



Reproductive Techniques in Conservation Biology

Proceedings from a CRU seminar at SLU, Uppsala, March 18, 2004

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Renée Båge (*editor*)

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Foreword

Welcome to the CRU seminar “Reproductive Techniques in Conservation Biology” at the Faculty of Veterinary Medicine and Animal Science, SLU, Uppsala, on March 18, 2004.

The objective of this seminar is to give an orientation about ”the state of the art” concerning reproductive techniques; what they can be used for in breeding programmes (within conservation programmes as well as new applications) in farm animals, pets and wild-life species.

This first seminar will provide a general background to the subject and present some of the knowledge available within CRU. Further seminars will follow on this topic, as well as postgraduate courses.

Uppsala in March 2004

Britt Berglund and Renée Båge
CRU’s Scientific Committee

The Centre for Reproductive Biology in Uppsala (CRU) invites to the seminar

Reproductive techniques in conservation biology

What is the state of art concerning reproductive techniques?
How can they be used in conservation of animal genetic resources?

Time: Thursday 18 March 2004, 9.00-15.15

Place: Room L in the Lecture Hall, Undervisningsplan 8, SLU, Uppsala

Registration: Before 8 March 2004 to Renee.Bage@og.slu.se. The seminar is free of charge.

Programme: Moderator *Britt Berglund, Dept. of Animal Breeding and Genetics, SLU*

09.00-09.30 Coffee and sandwich

09.30-09.35 Introduction
Britt Berglund

09.35-10.15 Reproductive techniques - a short general overview
Hans Gustafsson, Swedish Dairy Association

10.15-10.55 Achievable objectives and limitations of fresh and cryopreserved semen
Lennart Söderquist, Dept. of Obstetrics and Gynaecology, SLU

11.00-11.40 Achievable objectives and limitations of fresh and cryopreserved oocytes and embryos
Renée Båge, Dept. of Obstetrics and Gynaecology, SLU

11.40-12.20 Sperm collection and cryopreservation in carnivores (Leopard at Nordens Ark)
Eva Axné, Dept. of Obstetrics and Gynaecology, SLU

12.20-13.20 Lunch

13.20-14.00 Conservation of genetic resources - farm animals
Birgitta Danell, Dept. of Animal Breeding and Genetics, SLU

14.00-14.40 Cryoconserved semen in cattle genetic research.
Mia Holmberg and Anne Lundén, Dept of Animal Breeding and Genetics, SLU

14.40-14.50 Closing
Britt Berglund

14.50- Coffee

Welcome!

CRU's Scientific Committee / Britt Berglund and Renée Båge
<http://www-cru.slu.se>

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Reproductive techniques – a short overview

Hans Gustafsson

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Assisted reproductive techniques (ART) are biotechnical methods, by which nature is assisted in producing offspring. The objectives for ART can be as in the humans to overcome some forms of infertility or as in farm animals to increase litter sizes or for genetic improvement. Preservation of gametes and zygotes by freezing simplifies the international movement of genes and makes it possible to establish gene banks.

Although the application of ART to rare species has met with only limited success, the benefits achieved in domestic livestock suggest that ART also have a potential for conservation biology. Among domesticated animals, ART has been used most extensively in cattle, which will be exemplified in the presentation.

Artificial inseminations (AI) is the oldest ART, first performed in the dog by the Italian monk Spallanzani in 1784. AI meaning placement of semen in the genital tract of a female by a technical route involve several important steps such as collection of semen from the male, extension of semen and placement of the semen in the genital tract. Methods for cryopreservation of semen was developed in the late 1940s which in most countries are standard routines for AI in cattle today. The AI-technique makes it possible to increase the number of offspring from a male geographically independent, which in dairy cattle has reduced venereal diseases and greatly increased the genetic merit. Timing of AI is critical and oestrous detection is a problem related to the use of AI. Several technical aids have been developed to detect cows in oestrus.

