Session 2 Animal growth and evaluation

L 2 BIOTECHNOLOGY IN MEAT PRODUCTION

Fredi Christian Schwägele

Institute for Chemistry und Physics, Federal Centre for Meat Research, E.-C.-Baumann-Str. 20, D-95326 Kulmbach

Key words: Meat production, biotechnology, gene technology, gene diagnostic, reproduction technology

Introduction

Biotechnology is considered as one of the key technologies in the 21st century. With respect to a broad spectrum in different fields of research and development it seems to be a promising discipline for science as well as industry. There are manifold areas of its application like medicine, pharmacology, environment, agriculture and food production. In general **biotechnology is defined as any technical use of biological systems for the benefit of human beings**.

In this sense agriculture and food production are using plants, animals or microorganisms for a long time to improve both their quality and quantity. The topic of this contribution deals with "biotechnology in meat production", especially with recent developments in **genome analysis** or **gene diagnostics, gentechnical methods for DNA-recombination** and **modern reproduction technologies** in particular with pig and cattle as domestic animals. Conclusions will be drawn concerning possible **consequences of biotechnology for animals and consumers** as well as arising **ethical questions**.

Modern biotechnology comprises three main challenges

According to Winnacker, 2000 there exist three main challenges in biotechnology:

1) **The genomic challenge:** The genome is the sum of all genes describing an organism. Genes are the carriers of information of the different components in a cell like proteins, polysaccharides, lipids and other compounds. At the beginning of the last century the term "gene" was created by the Danish botanist Johannsen, who was interested in the correlation between genotype and phenotype of an organism. Up to the present the genetic information of several genomes of prokaryotes and eukaryotes inclusive parts of the human genome are available. Biology has made a dramatical change from a science with only little genetic data to a science with plenty of genetic data. For this reason a new branch, the so called bioinformation science has come into being with the aim to improve the administration of the flood of information.

2) **The economic challenge:** The possibility of commercialisation of genomic research was and will be the essential driving fore of progress for the future. As classical pharmaceutical companies hesitated and showed that they were unable to react on the new genomic challenge scientists themselves took their chance. In the end of the 20th century there existed 1400 new biotech-companies in Europe. The development of genomic research did not take place in the classical pharmaceutical industry, but mainly outside of the traditional domains of financial research support. One of the most outstanding ideas in this area was the human genome project and the foundation of HUGO (Human Genome Organisation) as well as Celera Genomics.

3) **The challenge of genomic projects:** What are the risks of genomic projects? There are two important points to be discussed:

a) The genome analysis and b) the handling of embryonic stem cells.

Genome analysis is a convenient procedure allowing to find out so called positive and negative characteristics of an organism. At first sight such a possibility seems to be utmost beneficially. But with respect to living beings this means that only a few specialists would have the insight and the choice to decide what kind of genes are worth to be conserved or deleted, respectively over expressed or suppressed. In this case man could more and more tend to replace god in his role as creator, although it seems not clear at all, if he would be really successful.

Another very interesting field is the technique of cloning, which is using embryonic stem cells. Four years ago it was for the first time to produce totally identical organisms, so called clones, applying cells of adult organisms. The innovation with respect to the produced sheep "Dolly" was the transplantation of a nucleus originating from an entirely differentiated cell to an embryonic cell, where the nucleus was removed. In this way a nucleus in an already highly specialised state could be reprogrammed into a very early

state of cell cycle allowing the formation of a whole new organism. Cloning per se does not seem to be alarming in this concern, but the fact of open possibilities of directed cell programming according to the intention of some highly skilled specialists.

Analysis and mapping of genomes of domestic animals

Domestication of animals can be considered as the onset of genome analysis with our most common farm animals like cattle, pig, sheep and poultry. At that time man started programmed sustainable breeding to influence the genomes of domestic animals and to improve their properties in various respects. About forty years after the investigation of the molecular structure of the DNA by Watson and Crick (1953) different reliable techniques were developed for the analysis of the genomes of organisms. In the very beginning genome analysis was performed in the direction from the protein and his function to the gene (top down), whereas today the more efficient way from gene to function (bottom-up) is favoured. The DNA-sequencing method of Frederick Sanger (1977) opened the way to decode whole mammalian genomes. In contrast to the sequence data gathered in the human genome project, the analysis and mapping of domestic animal genomes is much less developed.

