numbers of insemination doses of an adequate number of males per breed is difficult to organise and is time-consuming and costly. Current gene bank semen stocks are absolutely insufficient for recovery of a lost breed. Furthermore, seven generations of back-crossing with adequate male and female population sizes would be extremely costly. Thus, we would state that recovery of a lost breed from gene bank semen stocks is a theoretical possibility but will never be a practical reality.

Recovery of a breed in one generation can be achieved when using the combination of cryopreserved oocytes and sperm cells, or embryos. Collection or *in vitro* production of embryos, cryopreservation and embryo transfer are all practical, or even routine, possibilities in cattle, and a few other mammalian species. However, freezing of embryos (or oocytes) is impossible in bird species.

Gene banking and grafting of gonads (e.g. ovarian tissue) or PGCs are emerging alternative strategies. For chicken, IMAGE confirmed earlier demonstrated applicability of these strategies. For other mammalian species in which gene banking of semen and embryos is not (yet) a cost effective practical option (e.g. for pigs), gonad transfer could also potentially be developed.

## Cryobanking and transfer of gonads or PGCs; state of the art

Ovarian transplantation is already in use as a very effective and often indispensable means of preserving laboratory animals. The technique is used by world-wide operating companies like The Jackson Laboratory[2] and Charles River[3], and probably also by other suppliers (Harlan Sprague Dawley Inc., Wistar). The method is also 'ethically sound'[4], as it very strongly reduces the number of animals that need to be kept in captivity for the thousands of different strains of laboratory mice and rats. In humans, cryopreservation of ovaries is used routinely to preserve fertility in women that need to undergo chemotherapy.

In poultry species, similar methods were shown to be efficient for preserving ovarian and testicular tissue and for using that reproductive material to generate offspring [5] [6] [7]. The Canadian and USA national gene banks maintain stocks of cryopreserved ovaries and testes from various poultry species[8].

Another viable alternative in bird species is transplantation of primordial germ cells (PGCs). These are the gonadal cells in an embryo that give rise to the (adult) gametes, i.e. oocytes and spermatozoa. PGCs can be harvested from eggs, and can be proliferated in *in vitro* culture, and subsequently be cryopreserved. PGCs can be used to generate offspring by injecting these cells into recipient eggs [9]. When these eggs are incubated, the gonads of the developing recipient will contain donor germ cells. The recipient can, after reaching adult fertile age, produce gametes and offspring of the donor-genotype can thus be obtained.

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[2] https://www.jax.org/news-and-insights/jax-blog/2015/january/ovarian-transplantation-to-overcome-breeding-difficultiesblog-post-detail-p/
[3] http://www.criver.com/products-services/basic-research/transgenic-colony-services/line-rescue
[4] Huang et al., 2010. Cryobiology 60 (2010) 129–137.
[5] Song Y. and Silversides F.G., The Technique of Orthotopic Ovarian Transplantation in the Chicken. Poultry Science (2006); 85 (6): 1104–1106
[6] Liu J., Song Y., Cheng K.M., Silversides F.G. Production of donor-derived offspring from cryopreserved ovarian tissue in Japanese quail (Coturnix japonica). Biol Reprod 2010; 83:15–19.
[7] Silversides F.G. and Robertson M.C. Cryoconservation of avian gonads in Canada. Poultry Science 2013; 92(10): 2613–2617.
[8] Personal communication USA animal germplasm program
[9] Park et al., 2003. Biol. Reprod. 68:1657–1662

## Legal EU framework regarding gene banking and use of gonads and PGCs

Collection of gonads can be done after humane sacrifice of juvenile or new-born animals. The use of gonads, e.g. ovaries, for generating offspring involves a surgical procedure under general anaesthesia, in which the ovary or ovaries of a juvenile recipient animal are (partly) removed, and a donor ovary is placed at the appropriate site. The risk for morbidity and/or mortality following such procedures is small and animals generally recover quickly from this procedure.

The use of animal procedures is subjected to EU and national regulations. General guidelines that apply to farm animals are described in Council Directive 98/58/EC[10]: Breeding procedures which are likely to cause suffering or injury must not be practised, but procedures/interventions causing minimal or momentary suffering or injury, are permitted if allowed by national provisions. Other, species-specific, directives apply, such as Council Directive 2001/88/EC[11] and Council Directive 2007/43/EC[12], provide specific guidelines for pigs and chickens respectively, and describe a number of permitted interventions, such as ear marking, or teeth trimming in pigs.

Generally, EU directives prohibit interventions that damage, or hurt animals, unless specifically allowed in national legislation. In the Netherlands, in compliance with these directives, the law on animals ("wet dieren') prohibits all bodily interventions, unless: a) For a veterinary necessity; b) If indicated in general administrative regulation ("algemene maatregel van bestuur"); or c) If mandatory or permitted by any legal requirement. Procedures for reproductive technology that are specifically allowed (Dutch 'Decision Veterinarians' and 'Decision Animal Keepers') include caesarean section (for producing SPF animals) and transvaginal follicle puncture in mammals. Currently, there are no provisions for the procedures involved in collection or transplantation of gonads or PGCs, but national governments may provide specific derogations if specifically asked for, and if the government considers that the provided reason and justification of such procedures clearly outweigh potential animal discomfort or suffering.

The collection of PGCs is not seen as an animal procedure *per se*, as the developing embryo in early egg incubation is not considered an 'animal'. However, national interpretation may differ regarding injection of PGCs into chicken embryos. For instance, the Dutch minister considers the resulting hybrid offspring as 'genetically modified', which is prohibited.

A separate issue that may be addressed is the production of subfertile or infertile recipients for transfer of gonads or PGCs. Chemical ablation of gonad development may be a restricted 'animal procedure', or may raise ethical questions. And the same is true for production of infertile animals by making interspecies hybrids or by genetic modification. These techniques may need to be addressed, although they may not be essential for successful transfer of gonads or PGCs *per se*.

## Cryopreservation of gonads: yes or no?

National government and cryobanks have to decide about their needs and the acceptability of gonad cryopreservation for long term conservation purposes. Following the rationale in favour of gonad cryopreservation, national policies and regulations have to be put in place for this purpose.

[10] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A31998L0058
[11] https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32001L0088
[12] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32007L0043