Induction of oestrous and ovulation by hormonal treatment makes it possible to timed insemination without the need of oestrous detection.

Sexing of semen as determined by DNA content in X- and Y-chromosome-bearing sperm has become possible since the early 1990s. Bull-semen can be sorted with approx. 90% accuracy however with a low yield.

Embryo transfer (ET), first performed in a rabbit in Cambridge 1890 by Walter Heap, is today another routinely used reproduction technique in livestock. Embryo transfer means transfer of an *in vivo* fertilised embryo from a biological mother to the uterus of a fostermother, which finally gives births to the young. Embryo transfer mostly involves also methods for increasing the ovulation rate (superovulation), increasing the final efficiency. Since 1970s embryos are possible to freeze and store in liquid nitrogen for future use.

In Vitro Production (IVP) of embryos is an alternative method to produce embryos for ET. The method involves collection of oocytes (ovum pick up = OPU), maturation of oocytes, capacitation of sperm, fertilisation and culture to a transferable stage. Collection of oocytes can be performed from ovaries of slaughtered or from a living animal by the aid of ultrasonography. The method has the potential to decrease the generation interval by collecting oocytes from pre-puberal animals and even from foetuses. The IVP - technique still has some problems, which need to be solved. In vitro systems make it possible to add other technologies e.g. freezing of oocytes or gonadal tissue for future fertilisation, intracytoplasmic sperm injection (ICSI), and gene tests but most of these methods need further development for practical use.

Cloning technology is still at an experimental stage, although commercial companies producing bovine clones for agricultural purposes exist. Embryo cloning in its simplest form, splitting of embryos, has a potential to produce a litter of 2-4 offspring from one embryo. It has been used to a limited extent in the bovine – ET since the 1980s. By using a more complicated technique, nuclear transfer, the embryo clones can, at least theoretically, be unlimited in size. Since the birth of the sheep Dolly it has also become evident that somatic cloning is possible.

Achievable Objectives and Limitations of Fresh and Cryopreserved Semen

Lennart Söderquist

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Artificial insemination (AI) is a process of collecting semen, extending it with appropriate fluids for either short- or long-term preservation, and then placing it into the reproductive tract of sexually receptive females. By avoiding direct contact between animals, the risk of spreading infectious diseases is reduced. Artificial insemination used in a proper way increases the breeding capacity of the males, permitting a higher degree of selection and an extended use of animals with a high breeding value and has, throughout the last half century, substantially contributed to the breeding progress seen in farm animals. A prerequisite for the use of AI has been the development of procedures for semen preservation. Today long-term preservation of spermatozoa allows the banking of genetic resources, the exchange of genetic material across national borders and to help in conserving threatened or endangered species

However, to be able to utilise AI in conservation biology, we need to gain a deeper knowledge of the influence of different steps in the processing and handling of semen from different species. Cryopreservation of semen involves subjecting the spermatozoa to a series of closely related steps like dilution and sometimes reconcentration, temperature reduction, cellular dehydration, freezing and thawing. A great variation is seen between species and individuals in how their spermatozoa can endure these different steps in the cryopreservation process. The accumulated cellular injuries that are seen are mainly caused by harmful formation of ice crystals and solution effects, resulting in a shorter life span in frozen-thawed semen than in its liquid-preserved counterpart. But the advantage is that cryopreserved semen can be stored for a very long time in a frozen state in liquid nitrogen (-196°C).

Use of liquid (fresh) semen implies a somewhat simpler handling procedure and also gives higher fertility results. Disadvantages are the high demands on a fast distribution system, due to the short survival (often only some hours) of the diluted semen, and therefore it is more difficult to inseminate liquid semen at an optimal time. The use of cryopreserved (frozen-thawed) semen makes the planning of AI easier since deep-frozen semen can be stored in liquid nitrogen until use. The semen can then be thawed and inseminated at an optimal insemination time. Drawbacks are the more time-consuming semen processing and handling procedure, the need for more expensive equipment and often poorer fertility results.