The analysis of complex genomes can be performed in three steps, by **genetic, cytogenetic** and **physical** mapping (Brenig, 2000):

Genetic maps are based on the transmission of coupled and independent gene loci. If there are two very distant located genes the probability of recombination is much more higher than in the case of two genes of the direct neighbourhood. The distance of such gene loci is measured in centiMorgan (cM), expressing the frequency of possible recombination during meiosis. Highly polymorph repetitive DNA-regions, so called microsatellites are serving as specific markers for this process. But also other polymorph loci can be used. In the frame of the international genome analysis projects of agriculturally used domestic animals rather well developed gene maps are available.

In the case of **cytogenetic** mapping the location of DNA-fragments on a chromosome is determined (chromosomal painting). At a very high resolution mapping can be performed in the range of some kbp (kilo base pairs).

Physical mapping allows a detailed and complete analysis of the whole genome of an organism, describing the nucleotide sequence of the DNA which is the carrier of the genetic information determining the characteristics of an individual in the different specific genes. For this purpose it is necessary to produce defined genome-fragments, which can be amplified in so called gene libraries. In the following step the absolute and relative arrangement of the various fragments is analysed by means of partial sequencing techniques. Repetitive DNA-sequences represent a specific problem, which has to be overcome by heuristic methods. After the first assembling of the existing fragments the whole DNA can be sequenced. The genome analysis is finished after the identification of structural and regulatory genes, coding (exon) and non coding (intron) sequences.

State of the art of genome analysis of domesticated animals

In the course of the discussion about genetic engineering in food a lot of diagnostic techniques are playing a substantial role. These methods applied in genome analysis are called secondary genetic engineering (Brem, 1997) because there is no direct intervention in the nucleus of living cells. This is in fact an area, which we are using more or less extensively and will perhaps use even more extensively in the future. There are many reasons for the increasing interest in genome analysis of domesticated animals:

The investigation of molecular interactions in correlation with the functionality of genes is of growing importance for practical animal breeding and is in some respect already economically profitable. This development will not change for the next future as the analysis of the genomes of domestic animals is a very decisive step forward to elucidate all the available possibilities in modern breeding.

The pig genome

Considering the progress of genome analysis in the case of the main domesticated animals it is obvious, that pig played a particular role especially during the last years, because of the manifold molecular genetic similarities between man and pig. Nevertheless there are also a lot of benefits for the economic interests of man in the field of animal production. The pig genome consists of 38 chromosomes with about $2{,}72 \times 10^9$ bp and is estimated to about 50.000 different genes.

Selection for improved meat quality within the different lines has been limited by the absence of relevant measures that can be taken on live pigs. The knowledge about so called major genes, however, together with the development of DNA-technology in particular

and the possibility of molecular genetic analysis may overcome this problem (De Vries et al., 2000). Geneticists consider that a gene can be defined as a major gene when the difference between the mean value of the individuals homozygous for this gene, is equal or superior to one phenotypic standard deviation of the trait of interest (Sellier and Monin, 1994). Genes with such large effects can usually be detected by analysing phenotypic data across families where the gene segregates.

The Halothane gene

The Halothane gene is also referred to as the porcine stress syndrome (PSS) gene, and has been studied and discussed extensively (Simpson and Webb, 1989; Sellier and Monin, 1994). The gene started to become relevant for breeders when Christian (1972) speculated the existence of monogenic variation in stress-susceptibility and when Eikelenboom and Minkema (1974) showed that PSS could be triggered by halothane gas. Pommier and Houde (1993) reported that the PSE condition does not result directly from the Halothane gene, but the gene often exacerbates the problem. Since 1991 all three Halothane genotypes can be separated by means of PCR. Fujii et al. (1991) found the causative mutation for porcine stress syndrome in the gene encoding the fast twitch fibre ryanodine receptor isoform or calcium release channel (RYR1 or CRC1): a single point mutation in the gene at position 1843 (T-C Transition). The gene definitely improves carcass lean content, but reduces meat tenderness and juciness. No general conclusion is possible in the end, since the optimum approach depends on slaughter conditions and the type of processing.

