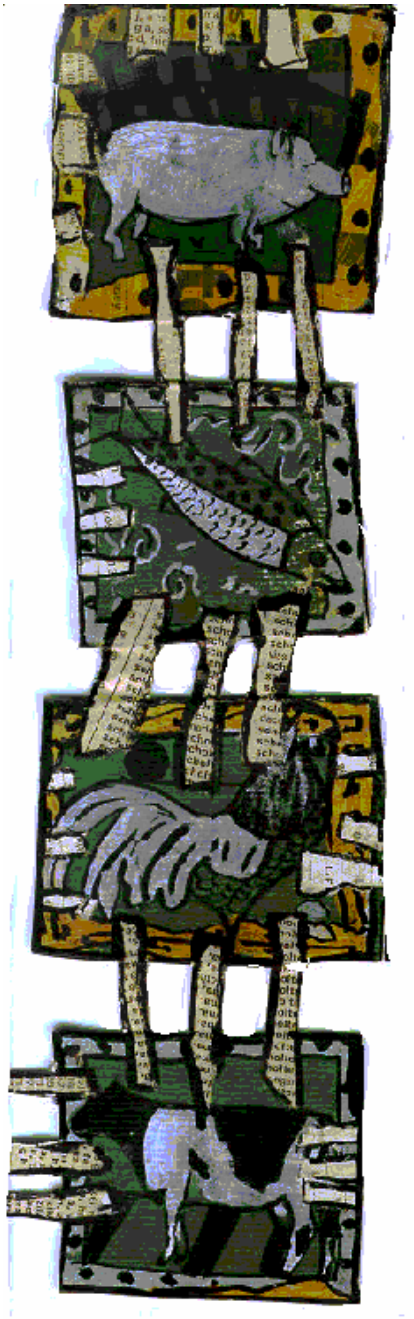
Farm Animal Industrial Platform *(FAIP)*



Reproduction and Selection Technologies

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Reproduction and selection technologies

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ARTIFICIAL INSEMINATION (AI) AND SEMEN TECHNOLOGIES

Introduction

Artificial insemination (AI) has been used for fifty years, in different species and breeds, including humans. In 1780, Lazzaro Spallanzani, an Italian physiologist, was the first who used this technique to obtain pups from his dog. Nowadays AI is the most important reproduction biotechnology for the genetic improvement in domestic animals. A few selected males produce enough spermatozoa to inseminate thousands of females per year. Methods have been developed for semen collection, semen freezing and insemination in different species (cattle, sheep, goat, swine, poultry, rabbits, horses, dogs, cats, and a variety of laboratory animals). Only healthy and selected males are used; therefore males are always tested for disease and typed for identification before using them. The major advantages of AI are 1) genetic improvement: excellent animals can be used more often and more efficiently, 2) control of diseases: a) instead of males, their semen is transported, b) extermination of venereal diseases, 3) availability of accurate breeding records (Salisbury, 1978; Foote, 1980; Colenbrander et al., 1993; Perret, 1997; Cunningham, 1999).

Description of the technique:

Collection of semen and preparation

For the collection of semen in mammals an artificial vagina is used. Amantea first applied this in dogs (1914). The artificial vaginas differ according to the species. The volume of sperm produced varies according to species, age, season, behaviour and libido. A bull produces 5-15 ml, a ram 0.8-1.2 ml, a boar 150-300 ml, a cock 0,2-0,5 ml and a turkey 0,25-0,4 ml (Foote, 1980; Hartigan, 1995; Surai & Wishart, 1996). Sperm is collected at body temperature. After collection it must be kept in a water bath at 37°C, in order to maintain its initial quality and fertilising ability. After the collection semen gets evaluated for quality (volume, motility, and concentration). Rapid and effective evaluation of the collected samples is necessary in order to preserve semen quality. Ejaculated sperm does not survive for long periods, unless various agents (extenders) are added. Extenders provide nutrients as a source of energy; they protect sperm cells during rapid cooling and freezing; and they increase the volume of the semen so that it can be used for multiple inseminations. Usually, extenders contain egg yolk, heated milk or a combination of the two as basic ingredients. Semen is diluted at specified rates so that the volume of frozen or fresh semen used in insemination will contain sufficient spermatozoa to give high probability of conception. The technique has proved to be successful for cattle: from an ejaculate of a bull it is

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possible to obtain 300 frozen doses containing on average 10.000.000 spermatozoa. A ram produces 15 doses and a boar produces 10 doses per ejaculate. Semen collection in poultry takes place by massaging a cock on belly and back. The semen is collected in an ampoule with an extender at 15°C. Diluted sperm, preserved at 5 to 7°C, can be used until 48 hours after collection. A cock produces 7 to 20 doses per ejaculate (van 't Hoog, 1997). Semen collection in fish farming takes place in massaging flanks of males. The volume of semen collected varies from 0,1 ml /kg in turbot and silurid species (oligospermic) to few ml/kg in salmonids, sea bass, sea bream and cyprinids to 10 to 20 ml/kg in sturgeons. Sperm can be stored at low temperature (0 to 4°C) between a few hours to a few days depending on species (Billard, 1992). In some species (catfishes, sex reversed males of salmonids, oysters), sperm needs to be extracted from the testicles by dissection in specific extenders after slaughtering of the males.