The beginning of a new life is the final result of a series of subtle changes and events. Besides finding successful methods and techniques to collect the semen that is to be used, there are many factors that can influence the fertility results. One prerequisite is to find means to detect the heat and ovulation time (which varies considerably between species) to be able to inseminate the female at the optimal time. This might involve the need to develop new methods to be able to detect necessary hormones or metabolites in i.e. blood or faeces.

The site of semen deposition as well as that the AI-dose contains an appropriate number of spermatozoa of good quality (sperm motility and morphology) also influences the fertility results, and demands that optimal protocols and handling procedures are established for the species in question. Reports have shown that better fertility results can be achieved the further into the female genital tract that the semen is deposited. The laparoscopic technique (deposition of semen into the uterine horn) is, however, rather complicated and prohibited to use in unanaesthetised animals according to Swedish animal welfare legislation.

The inseminator's technical and hygienic performance of the AI and the stress that the female is exposed to during the time of insemination also adds to the factors that can influence the final fertility results. In conclusion, a careful planning is a prerequisite to be able to perform a successful AI programme.

If we can develop suitable techniques to collect and process semen along with well adapted protocols, techniques and routines, which lead to an acceptable sperm viability and fertility, AI might constitute an alternative to natural mating and cryopreserved long time stored semen might contribute in conserving threatened or endangered species.

Achievable Objectives and Limitations of Fresh and Cryopreserved Oocytes and Embryos

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The aim of assisted reproductive technologies (ART) is to produce offspring, usually in higher numbers than during normal, physiological conditions. In conservation biology programmes, these technologies can make it possible to maintain endangered breeds or species. Inbreeding is avoided if not only sperm cells are used in high numbers but also female gametes, oocytes. Further, gene banking of cryopreserved embryos allows storing of the full genetic information from a certain species. The development of ARTs has mainly been done for livestock species and although some of the technologies have been in commercial use for decades now, the efficiency as well as the importance is limited. Artificial insemination (AI) and natural mating are still the dominating breeding methods. The knowledge and experience from ART in farm animals can be applied to other species, but before doing so, the methods must be modified in order to be effective and repeatable. For this, it is absolutely necessary to have basic knowledge of the unique reproductive physiology in each animal species. Concerning wildlife, it is also necessary to balance the development of ART with preservation of habitats.

Collection of oocytes from antral follicles can be done either from live or dead animals, and the oocytes can either be cryopreserved for future use or used for *in vitro* production of embryos. The anatomy of the animal dictates the method of collection. In large animals, the ovaries can easily be reached and manipulated via rectum and vagina, which enables repeated collections from individual animals. When surgery is required to reach the ovaries, the method is no longer as repeatable. The developmental capacity of the harvested oocytes varies because follicles of different developmental stages are present at the same time in the ovaries. Collection of oocytes by the ovum pick-up method can be done twice a week and gives on average 7 oocytes per session, with a resulting number of transferable embryos varying from 0.4-4.7. Oocyte cryosensitivity varies greatly between species, mainly depending on lipid contents.

Embryo production *in vivo* involves the following key procedures: Superovulation of the donor animal, oestrus synchronisation of recipient animals, AI, embryo transfer and embryo cryopreservation. Each of these steps must work before the method can be introduced in a new animal species. In cows, the average number of embryos produced are 4-5, but the variation is huge (0-25), with 20-30% of the donors never responding to the stimulatory treatment. There are unwanted side effects from the hormone treatment, and since the outcome is very unpredictable, it is difficult to estimate how many recipients should be prepared.

Embryo production *in vitro* requires skilled operators, both at oocyte collection and, particularly, in the laboratory. Expensive equipment is necessary and very specific laboratory conditions must be fulfilled. Compared to *in vivo* produced embryos, the embryonic, foetal and neonatal losses are higher with *in vitro* produced embryos, and the offspring produced may have defects (“the large offspring syndrome”) due to suboptimal *in vitro* culture conditions. The embryos are more cryosensitive, although gradually improved vitrification protocols (rather than the traditional slow-rate freezing) seem to partly overcome these problems.