The RN^- gene

The RN^- gene was first suggested by Naveau (1986) as being responsible for "acid meat", and later confirmed by segregation analysis in two French composite lines of pigs (Le Roy et al., 1990) as a gene with dominant inheritance. The gene is named after the Rendement Napole (RN) test, predicting cooking yield. The dominant allele leads to a decreased technological quality due to a lower protein content and reduced ultimate pH, resulting from an increased glycogen content in the white (fast glycolytic) fibres. The gene has been found segregating only in populations with Hampshire influence. The gene is located on chromosome 15 and later DNA marker studies (Milan et al., 1996) mapped the gene more accurately. The most recent published results place RN^- between Sw2053 and Sw936, a bracket of approximately 8 cM and a commercial DNA-marker test for detecting the gene was developed and validated (De Vries et al., 1997). The test successfully predicted large differences in ultimate pH and phosphate free ham processing yield. The study also showed that the yield differences completely disappeared when phosphate was used. Although the test is an excellent tool to reduce quickly the incidence of the gene, its complete elimination requires a DNA probe that detects the causative mutation.

Intramuscular fat (IMF)

Intramuscular fat plays an important role in the eating quality of pork. A segregation analysis on meat quality data of F2 crosses between Meishan and Dutch pig strains results in a recessive major gene for IMF, originating from Meishan. Animals with two copies of the gene had an average of 3,9 % IMF in the loin, whereas carriers of homozygous negative animals had 1,8 %. Research is underway to look at the existence of this gene in purebred populations (e.g. Duroc). This could eventually lead to DNA tests that allow better control of the marbling level of pork. With this tool breeders will have a large influence on the level of this trait, since its heritability is around 50% (De Vries et al, 1994; Hovenier et al., 1993). The challenge is to achieve a higher IMF without increasing the levels of the other fat depots. An example for a candidate gene or meat quality is provided by the gene for heart fatty acid binding protein (H-FABH). This gene maps to pig chromosome 6. More recently a larger effect on IMF was found with the related gene adipocyte (FABP) (De Vries et al., 2000).

Androstenone gene

Another trait with a high heritability is the level of androstenone (Willeke, 1993), which is one of the causes for the so-called "boar-taint" problem in meat from entire males. Applying the segregation analysis a major gene for androstenone level was found in Large White populations that were selected on this trait. The gene giving rise to low androstenone level was dominant, and carriers of this gene had 3 standard deviations lower level than non-carriers. In the same data set a major gene for the development of the bulbo-urethral glands was found.

Boar taint is also caused by skatole. Genetic work on skatole is limited. However this traits also show some genetic variation, and Lundström (1994) suggested, based on work in experimental Yorkshire lines, that the effect on skatole may be due to a major gene

with a recessive mode on inheritance. It is expected that the expression of the gene depends on certain environmental conditions like diet composition and hygiene.

Muscle fibre traits

Muscle fibre types differ phenotypically in that they express different subsets of myofibrillar isoform genes with different ATPase activities as well as different types and levels of metabolic enzymes. The different myosin heavy chains isoforms are coded for by separate genes, some of which are preferentially expressed in fast skeletal muscle and in slow skeletal muscle (Goldspink, 1996). The number of myofibers is prenatally determined, so the maximal number of myofibers available for meat production is formed during embryonic myogenesis, which seems to be under the gentic control of the MyoD gene family (Buckingham, 1992). Te Pas et al. (1994) reviewed for more detailed information on the MyoD gene family muscle regulatory mechanism.

The evidence for major genes was originally obtained using segregation analysis, i.e. without any DNA marker information. Afterwards molecular studies were performed to detect the location of the genes on the genetic map. DNA studies are required to dissect the genetic nature of most traits of economic importance.

DNA markers

DNA markers can be used to localize genes responsible for qualitative (coat colour, etc.) as well as quantitative (growth rate, IMF, etc.) traits. In the last mentioned case the approach is referred to QTL (Quantitative Trait Locus) mapping. In pigs QTL mapping is used for families created from crosses based on divergent lines (Large White x wild boar or Chinese Meishan).

With respect to an IMF major gene it is possible to apply the above mentioned technique using microsatellite markers. Recently it was reported evidence for suggestive linkages with markers on chromosome 1 and a marker on chromosome 3 (De Koning et al., 1998). The same region on chromosome 1 was also identified as containing a QTL for backfat.

A number of pig populations are now being used or have been created for the purposes of searching for meat quality QTLs. Traits measured include carcass composition, fat and lean distribution as well as meat quality measures such as water binding, shear force, IMF content and taste panel assessment. First results were published by Andersson-Eklund et al. (1998). For meat quality the authors did not find any QTL, however, significant QTLs for carcass characteristics, such as loin eye area and lean meat percentage were detected. The largest QTL was on chromosome 4 and is responsible for backfat and growth.

An alternative to the above approach would be to use markers to introgress favourable QTLs identified in a non-commercial genotype such as the wild boars into commercial line.