Freezing of Semen

Sperm can be frozen in all the species. The use of frozen sperm is limited to conservatory purposes and preservation of genetic resources (Maisse, 1996). At the beginning, mainly fresh or cooled (5°C) semen was used in AI. After Polge and co-workers (1950) discovered the protective effect of glycerol during freezing, the technique evolved to the use of the frozen semen. This technique is used in cattle and sheep. Before freezing, a mixture of semen and extenders are kept for almost six hours at 5°C. This enables sperm cells to spread uniformly into the extenders. Then semen is packed into small “plastic” containers (straws or pellets). Straws contain 0,25 to 0,5 ml of semen extended for bulls and 0,5 ml for ram. Pelleted semen is prepared by including some drops of diluted semen (0,1 ml) in a hemispherical structure made in a block of dry ice. Straws are first cooled to 5°C. Then, they are brought down at a controlled rate to -110°C before storage in liquid nitrogen at –196°C. Pellets are cooled quickly to a temperature of –79°C, and then they are transferred into containers immersed in liquid nitrogen at –196°C. Before semen is used, the samples (straw or pellets) must be thawed rapidly by placing them in a warm water bath (30-37°C) for e.g. two minutes. Then they must be used rapidly, because spermatozoa cannot survive for a long time after thawing. Each straws or pellets are marked with the indication of the animal indentification number and the date of processing.

Insemination or Fecondation

The semen is brought into the female reproductive tract (cervix or uterus) by a so-called sterile pistolet or catheter. Insemination must take place at the right place and at the best moment in order to enable spermatozoa to meet an ovum. In cattle, rectovaginal insemination is used. A gloved hand is inserted into the rectum of the female and used to locate the cervix. The insemination tube is brought into the vagina and carefully guided into the cervix by means of the gloved hand in the rectum. The technique requires practice and experience. Other techniques are used according to the species. A hen is inseminated in the cloaca by using an insemination pipette. Fertilisation of the eggs from aquaculture species is practised in specific extenders to allow longer duration of sperm motility in order to maximise success of fertilisation in fresh water fish or in sea water for marine fish and molluscs. 3 ml of semen can fertilise 1 litre of eggs (10.000 eggs in salmonids to 1 million eggs in marine species).

Detection of oestrus

Accurate timing of insemination is essential for achieving the best conception rate. Usually, the best way to detect the heat is when the females start to mount either male or females. A farmer must control a herd every day, or twice a day (morning and evening). There are many aids for detecting oestrus, such us using vasectomized sires with coloured crayons held on the sternum by an harness, so all of females colour-marked on the rump should be noted during each control. Odours can also be detected by trained dogs or by a 'snuffle boar'. For cows, pedometers placed on the legs can be used, because cows, while on oestrus, are more active than usual (Foote, 1980; Secchiari et al., 1998). Hens get inseminated every week in order to be sure of fertilised eggs.

Heat induction

This technique is usually addressed in extensive farming where heat detection is difficult or to synchronise deliveries in particular period of the year (e.g.: sheep and rabbit). In these species heat induction became really important due to its several advantages: 1) programming and concentrating parities taking a look to the market demand; 2) anticipating the first birth, and reducing distance between parities; 3) the chance to use better AI in those species with a seasonal breeding and 4) to increase litter size and improve fertility. To induce oestrus in females particular hormones are used. Administration techniques and hormones vary according to specie and phase of oestrus cycle.

Differences

In pigs AI has had a slower start than in cattle, but now it is rapidly increasing. In some countries more than 50% of breeding females are artificially inseminated (Wilmut et al., 1992; Visscher et al, 1998). The introduction of AI in pigs is changing the marketing strategy of breeding companies, from selling live boars to selling semen. The problem in pigs is the short effective life of AI sperms in the sow. Success with AI is highly dependent on good detection of oestrus and accurate timing of insemination. Optimum fertility is obtained when semen is used within 24 hours of collection. With modern long-life diluents semen doses can be stored at 16-18 °C for 2-5 days prior to use. However, over this period a 5-10% drop farrowing rate and a decrease in litter size of up to one pig can be expected (Colenbrander et al., 1993; Meredith, 1995).