Inter-species embryo transfer, with an embryo transferred from an endangered species to a surrogate mother of a related and more common species, was initially believed to be a model for rescue of species that were close to extinction. There have however been very few successful reports, and the biological mechanisms for the development of a transplanted embryo in the uterus of a foreign species are more complicated than expected.

Finally, three new and very advanced technologies can be mentioned, which in the future may result in as abundant female gamete production as in male animals:

Ovarian tissue can be collected and cryopreserved, giving access to the enormous supply of oocytes stored in ovarian preantral follicles. Much research is required before we fully understand how to activate these very immature oocytes in order to make them mature, fertilise and develop to embryos *in vitro*.

Somatic cell cloning is still largely experimental and extremely ineffective. Problems seen with *in vitro* produced embryos are reported to a much larger extent after cloning, making it impossible to implement the method for animal welfare reasons. Further, the offspring produced is only a nuclear copy of the donor animal, with the genetic information from mitochondrial DNA missing.

Embryonic stem cell differentiation into oocytes is the latest way to produce high numbers of female gametes *in vitro*. So far, this has only been accomplished in mice.

Cryopreservation of Avian Spermatozoa for Conservation

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Domestic fowl were the first species to have progeny produced from frozen spermatozoa - over 50 years ago. Since then, there has been little routine application of sperm-freezing technology in commercial poultry breeding or in the cryo-conservation of non-domestic avian species. The reason has been the relatively poor fertilizing ability of frozen/thawed chicken – and especially turkey – spermatozoa. This is also (probably) true for non-domestic species, with the added complication of the necessity to develop species-specific systems for bird husbandry, semen collection, artificial insemination and sperm cryopreservation.

It is likely that the main impediment for successful use of cryopreserved spermatozoa is the avian fertility system rather than a poor post-thawing sperm survival rate. For example, although frozen/thawed chicken spermatozoa have around 50% survival rate as judged by standard motility/morphological analysis, quantitative studies have shown that, even at optimal efficiency, the fertilizing ability of inseminated, cryopreserved chicken spermatozoa is less than 2% of that of unfrozen spermatozoa. The reason for this poor outcome may be the fact that in birds spermatozoa are stored within oviducal sperm storage tubules for days before fertilization.

Despite this, several fertile eggs can be obtained from chicken hens inseminated with frozen/thawed spermatozoa, provided that the basal fertility of the strain of birds used is highly efficient and that large numbers of spermatozoa can be inseminated in single, or a series of, inseminations. This has been achieved with a range of techniques and cryoprotectants, including slow freezing (1 to 7 °C per min), mainly using glycerol and dimethylsulphoxide, and fast-freezing by pelleting directly into liquid nitrogen, mainly using dimethylacetamide.

Progeny have also been produced from frozen duck and goose sperm and, on a few occasions with very limited efficiency, from turkey spermatozoa. Although, as stated above, the technology is not used in commercial poultry breeding, in the week of writing this abstract, the author has received two requests from companies interested in both applying and developing poultry sperm-freezing technology. Whilst there is a recognised need for sperm cryopreservation for conserving poultry genetic resources and an intention to set up 'sperm banks' in Europe and the USA, this has yet to become a reality.

Non-domestic species that have been bred from frozen spermatozoa include budgerigar, cranes, pheasants, bustards, falcons and eagles, each species having its own system for sperm collection, sperm freezing and insemination. Semen can be collected following by manipulation - by massage or electroejaculation - or as a 'voluntary' sample - caught by interruption of mating with a live or dummy female, or produced by an imprinted bird in response to the handler. Insemination can also require manipulation - to expose the vaginal opening - or can be performed on imprinted birds which will 'stand' for the handler. In terms of cryopreservation systems, pelleting of samples with dimethylacetamide works for pheasant and houbara bustard sperm but not for eagle or falcon sperm, which require slow freezing rates. Sperm from most other species have been frozen by slow freezing in the presence of glycerol or dimethylsulphoxide.