Candidate genes

With respect to existing polymorphisms the so called candidate gene approach can be applied to look for associations across populations. When associations are identified the resulting marker can potentially be used directly in breeding programmes. An example for a candidate gene for meat quality is provided by the gene for heart fatty acid binding protein (H-FABP). Gerbens et al. (1997) identified polymorphisms in this gene (chromosome 6) and found these to be associated with variation in IMF in Duroc. An even larger effect on IMF was detected with the related gene adipocyte FABP4, which seems to be independent of backfat and so offers promise for the manipulation of IMF by Marker Assisted Selection (MAS).

Another relevant candidate gene approach is the research on calpain, calpastatin and cathepsin (B, B+L and H). In beef and sheep there are many reports of the role of the calpain system (Koohmaraie, 1996). Many studies are also in progress to investigate the possible influence of pig genetic on cathepsin activity, which play a key role in the seasoned product (dry-cured ham) proteolysis (Armero et al., 1999).

Other markers which have been generated for meat quality based on the candidate gene approach include myogenin (increased muscle fibre number, which may impact overall pork quality) (Soumillion et al., 1997) and the dominant KIT gene leading to white coat colour in pigs (Johannson Moller et al., 1996).

The cattle genome

The cattle genome is composed of 29 pairs of autosomes and two sex chromosomes. The total amount of DNA per diploid cell is evaluated at 6×10^{-12}g. Like the genome of almost all vertebrates, the genome of ruminants, and among them cattle, is replete with various repetitive sequences. At least 50 % of the cattle genome is composed of repetitive sequences and about half of which are

highly repeated (D. Vaiman, 1998). The basic constituents of cattle repetitive sequences are short interspersed elements (SINES), long interspersed elements (LINES), microsatellites and SINES-associated microsatellites.

The identification of new unknown genes is a major endeavour for animal geneticists. With the development of fairly complete second-generation genetic maps for the bovine genome (Kappes et al., 1997), it clearly appears that any gene or even quantitative trait locus (QTL) is now attainable by genetic analysis of segregation. Global mapping would be very expensive. Therefore, the possibility of enriching some precise chromosomal regions in genetic markers is in particular of importance. For this purpose, genetic markers have been successfully isolated from three distinct DNA sources: flow sorted chromosomes, microdissected chromosomes and from somatic cell hybrids.

As it is not feasible to develop all the applications of molecular genetics to cattle in this contribution, only a few points will be discussed:

Among genes responsible for milk production three other genes were mapped in cattle: The White Heifer disease locus (Charlier et al., 1996a), the muscular hypertrophy gene found in the Blanc-Bleu Belge breed (Dunner et al., 1997) and the syndactyly locus (Charlier et al., 1996b).

There are also impressive results achieved in other ruminant species which are of economic interest. Applying PCR amplifying a large proportion of genetic markers developed for cattle in sheep and goats the cross-species conservation of microsatellites, although imperfect, made it possible to map various genes in sheep and goats (Cockett et al., 1994, 1996; Vaiman et al., 1996a). Numerous programmes are under way to achieve the next steps, i.e. the cloning of new gene localization.

Molecular diagnosis in cattle embryos

In cattle, the identification of genetic traits in preimplantation embryos is of high economic interest. Due to the fact that cattle are costly animals and have a long pregnancy, it is of particular interest to be able to determine the genotype status of precious embryos before implanting them in foster mothers. Two especially important traits exemplify the specific problems linked to bovine embryo transfer: the determination of the embryonic sex and the demonstration of transgenic embryos (Vaiman, 1999). Embryo sexing can be performed by means of different specific PCR-techniques with DNA targets in the X and Y chromosome. Several commercial kits are available. These probes can also be applied in some cases in other species, such as water buffalo (Rao et al., 1993), the bison (Cotinot et al., 1991), sheep and goats (Matthews and Reed, 1991; Rao and Totey, 1992).

Another matter of molecular diagnosis is **transgenesis**. Transgenic animals are obtained by the development of embryos into which foreign DNA has been introduced. Different techniques for transformation are available, which are discussed in the next subtopic.