Artificial insemination in sheep is not routinely used. Unfortunately, the anatomical structure of the cervix prevents intrauterine deposition of semen by insemination syringe (Cappai et al., 1998). Consequently, cervical artificial insemination with frozen/thawed-semen results in low fertility (Maxwell and Watson, 1996), due to impaired sperm transport through the cervix and the short survival of frozen-thawed spermatozoa in the female reproductive tract (Lighfoot and Salamon, 1970). From a technical point of view most artificial inseminations in sheep selection schemes are realised with fresh semen after oestrus synchronisation by cervical insemination. A common use of frozen semen by cervical insemination does not seem currently possible because of low conception rates. Therefore frozen semen is used only in a few cases for particular mating by intrauterine techniques (Sanna et al., 1994; Maxwell & Watson, 1996).

Artificial insemination in fish is different if compared with others farm animals. Females are manipulated with a little pressure on their belly. Mature oocytes are collected in a proper recipient. Males are massaged directly with their fin back, and spermatozoa collected in the same recipient of the oocytes are. This is a dry method because the gametes are mixed together without adding water: sometimes diluents can be added (Billard, 1992). Oocytes are let to rest for 10-15 minutes and all unfertilised oocytes are removed. Fertilised oocytes are put in an incubator. One hundred per cent of the salmonid, catfish and turbot production uses AI. In other marine fish and molluscs species, in which fertilisation is occuring naturally in the water by mass spawning after releasing of the gametes by the males and the females.
In poultry and more particularly turkeys, AI is used routinely by the breeding companies. In turkeys 100 % AI is applied.

Sperm sexing

The procedures is based on the identification of the minimal difference in DNA contents (about 3%) between X and Y spermatozoa using a flow cytometer (Pinkel et al., 1982; Cran, 1992) In mammals, the larger X-chromosome carries more DNA than the Y-chromosome and percentage differences are reported to be 3.0, 3.6, 3.8, 3.9, 4.1, and 4.2 for rabbit, swine, cattle, dog, horse and sheep respectively (Johnson, 1992). The potential application of sperm determination in farm animal can be seen in a reduction of costs, (e.g. to obtain heifer calves for dairy replacements or bulls for beef production) and therefore elimination of supernumerary male embryos in particular in export programmes and use of fewer female recipients (see embryo transplantation), all this will bring a big economic advantage.

Monosexing and Triploidism in Fish farming

In fish farming, males often have the disadvantage to mature before achieving the commercial size desired by the consumer (trout, charrs, carp, bass, bream, turbot, halibut). This maturation decreases the level of lipid in the meat, decreases growth and increases weakness. The opposite occurs in some other species such as tilapia and catfishes. In 99% of the animals (invertebrates, batracians, reptils, fishes), the phenotypic sex of the males or the females can be managed by external factors (temperature, density, social relation, hormones), particularly in hermaphrodites species that change sex naturally during their life or present simultaneously the two sexes (sea bream, groupers, oysters, scallops). This is used to manage the sex in order to produce monosex fry, in most of the case female, without any hormonal treatment of the animals eaten by the consumer. Such type of treatment is recognised by the EU regulation Directive 96/22/CE of 29 April 1996). Most of the European and American trout production, the Korean and Japanese hiramé productions are based on this technique. Field trials are in progress in Atlantic salmon, in common carp in Israel and in other marine species.

Almost ninety nine per cent of the animals (crustaceans, batracians, reptiles, molluscs) and 100% of the plants consumed by humans (weat, rice, banana, onion, sugar, tea, paprika, lemon, coconut, coffee, potato, etc.), except for mammals and birds, polyploids are observed in wild populations of fish and molluscs. Triploids present gonad or gametic sterility depending on their sex. In the 80's, research has

proposed standardised processes to induce triplody by thermal or hydrostatic treatments practised few minutes after the fertilisation (Chevassus, 1987). This type of genotypes are proposed by scientists to prevent contamination of wild populations by escaped population from farms, an original situation if compared to other domesticated animals for which wild ancestors have disappeared. Triploidisation, by preventing the maturation of the females avoids the decrease of meat quality (colour, lipid content) and allows diversification of the aquaculture production with fish of bigger sizes for smoking, filleting and processing that could not be achieved if sexual maturation occurred or in oysters without maturing gonads. Today 10.000 mt of triploids of rainbow trout are farmed in Europe. 80 % of the US oyster reproduction is triploïds and the first mt are tested in Europe. Ecological and quality reasons pushed the generalisation of this technique in the next future for the interest of the society.