The technologies for non-domestic birds have been developed and applied in conservation centres, such as Patuxent Wildlife Centre (cranes - USA), Centro de Estudios de Rapaces Ibéricas (peregrine falcon - Spain), International Foundation for the Conservation and Development of Wildlife (houbara - Morocco); or Muséum National d'Histoire Naturelle (pheasants - France). These centres have the backing of government programmes for conservation of particular species, of international organisations, such as the International Crane Foundation or World Pheasant Association - or, as for houbara, of funders interested in maintaining numbers of game birds.

Most of the work with these species has demonstrated that the technology is possible and might be, rather than has been, incorporated into breeding programmes. Although the French researchers state in their 2003 publication title that their work is 'the first step towards a cryobank for endangered avian species', it is not known when this is likely to become a reality. Private bird breeders are also interested in sperm cryopreservation for reasons that are part-hobby and part-commercial - for the purposes of breeding raptor species for falconry and pigeons for racing or showing. These are individuals who have a valuable contribution to make to the development of avian sperm cryopreservation technology, since they are able to be very attentive to a small numbers of birds and consequently achieve good results in terms of semen collection and successful insemination. A Belgian organisation advertises, on a commercial basis, pigeon semen collection, sperm freezing, storage and insemination of customer and donor bird semen, but details of their procedures and guarantees have not been forthcoming.

References provided on request to G.J.Wishart@abertay.ac.uk

Sperm Collection and Preservation in Carnivores

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Many species of carnivores are today endangered due to hunting and loss of habitats. Modern Zoos serve as genetic resources for threatened species. Captive breeding may, however, be problematic in some species due to partner preference and insufficient knowledge about reproductive physiology and behaviour. Because of the limited space available in Zoos it is not possible to keep self-sustaining captive populations of all species that are threatened in nature (Blomqvist et al.) Therefore there is an increasing interest in the possibility to use artificial reproductive techniques for conservation of threatened carnivore species. Gametes or embryos from genetically valuable individuals can be stored frozen as an insurance against loss of genetic variation. These gene banks are often referred to as “the Frozen Zoo” (Leibo & Songsasen 2002). To succeed with cryopreservation of gametes and artificial insemination, it is necessary to collect as much information as possible about each species basic reproductive physiology. Domestic species may serve as models for threatened wild relatives. While there has been more success in advanced biotechnologies in the domestic cat than in the domestic dog, methods for routine semen conservation and artificial insemination are not as well developed in cats as in dogs. In the domestic dog, sperm conservation and artificial insemination are today routine methods with pregnancy results similar to natural matings after non-surgical intrauterine insemination with frozen-thawed semen (Linde-Forsberg et al. 1999). Despite that artificial insemination and semen collection in cats have been reported for at least 30 years (Leibo & Songsasen 2002) and there has been success with advanced methods like IVF, ICSI and cloning (Farstad 2000, Shin et al. 2002) practical clinical application of artificial insemination is more on the experimental stage in cats. In contrast offspring have been generated after artificial insemination in more species of wild felids than wild canids. In felids births have been reported after artificial insemination in the ocelot, leopard cat, cheetah, snow leopard, clouded leopard and tiger and in the canids, in the wolf and red wolf (Farstad 2000, Goodrowe et al. 2000). From the domestic dog and farmed foxes, semen is usually easily collected by digital manipulation while electroejaculation is used for wild canids (Goodrowe et al. 1998). Semen can be collected by the use of an artificial vagina from some male domestic cats. Since this method cannot be applied on all males and since a period of training is required, electroejaculation under anaesthesia is a more reliable method for semen collection from cats and obviously the method of choice for wild felids. Collection of spermatozoa from the epididymis offers a possibility to collect genetic material from an animal even after its death. Motile spermatozoa have been retrieved from canine epididymides stored for as long as 8 days at 4°C (Yu & Leibo 2002). Epididymal cat spermatozoa offers an excellent material for research because of its availability due to routine castration of domestic cats (Axné et al. 2004, in press). Semen can be used fresh for insemination immediately after collection, chilled for short-term storage or frozen for long-term storage. Lowering the temperature slows down cell metabolism and therefore prolongs the sperm cells life span. Freezing spermatozoa and keeping them in liquid nitrogen at -196° interrupts cell metabolism allowing almost indefinite storage. Chilling can, however, harm spermatozoa by inducing alterations in the sperm membrane. Freezing induces further damages to the sperm cells. Therefore fertility of frozen-thawed spermatozoa is lower than that of fresh. Research to improve protocols for semen chilling and cryopreservation aims at reducing these damages (Watson 2000). The optimal composition of chilling or freezing extenders and the optimal rates of cooling and freezing differ between species due to for example differences in the composition of the sperm cell membranes. Differences in freezing sensitivity of spermatozoa between canine species have been observed (Farstad 2000, Leibo & Songsasen 2002). More research to improve protocols for semen conservation and artificial insemination would increase the efficacy of reproductive techniques in conservation biology and make “the Frozen Zoo” an important resource for future biodiversity.