Transgenesis, although widely used, largely exploits the egg as a black box, and little is actually known about the fate of the microinjected DNA (Bishop and Smith, 1989). Some copies are integrated, sometimes in long head-to-tail-arrays, at different places in the egg DNA, while other not integrated copies remain present even at the morula and blastula stage of the developing embryo (Jänne et al., 1994). Not integrated DNA is a permanent problem for screening embryos by PCR before implantation. Eukaryotic cells do not methylate their adenine residues in contrast to bacteria. Thus, demethylation is thought to occur while eukaryotic cells replicate. The fate of a methylated transgene after microinjection differs according whether it is integrated or not. When it remains extrachromosomal, it seems to escape demethylation and thus can be degraded by DpnI, recognizing adenine methylation. This ingenious technique was successfully used to discriminate transgenic embryos from non-transgenic ones in cattle (Hyttinen et al, 1996). Transgenesis could potentially accelerate the improvement of cattle breeds, once the uncertainties of the choice of the embryo to be implanted are efficiently controlled.

Gentechnical methods for DNA-recombination

All methods using a direct intervention into the nucleus of living cells are belonging to so called techniques of primary genetic engineering. The term transgenic refers to organisms which carry in their genome and/or express *in vitro* manipulated gene constructs. For animal breeding the three crucial aspects of transgenesis are integration, expression and transmission of the gene construct(s), i.e. gene transfer into the germ line. Somatic gene transfer approaches result in mostly transient gene expression with the longest duration being a life span (gene therapy).

The generation of transgenic farm animals was first reported more than 15 years ago (Brem et al., 1985). At this time gene transfer was carried out by microinjection of DNA constructs into the pro nuclei of zygotes. Up to now there exists more than only this method reported to be available for germline manipulation of livestock, e.g. infection with (retro-) viral vectors (Brem, 1993) and the application of embryonic stem cells (Dove, 2000). Alternative techniques whose efficiency remains to be established are sperm-mediated gene transfer i.e. the mixing of spermatozoa with DNA constructs prior to fertilization (Lavitrano et al., 1989) and several transfection methods (Brinster and Avarbock, 1994). Germline gene transfer requires early embryonic stages. It offers the exciting prospect of completely new breeding strategies and novel applications.

Gene transfer into farm animals is facing several obstacles as only a few applications have been successful in contrast to experiments in mice. DNA microinjection results in random integration of the gene constructs in the host genome. Thus the transgene expression often underlies "chromosomal position effects" (Brem et al., 1996). Also totipotential cell lines derived from farm animals are currently not available for routine gene transfer experiments. Without doubt a very exciting development in pig embryology will be the *in vitro* successful establishment of embryonic stem cells from this species.

Somatic gene transfer experiments do not aim at the integration of the gene construct into all cell types. Thus there is no requirement of transferring the DNA in early embryonic stages and "transient" transgenesis can be achieved by all passive and active transfection methods. In animal production somatic gene transfer might be used for the improvement of disease resistance or the production of proteins of high value (Archer et al, 1994).

The improvement of production efficiency and quality of animal products

With respect to the improvement of pigs in production efficiency and quality of animal products by transgenic means the following example on transgenic animals carrying gene constructs altering growth related functions has to be mentioned:

Growth is a very complex process influenced by the interaction of hormones and autocrine/paracrine factors, nutritional conditions and environmental factors. The generation of the first transgenic pig was based on the assumption that insertion of additional copies of the *GH* (growth hormone) gene controlled by a heterologous promoter would result in an enhanced growth performance. However, after the first experiments it was obvious, that other promoter elements had to be used to provide lower constitutive or inducible expression of *GH* constructs, resulting in the desired increase of carcass leanness and reduction of detrimental side effects (Pinkert et al, 1994).

Similar effects were achieved in the growth performance of muscle cells themselves, transferring the chicken proto-oncogene (c-ski) to pig. An accompanying side effects was a high degree of vacuolic degeneration of the muscle tissue.

Therefore it appears to be questionable whether the efforts required for optimising transgenic approaches to alter growth performance and carcass composition are justified by its potential benefits. Moreover, the production of transgenic meat depends on the consumer acceptance (Pursel and Solomon, 1993).

Approaches to reduce disease susceptibility of livestock will be a benefit in terms of animal welfare and will also be of economic importance. The costs of disease and health control have been estimated to account for 10 – 20 % of total production costs. In pigs, two approaches to improve health have been tested: the transfer of a specific disease resistance gene (Mx1 gene) and of antibody encoding genes (expressing monoclonal antibodies specific for a pathogen). Although it was shown that the Mx1 gene construct was tightly regulated in mouse cell lines, there was only a basal low-level transcriptional activity in tissues of transgenic pigs. The gene transfer experiments in pigs demonstrated that the choice of the regulatory elements controlling Mx1 expression is crucial (Müller et al, 1992). As shown in many investigations the expression of gene constructs encoding mouse monoclonal antibodies in transgenic farm animals revealed some unexpected findings, e.g. aberrant sizes of the transgenic antibody or only little binding capacity (Weidle et al., 1991). It remains to be investigated, however, whether the efforts required for optimising the concept of "congenital immunization" are justified by its benefits in terms of increasing disease resistance in a certain species.