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EMBRYO TRANSPLANTATION (ET)

Introduction

Embryo transplantation (ET) has been used during the last twenty years in different livestock species such as horses, cattle, sheep, goats and pigs. The main benefit of ET is the increase in number of offspring per female, thus increasing the reproductive rate of females and their contribution to the breeding programme. Next to this, the breeding efficiency within the herd is improved. For ruminant breeders the economic benefit from the sale of animals with high genetic value is an important advantage. Further, for all species, ET permits an easier and more rapid exchange of high genetic material between countries, and reduces the cost of international transport by flying embryos rather than shipping live animals. ET can limit the transport of live animals between farms, thus decreasing the risk of infectious diseases. Especially in pig production this offers possibilities for the safe exchange of genetic material down the breeding pyramid. The technique can also be used for the storage and the expansion of a rare genetic stock. ET is fundamental for all new biotechnologies used today in animal breeding. A disadvantage of ET is the high cost of the technique, varying from one country to another, but are always high. Therefore this technique is usually used for breeding animals (Ruane, 1988; Woolliams & Wilmut, 1989; Polge, 1995; Nicholas, 1996; Wilmut, 1996; Cunningham, 1999).

Description of the technique

The technique consists in the recovery of embryos, usually from an elite female (donor) and to transfer them to recipient females. Embryos can be collected non-surgically in horses, cattle, and sometimes sheep and goats. In pigs, the non-surgical collection of embryos is developing rapidly and close to its routine utilisation. The recovery of embryos includes the induction of multiple ovulation by hormonal treatment. This increases the ovulation rate so that simultaneous collection of a large number of embryos per female compared to natural reproduction is possible. The induction of superovulation is almost similar in all the species where ET is used, with the exception of mares. After ovulation, the animal gets inseminated, usually by artificial insemination. A few days later - e.g. 7 days in cattle and sheep, fertilised eggs are collected from the uterus with a sterile catheter. Before transferring embryos to a recipient female, they are evaluated at the microscope according to morphological criteria and only selected embryos can be implanted. Recipient females must be on heat at the same time of the donor females. To synchronise donor and recipient, the procedure used is similar to the one described in the artificial insemination session. In cattle and horses a non-surgical method nowadays is a routine method like the AI method. In goat, embryos are transferred by laparoscopy, but the cervical canal is large enough to permit non-surgical transfer. In sheep, embryos usually are transferred by mid-ventral laparoscopy although in the last years there was an increasing use of laparoscopy. In pigs a surgical method was mainly used until recently; non-surgical embryotransfer in pigs is developing quickly. The main practical problem of ET is the enormous variability of the superovulatory response. The cause of this variability depends, among others, on the specific physiological effects of the hormone treatment. In cows, for example, on average two or three calves born from each recovery. However, the success can vary from zero to more than ten calves.

The technique has a few fundamental stages: selection of donors and recipients, synchronisation of donor and recipient females, superovulation and insemination of donors, collection of embryos, their evaluation and transplantion in recipient females.

Freezing of embryos

After collection, the embryos are packed in straws. These straws are immersed into a freezing medium, containing a cryoprotectant for 5 to 10 minutes. Then the straws are transferred into an alcohol bath at –6°C, and after a few minutes of adaptation, cooling passes slowly to a temperature of –32°C. The straws are then transferred directly to liquid nitrogen and stored in a container at the temperature of –196°C. When frozen embryos have to be used, straws are carried out to a temperature of 20-30°C. The cryoprotectant has to be removed. Then the embryos are placed into a normal medium and transferred. Freezing of fish eggs is still impossible even if a short decrease at -4°C give live fry (Lubzens et al., 1996). Freezing of blastomeres of rainbow trout is possible (Leveroni & Maisse, 1998) but their reimplementation still needs experiments. As in other species, cryopreservation of blastomeres or embryos could open new applications for establishing gene banks in the context of aquaculture or ichthyodiversity preservation.

IN VITRO EMBRYO PRODUCTION (IVEP)

Introduction

As mentioned before, the ET success rate is highly variable. An alternative to embryo production *in vivo* is to use the ovaries of females to collect oocytes (ovum pick up-OPU). This technique involves three steps: collection and maturation of the oocyte (in *vitro* maturation-IVM), fertilisation (in *vitro* fertilisation-IVF) and culture of the resulting embryo to a stage at which it can be transferred into the uterine horn of a recipient female. Females after puberty can be potential donors of oocytes; donors in the immediate post-partum or in late pregnancy stages are not suitable (Ball et al., 1984; Naitana et al., 1992; Wilmut et al., 1992). IVEP was first developed for laboratory, domestic, primate, avian, and aquatic species. Nowadays, it is a technique used on farm animal species such as cattle, pigs, sheep, goats, rabbits, and equine. In the last decade, IVEP has been used in human too. The first success was on rabbits by Chang (1959). One of the advantages of this technique is the increased possibility of selection through the female line. The commercial application of IVEP depends on the type of embryos produced. In cows, for example, only 30% of cultured oocytes develops into embryos to be transferred (Cunningham, 1999). The disadvantage of this technique is that it can be done only in laboratory and moreover it is a really costly technique.