Axné E, Hermansson U, Linde-Forsberg C. *Anim Reprod Sci* 2004, in press.

Blomqvist L, McKeown S, Lewis JCM, Richardson D. EEP Felid regional collection plan & veterinary guidelines 1st ed. European Association of Zoos and Aquaria.

Farstad W. *Anim Reprod Sci* 2000, 60-61, 375-387.

Goodrowe KL, Hay MA, Platz, CC, Behrns SK, Jones MH, Waddell WT. *Anim Reprod Sci* 1998, 53, 299-308.

Goodrowe, KL, Walker SL, Ryckman DP, Mastomonaco GF, Hay MA, Bateman HL, Waddell WT. *Anim Reprod Sci* 2000, 60-61, 389-403.

Leibo SP, Songsasen N. *Theriogenology* 2002, 57, 303-326.

Linde-Forsberg, Ström Holst B, Govette G. *Theriogenology* 1999, 52, 11-23.

Rota A, Ström B, Linde-Forsberg C Rodriguez-Martinez H. *Theriogenology* 1997, 47, 1093-1101.

Shin T, Kraemer D, Pryor J et al. *Nature* 2002;415(6874):859.

Yu I, Leibo SP. *Theriogenology* 2002, 57, 1179-1190.

Watson PF. *Anim Reprod Sci* 2000, 60-61, 481-492.

Conservation of Genetic Resources – Farm Animals

Birgitta Danell

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The Convention on Biodiversity calls for plans of action for the conservation and sustainable utilisation of the existing biodiversity, be it wild or domestic, to be developed by each country. In this process the national priorities for domestic animals should recognise the framework prepared by FAO in the proposal for a Global Programme for the management of Animal Genetic Resources (AnGR). The elements in the FAO-framework (listed below) requires activities in many different areas. The emphasis on each activity can vary greatly and has to be chosen by each country in accordance with priorities following from the national policies.

1. The identification and characterisation of existing breeds in the country
2. The identification of present areas for use of animals and the likely development of existing and new areas
3. Utilising and developing AnGR in commercially viable livestock production
 - a. Access and development, breeding programmes, dissemination tools, AI, recording, etc.
4. Monitoring of AnGR
5. Conservation of AnGR of low immediate economic interest
 - a. In-situ conservation, stimulating the development of old and new areas for active use
 - b. Ex-situ conservation – cryo preservation of semen, embryos etc
6. Information and education, human resource development

This seminar addresses the use of reproductive techniques (RT) in conservation programmes (elements 3, 5 and 6). Artificial insemination (AI) and embryo transfer (ET) are widely used today, but these and coming methods are of relevance both in a conventional breeding programme with the purpose of developing the breed and in a programme for preserving breeds at risk. Cryopreservation including collection and storage of genetic material for risk management (security store), for long term storage as well as for support of the daily operations is a valid activity for all types of breeds.