Description of the techniques

Oocyte collection and maturation

The immature viable oocytes can be collected from animals immediately after slaughter by dissection of the ovaries or by simple aspiration from live animals (*ex vivo* ovum pick up- Pieterse et al., 1988; Brackett, 1992; Besenfelder et al., 1998). Due to this technology limitations in female reproduction may be overcome. It is possible to have oocytes from females that do not superovulate such as pregnant or sterile females (Leitch et al., 1995; Galli & Lazzari, 1996). This technique can fundamentally increase the number of offspring produced per donor anima without any hormone treatment. In cattle, for example, it is possible to produce up to 150 embryos per year per female. Genetic improvement rate in a population can be increased thanks to the use of young heifers of 6-8 months of age as parents (shorten generation interval).

Immature oocytes are placed under suitable culture conditions for *in vitro* maturation (IVM). The optimum culture environment requires a temperature not below 39°C and a medium supplemented with foetal calf serum and some hormones. After 22-24 hours, the oocytes turn to the mature stage (Naitana et al., 1992; Galli & Lazzari, 1996).

Fertilisation

When the oocytes are mature, they must be fertilised (IVF). In cattle, for example, commercial frozen semen is used for fertilisation. The sperm must be separated from the extenders, e.g. by washing or centrifugation on discontinuous Percoll gradients. The latter ensures a high recovery of motile spermatozoa (Lynham & Harrison, 1998). Then the spermatozoa are diluted in a medium, and prepared in microdrops under paraffin oil. The mature oocytes are introduced in the fertilisation medium for 18-24h at 38.5°C. Following fertilisation embryos can be transferred either into a temporary recipient

female (rabbit or sheep) or cultured *in vitro,* with or without somatic cells, in a particular medium. Embryos developed in a temporary female recipient have a better sensitivity to freezing and thawing; they behave like embryos recovered from superovulated donors; they can be frozen with the same protocol, and moreover a high pregnancy rate will result after thawing and transfer to the recipient female. If frozen-thawed embryos, cultured in vitro, are used, on the contrary, a lower pregnancy rate is achieved (Galli & Lazzari, 1996). Fish and molluscs eggs are incubated at optimal natural temperature for the development of the embryo. Their transport is possible after the development of the neural and axial systems in Salmonids and all the time in species with planctonic eggs. This has allowed transportation of Salmonids from Europe to Chili by boat and across the Andes Cordilier at the end of last century. As for sperm, this way is used to disseminate genetic material. This is particularly interesting as fertilised eggs can be disinfected to prevent disease risks.

EMBRYO SEXING

The possibility to determine the sex of the offspring will have an important influence on the livestock management, productivity and for the genetic improvement of all domestic animals, although more used in cattle. Dairy farmers are more interested in female offspring production. A large number of female offspring guarantees maximum selection through replacement within the herd, resulting in a faster breeding progress. Moreover, sex predetermination reduces the number of recipient female required. After embryos have been recovered from the reproductive tract of a donor female and before they are used for ET procedures, they can be sex determined. The method of embryo sexing is based on the determination of the presence of the Y-chromosome, specific to the male, inside some cells of the embryo. Some cells are removed from the embryo and their DNA analysed to mark a known DNA sequences present in the Y-chromosome (Nibart, 1992; Powell & Wilmut, 1995).

CLONING (embryo splitting, nuclear transfer)

Splitting (cutting) embryos at a really early stage creates identical animals. In order to cut embryos, simple equipment is used, such as a hand-held blade. In this way, it is possible to obtain two embryos from one. Embryos can also be cut into smaller parts using micromanipulation. However, this technique is very expensive and the maximum number of identical individuals obtained is four. The embryo splitting technology is now used commercially to increase the number of lambs or calves born following routine embryo transfer or *in vitro* embryo production. Reports for pigs are really scarce and only few identical twin piglets have been generated after transfer of bisected pig embryos (Niemann & Reichelt, 1993). An alternative method of making clones involves the use of another embryo manipulation procedure called nuclear transfer. The first successful nuclear transfer in vertebrate animals was reported in frogs in 1952. Furthermore, it has also been used in domestic animal such as sheep (Willadsen, 1986) and cattle (Prather, 1987). Nuclei (=DNA) are taken from embryos at an early stage (donor). These are transferred, by injection, into unfertilised ova from which nuclei have been removed (Heyman & Renard, 1996; Wilmut, 1996). This technique has been proved in several livestock species:

cattle, sheep, goats, swine and rabbits. This technology has had limited impact however because the adult performance of an embryo is not known and only limited number of clones can be made from a single embryo. (Wells et all., 1998). Splitting or taking the nucleus from an embryo creates not clones but identical twins. A clone is a set of genetically identical individuals, whereas an embryo carries 50% of genes from his mother and 50% of genes from his father (Visscher et al., 1998; Cunnigham, 1999). Wilmut et al. (1997) proposed a different way of nuclear transplantation, in contrast with embryo cloning, adult cloning (from a somatic cell), that can be done with an animal that has already proven its production potential and, theoretically at least, an almost infinite number of clones can be produced from a single animal.

Description of the technique

Cloning from somatic cells

Wilmut and colleagues took cells from the udder of a 6 years old Finn Dorset sheep and placed them in a culture with a very low concentration of nutrients, so to stop cells dividing. At the same time an unfertilised ovum was taken from a Scottish Black-face sheep. The nucleus (with its DNA) was extracted, leaving an empty ovum cell. So both cells (the nucleus one and the one without nucleus) were placed next to each other and an electric pulse let them fuse into one cell. Then the new cell (fertilised cell) started the division. Six days later, an embryo was implanted in the uterus of another Black-face sheep. After 150 days of gestation, from the Black-face sheep was born a Finn Dorset lamb named Dolly. Dolly was genetically identical to its original donor. Recently, in Italy, a calf was cloned from a leukocyte cell of an adult bull (Galli, 1999, personal communication).

Acceleration of the genetic progress by the diffusion of high genetic values individuals by cell transfer or nuclear transplantation is at research level in fish (Hong & Schartl, 1996). Most of the progress was achieved in model fish like medaka Orezia latipes or zebra fish Brachydanio rerio. ES totipotent cells can be cultivated and stored. Their injection in host embryos or the injection of their nucleus in enucleated eggs is possible. Further research is necessary before this technique can be used by the industry.

Moreover, as in plants, gynogenetic (only from egg) and androgenetic (only from sperm) has been performed in batracians and fish (Rostand, 1934; Purdom, 1969). As in plants, 100% homozygote clones, 100% heterozygote clones and hemiclones, are produced only at a laboratory scale by gyno or androgenesis for more than 10 years. They are particularly interesting in order to identify traits genetically determined by a few genes as quantitative trait loci (QTL – Quillet, 1994; Tanigushi *et al.*, 1994). One of their other possible applications is to develop reference lines (Bongers *et al.*, 1998) to study environmental factors (pollution toxicology), genetically determined diseases (cancer, etc.) in ordere to develop resistant strains or to develop vaccines. The absence of genetic variability of such genotypes could also be used to reduce the variability of the performance in farm animals.

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GENE MAPPING & MARKER ASSISTED SELECTION (MAS)

Introduction

Molecular technologies developed in the last decade have permitted to locate several genes of some interest in different species. The purpose of the gene mapping in livestock is to identify and locate the genes along the genome (pigs, cattle, sheep, goats, poultry), in order to identify genes controlling traits of economic interest. Nowadays almost 100% of the mouse genome is mapped (more than 14000 genetic markers). Mapping in humans actually covers 95% of the genome (more than 15000 genetic markers) (Cunningham, 1998). A complete genome map of domestic species will not be available in the near future. However, because a homology exists between mammals, geneticists can benefit from the knowledge of other well mapped species (mouse and human genome). This kind of approach is called comparative genetics (Archibald, 1998; Cunningham, 1998; Georges, 1998).

Terminology

Individuals are composed of a variety of cells. Inside each cell is the nucleus with a complete number of chromosomes, which differs according to species. Every cell contains two copies of every type of chromosome (chromosome homologous), one coming from the mother and one from the father. A long strand of DNA (deoxyribonucleic acid) which is composed of different sequences of 4 molecules (bases), adenine (A), cytosine (C), guanine (G) thymine (T) make chromosomes. Genes are sequences of bases carrying specific information for the synthesis of a given protein. A gene is a fundamental unit of heredity, which carries the genetic information from parents to their progeny. The genetic differences between individuals come from all the different genes carried in the genome. The genome represents all the genes of an individual. Genes have two or more different forms called alleles, coding for different characteristics (e.g. blue eyes, brown eyes). Each individual carries two alleles, one from the father and one from the mother. The two alleles present at the same locus (the position on the chromosome) can be identical and in this case the individual is called homozygous at that locus. Or they can be different at that locus, then the individual is called heterozygous. In order to better understand the terminology we use an example in humans. If a new born child inherits an allele coding for brown eyes both from the father and the mother he/she will be homozygous - brown eyes. He/she will be able to transmit to his/her progeny only brown eyes. If a baby inherits an allele coding for brown eyes from the mother and an allele coding for blue eyes from the father he/she will be heterozygous and having brown eyes (the brown is dominant over the blue). The progeny will receive the blue allele or the brown one. If the blue allele is inherited and coupled with a blue allele from the other parent the progeny will have blue eyes. It is estimated that each individual (e.g. human, mouse, or cattle) has about a hundred thousand of genes. Unfortunately genes of economic interest, i.e. genes coding for production traits (milk/meat production), product quality, health traits, fertility traits and heredity diseases, represent a group of genes (polygenes) which add their contribute to have a final single effect. For this reason it is really difficult to identify any single gene that gives its single contribution to a particular trait. The genes or loci which code for these kind of quantitative traits are called “quantitative trait loci” (QTL).