Reproductive technique	Breeding programme		Commercial use	Long term storage, risk management, security stores, gene banks	Trading and transfer of AnGR to replace live animal trade
	Efficiency of selection, mating designs and testing	Controlling the development of inbreeding	Dissemination of genetic progress, selection of AnGR for optimal use		
AI (artificial insemination)	++	++	+++	+++	
ET(embryo transfer)	++		++	+++	+++
OPU (ovum pickup)				+	
IVP (OPU + in vitro fertilisation)	++		++	+	+
Cloning			+	+	+
Sexing of semen	+		+++		

The importance of each technique (when functional) depends on the design and of the programme and on how well the technique is being used to serve the objectives. A very approximate indication is given in the table with 0, 1, 2 or 3 +. All techniques can improve the efficiency of the selection for genetic progress, but some of the techniques are much more important for the access and dissemination of AnGR. A programme for preservation of breeds at risk with no emphasis on selection at all may operate with a live population as one element and a gene bank of frozen semen, embryos etc as another activity. For the management of the live population the access to AI and stored semen is of great advantage, although not indispensable. For a gene bank to get established the relevant techniques must be in place, a proper sampling of genetic material must be done from the right and many enough animals and the legal issues of ownership and use rights of the stored genetic material must have been solved. Establishing and operating a gene bank is also associated with costs.

Cloning is nowadays by several scientists presented as a technique, which will be operational within reasonable time. The collection and cryo preservation of tissue is therefore again advocated. The advantage is a cheaper and simpler technique for sampling, but the outcome cannot yet be guaranteed. Different methods for saving AnGR from infertile

animals have been proposed as well as reconstruction of already extinct breeds by cloning. The genetic impact on the quality of the conserved AnGR from such activities is most likely small.

Cryoconserved Semen in Cattle Genetic Research I

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Semen samples from cattle have been collected and cryoconserved (frozen in liquid nitrogen) by Svensk Avel (Swedish major cattle breed organization) since the 1960's. At present 150 AI-doses from each of the bulls that enter the progeny-testing scheme are cryoconserved for the future. The extensive use of AI in dairy cattle breeding has created a population with large half-sib families and the many daughters of a bull are geographically spread, both within and over countries. This has enabled accurate genetic evaluations of AI-bulls since there are large amount of data collected from all their daughters. In addition, this extensive material creates great opportunities for genetic research such as gene mapping studies.

Most reproduction- and health traits in cattle are quantitative by nature, i.e. regulated by a large number of genes and to a great extent by environmental factors. The underlying genes or chromosome segments affecting such traits are called quantitative trait loci (QTL). Although the effect of each of these genes on the genetic variance is low, QTL significantly affecting a number of traits have been identified in several cattle populations. By using traditional methods of selection it has been difficult to improve traits like mastitis resistance and female fertility due to their low heritabilities. Traits with low heritability are difficult to measure properly due to the large environmental influence and consequently require an extensive recording to enable an accurate genetic evaluation. In addition, some traits are only expressed by one sex or can only be recorded after slaughter, which limits the rate of genetic progress. If we can identify QTL responsible for a significant proportion of the genetic variation in these low heritability traits, or detect closely linked markers that are coinherited with the QTL, the genetic progress could be enhanced by using marker-assisted selection.

The principle in QTL mapping is to search for associations between genetic variation at marker loci and the phenotypic variation for a trait. A significant difference in phenotypes among groups of offspring that inherited alternate marker alleles from their common parent indicates linkage between the markers and a QTL affecting the trait. The most commonly used design in QTL studies in dairy cattle is the grand-daughter design (GDD). In the GDD the marker genotype is determined on sons of the grandsires and the quantitative trait value is measured on daughters of the sons (granddaughters). The GDD is suitable to use in commercial dairy cattle populations where large half-sib families exist due to the breeding structure. The DNA necessary for genotyping of genetic markers is available through the extensive collection and cryoconservation of semen samples from all AI-bulls in the population.