Genetic markers

In domestic species the genes mapped do not directly affecting productive characteristics, but they may be linked to genes directly affecting desired characteristics. However, if a given gene is located (same chromosome) close to a gene of economic interest it is very likely that these can be inherited together. The mapped and known gene can be used to “mark” the gene of economic interest. For this reason they are called “genetic markers”. These genes are easy to be identified with a laboratory analysis. Very often, there are more then two types of alleles possible at the same locus. This is called genetic polymorphism. The purpose is to locate as many gene markers as possible along the genome, to verify their association to a trait of economic importance and subsequently use this information for the selection of reproducers carrying the favourable allele. Genetic markers used nowadays for the construction of genetic maps are divided into two classes (O’Brien, 1991). The first one are markers associated with gene sequences observed across mammalian species (type I). The types II are markers highly polymorphic but usually they present anonymous DNA sequences. It means, these markers are distributing throughout the genome and their function is mainly unknown. Moreover they don’t have a direct effect on a trait of economic interest, but it might be possible, located close to a useful functional gene (e.g. milk yield trait). They are inherited together (Montgomery & Crawford, 1997; Cunningham, 1998).

The main advantage of molecular markers is that they can be typed on animals of both sexes at any age, e.g. boars can be measured for litter size markers and moreover carcass quality can be measured on live animals (Visscher & Haley, 1998; Fernando; 1998).

Marked Assisted Selection (MAS)

The first method discorved for the detection of DNA polymorphism is the restriction fragment length polymorphism (RFLPs) published in 1980 by Botstein and colleagues. Among the new DNA markers discovered the variable number of tandem repeats (VNTRs), are most commonly used nowadays. The genome presents several regions with tandemly repeated sequences (e.g. bovine presents 40% repititive sequences). According to the number of base pairs repeated these markers are called mini- or micro-satellites loci: with more than five bases repeated markers are mini-satellites, with 5 or even less base pairs repeated they are microsatellites. The latter appear frequently throughout the genome and moreover they represent the majority of the genetic markers mapped now for all species.
In the 1990’s this “technique” started to be practically reliable thanks to the development of livestock genome maps based on highly polymorphic micro-satellites DNA markers. The idea of making selection by means of the directly genetic information came earlier in the 1970’s and 1980’s (Soller & Beckman, 1983). This process where marker genes, pointing to the presence of desirable genes, are used is called marker assisted selection (MAS-Mcclintock, 1998; Well et all., 1998). The purpose is to combine all genetic information at markers and QTL with the phenotypic information to improve genetic evaluation and selection. The advantage is that the effect of genes on production is directly measured on the genetic make up of the animal and not estimated from the phenotype. Traditional selection methods will not be replaced by molecular genetics but an integration of the two selection methods could be beneficial to the selection response.

Using genetic markers it is possible to identify earlier in the life of the animal which can be considered of high genetic value and sent to normal ttesting procedures. At present studies are at a really early stage, until now researchers know little about which variants of the genes are desirable.

Development of efficient selection schemes in Atlantic salmon and in trout is in progress in Europe. Multitrait selection on family value will be possible for many other species if research will be able to propose to the farmers selection schemes at lower cost of investment and function. Development of less expensive selection programmes could be achieved by the use of fingerprints to set up marked assisted selection. This will need further research in order to optimise their use and to measure genetic parameters. Some traits are determined by a few genes. Programmes of mapping of these genes are initiated under EU financial support on salmonids in the SALMAP programme. Development of other programmes for other species is of interest to determine genes responsible for disease resistance.

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TRANSGENESIS

Introduction

Transgenesis is a technique used for over twenty years, since the first success with mice by Gordon and colleagues (1980), several different transgenics have been inserted into farm animals. Technically gene transfer means the stable incorporation of a gene from another species in such a way that it functions in the receiving species and is passed on from one generation to the next (Cunningham, 1999). The transgenic technique offers several possibilities such as: 1) improve animal production (milk, meat, wool), product quality and disease resistance (reducing cost), 2) produce recombinant proteins in the mammary gland, and moreover 3) create a source of organs to be transferred into human beings (xenotransplantation) (Mcclintock, 1998). In order to produce transgenic animals, several steps are required (see below). For this reason the success rate is not high (1-2%). Therefore the technique is mainly used in mice, sheep and pigs and, very limited in cattle (Cunningham, 1999). Progress has been rather slow mainly because of a lack of basic understanding of gene regulation and gene expression. The research has focused primarily on the alteration of growth and development, but recently has also included the generation of foreign proteins in milk and blood and the increase of disease resistance (Niemann & Reichelt, 1993).

Transgenic technique, nowadays, is less directly applicable, and slower than it was thought at the beginning when it was discovered and proposed. This depends on several reasons: 1) most economically important traits are controlled by multiple genes, which are for a good part unknown today, and therefore hard to be manipulated; 2) the low efficiency of gene transfer in farm animal makes the technology really expensive, therefore preliminary tests are conducted on mice. For this reason results may not be directly applicable to livestock species.

Description of the technique

The technique widely used to produce transgenic livestock is the pronuclear injection. Hammer and colleagues (1985) generated the first transgenic livestock (rabbits, sheep and pigs) by pronuclear injection. The methods used to create transgenic farm animals are based on those developed in mice by Gordon and colleagues (1981), with some changes according to the species (Eyestone, 1998). To produce transgenic embryos, oocytes are first collected from the oviduct of superovulated females (donor) (see embryo transplantation techniques) and cultured *in vitro*. Oocytes are then fertilised with frozen-thawed sire semen and again collected 18-24 hours after insemination. Once the pronucleus is visible, a given quantity of solution containing 200-300 copies of DNA fragment is injected directly into the pronucleus by means of a fine glass needle. Then the early stage embryo is first cultured in a medium for 6-7 days (cattle) and then transferred into the uterus of a recipient female. The pregnant rate and therefore the calving rate are lower than with non-modified embryos (Eyestone, 1994), probably because of a chromosomal damage or a mutation that can occur during the pronuclear injection. The technique is quite simple; the success depends on the operator, the quality of the injection needles, the quality of the DNA, the quality of the oocytes and the ability to visualise the pronuclei. Once the offspring are born, they must be evaluated to see if the transgene exits in their genome. The PCR

procedure is used to check the transgenic integration. This is a process that can duplicate (amplify) any desired DNA sequence. DNA can be obtained either from blood, hair, saliva, semen or milk samples. Later, duplicated segments are separated by electrophoresis; single alleles will appear as single bands and according to the repitition length polymorphism can be detected. The technique basically needs just small amounts of DNA to be analysed (before amplification). Moreover, several individuals can be typed simultaneously.

Examples

Several of the protein products of livestock genes have been shown to dramatically affect the performance of farm animals. The best example is the growth hormone (GH). A wide variety of data have shown that an increase in the growth hormone level in meat-producing animals, increases growth rate and feed efficiency and, in dairy animals, increases milk production (Machlin, 1972, 1973; Niemann & Reichelt, 1993 Pursel, 1998). The effects of exogenously added growth hormone on growth rate have been known for more than 50 years since the original studies of Evans & Simpson (1931) and Lee & Shaffer (1934) who demonstrated that extracts from pituitary glands caused a dramatic increases in the growth rates of experimental animals.

The earlier transgenic animal was created using the growth hormone (GH); the first GH transgenic mice were produced in 1982 (Palmitier et al., 1982). These transgenic animals presented an enhanced growth performance: the growth rate increase 4 times and the final body weight increased twice. Later a number of GH transgenic pigs and sheep were created with human, bovine, rat growth hormone (GH). In pigs with bovine GH (bGH), for example, it was possible to see an increase of a faster growth rates and an increase of feed efficiency, and moreover there was a big reduction of fat carcass (Pursel et al., 1989). Unfortunately transgenic pigs presented a variety of health problems: susceptibility to stress, gastric ulcers, and reduced fertility (Pursel et al., 1989; Pinkert et al., 1994). GH transgenic lambs in some studies did not grow faster or utilise feed more efficiency carrying elevated doses of ovine GH (oGH) or bGH, but they were thinner than usual (Murray et al., 1989; Rexroad et al., 1989). Furthermore the absence of body fat could cause a hyperglycaemia and glycosuria (Rexroad et al., 1989, 1991). Moreover these animals presented anatomical abnormalities (Vernon & Pursel, 1998).

Transgenic fish are produced in experimented situations - since the 90's. Increase of growth reported twice in more than 20 experiments of Devlin et al. (1994) report that transgenic Coho Salmon Onchorhynchus Coho were more than 11 times heavier than non-transgenic controls. The production of such type of genotype is not well controlled and many steeps need to be improved before achieving possible farming of transgenics for human food. Many traits could be improved as growth rate, their ability to consume plants, to decreasing of production cost and capacity to resist to diseases. One of the more important problems to consider is that such type of fish can escape and reproduce eventually with wild animals. Sterilisation by triploïdisation, hybridisation or transgenesis or farming in completely closed systems could solve this problem but the main key factor will be the consumer acceptance.

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