Today, QTL analysis is a technique that is used worldwide to identify the genes behind traits of economic importance to livestock production, and cryoconservation of semen is a prerequisite for these studies.

Cryoconserved Semen in Cattle Genetic Research II

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Animal breeding is a field that has developed rapidly in recent years, partly thanks to the progress that has been achieved in molecular genetics. Traditional breeding programmes have been supplemented with information on major genes and QTL, which contribute to increase the accuracy in the breeding evaluation. Much of the knowledge about genes lies ahead of us but sometimes we need to go back in history to find information, e.g. genotype data on ancestors of a particular animal. One example when this information is useful is when you try to trace back a mutation to the founder animal. This is of importance when estimating the possible distribution of the mutation in different populations or breed. Another example is when you want to analyze if a phenotypic trend coincides with an increase/decrease in frequency of a particular candidate gene variant. Going back in history generally means that we need to retrieve DNA from animals that have been dead for several years. This is where frozen semen can be of use.

Tracing the origin of a mutation

Fishy off-flavour in milk is a recently observed quality defect characterized by a taste and smell of rotting fish. Milk from a few affected cows in a herd is sufficient to impart a fishy off-flavour to the whole bulk milk. We have identified the gene, *FMO3*, behind the off-flavour. This is one of the very few identified genes influencing a production trait of economic importance. We have now developed a DNA based test whereby this genetic defect can be eliminated in carrier breeds by genotyping of breeding animals.

We only found the mutation in the Swedish Red and White breed (SRB), but at a relatively high frequency, 0.155. Pedigrees of affected cows indicate that the mutation may exist in cattle populations of Ayrshire origin also in other countries, e.g. in Finland. The major dairy breeding company in Sweden, Svensk Avel, is now genotyping all potential breeding bulls as calves, before purchase. Moreover, they have genotyped many of their bulls that were used 30 years back or more. One reason is to try to trace the origin of the mutation, another reason is to provide the dairy farmers with information on matings that may result in affected offspring. This task is feasible due to the routine of storing frozen semen from all bulls that have entered the progeny testing procedure.

Phenotypic trends that coincide with changes in gene frequencies of candidate genes

During the last decades, a continuous decrease in cheese yield from milk has been observed by the dairy industry in both Sweden and Finland. This seems to coincide in time with an increase of the genetic variant *E* of the milk protein κ -casein, as a recent Finnish study reported an unexpectedly high frequency of the *E*-variant (0.307) in the Finnish Ayrshire breed (FAy). This variant has been traced back to a single FAy bull that was very popular in both Finland and Sweden, but the gene exists also in other breeds, e.g. Holstein. The *E*-variant has in several studies been associated with poor milk coagulation properties, why means to control the occurrence of this allele in dairy cattle breeds are likely to be of interest to the dairy industry. Because of the large influx of genetic material from FAy to SRB during the last decades, it was considered urgent to get an estimate of the current distribution of this allele among SRB breeding bulls. DNA was extracted from sperm from altogether 300 proven and unproven bulls whereby the bulls were genotyped for the κ -casein locus. The observed frequency of the *E* variant of κ -casein was 0.220. In an international perspective, the frequency of the *E*-variant in the sample of SRB breeding bulls is high. However, we do not know for sure that the high frequency was not there already decades ago. In order to establish if there has been an increase of the *E*-variant during the period of decreasing cheese yield, we would need to genotype the breeding bulls that were used 30 years ago. Hereby we would at least get a rough estimate of the *E*-variant at that time. We are currently investigating the coagulation properties of κ -casein *E*, relative to the A and B variants of the protein. Although retrieving genotype information from the old bulls is not a prerequisite for the success of the project, it would nevertheless be interesting to know the “historic” frequency of the κ -casein *E* variant.