Modern reproduction technologies

The ability to copy the *in vivo* development of pre-implantation embryos to an extracorporal *in vitro* system provides powerful possibilities for modern reproductive technologies in research and animal production. The development and improvement of such

technologies are concentrating on gamete and embryo collection and preservation, *in vitro* production of embryos, culturing and manipulating of embryos (splitting, nuclear transfer, production of chimeras, establishment of embryonic stem (ES) cells, gene transfer) and embryo transfer (ET).

Basic understanding of the reproductive physiology and the development of novel techniques is facilitated by modern equipment for ultrasonography, endoscopy and cryopreservation (Besenfelder et al., 1998). Sex determination of semen is of major interest for all species used for modern reproduction technologies. Intact male and female sperm cells can be separated by flow cytometry into purities up to 90 % (Johnson, 1996).

Embryo collection and production

Porcine embryos for instance can be collected *ex vivo* from living donors by either surgical or endoscopical flushing of oviducts or from slaughtered animals. Another possibility is the *in vitro* production of embryos from collected oocytes. Superovulation of sows results in about 30 collectable oocytes or embryos per donor. While both the quantity and quality of embryos isolated from prepuberal gilts are higher than from older animals, multiparous sows offer notable advantages as embryo recipients in terms of pregnancy rates, embryo and offspring survival (Brem and Müller, 1994).

It is expected that the use of endoscopy for reproduction of animals will represent a powerful tool for embryo recovery and embryo transfer for the future, because it provides free access to the ovaries, oviducts and uteri, allows visual control without surgery and avoids unnecessary movements or distortion of the organs.

Ovum pick up and in vitro production of embryos

Since the first reports of *in vitro* fertilization of pig (Harms and Schmidt, 1970) great attempts have been made to produce porcine embryos *in vitro*. Oocyte recovery is performed by flushing of ovulated oocytes from oviducts or by puncturing of follicles and aspiration of the oocytes. The latter can be done from untreated donors (immature oocytes) or after gonadotrophin treatment of the animals in which follicle maturation already has been started. *In vitro* production (IVP) of embryos can utilize follicular oocytes which normally undergo atresia. The IVP of embryos includes numerous *in vitro* steps which try to mimic the *in vivo* development of embryos: oocyte maturation, fertilization and culture until transfer or cryopreservation. The different steps for *in vitro* fertilization (IVF) have been developed separately. Finally, IVP of porcine embryos resulted in normal pregnancies with the birth of living pigs (Wu et al., 1992).

Embryo splitting, nuclear transplantation, embryonic stem cells

There is a great interest in the production of two or more genetically identical individuals (clones). In animal production and breeding clones guarantee the maintenance of single animals or lines with a high genetic value for commercial use and of rare breeds. In the future, the ability to clone embryos should compensate losses occurring during the procedure of embryo manipulation and transfer including pre- and postnatal mortality.

Embryo splitting is performed by microsurgical (bi-)section or early tubal stage embryos, morulae and blastocyst stages. The split embryos can be either cultured in vitro or transferred into foster animals after micromanipulation. Pig embryos seem to be much more sensitive to the splitting procedure than bovine embryos. The procedure of nuclear transfer is performed by dislocating pronuclei or blastomeres from an embryo into enucleated oocytes. Pronuclear exchange embryos were produced by positioning pronuclei containing karyoplasm in the perivitelline space of enucleated zygotes and subsequent fusion in an electric field. The transplantation of blastomeres into recipient oocytes is normally performed by electrofusion. The reasons for the poor development of reconstituted porcine embryos are unknown. It is suggested that nuclear transfer leads to a lack of RNA synthesis (Niemann and Reichelt, 1993).

A chimera is , by definition, an individual which generated from cells developing out of two genetically different zygotes. Chimeras are usually produced by aggregation of two early stage embryos (mainly morulae) or the injection of blastomeres or embryonic cells into the cavity of blastocysts. The production of germline chimeras was reported in two studies (Anderson et al., 1994; Onishi et al, 1994).

The handling and manipulation of blastomeres per se is required for molecular genetic analysis of pre-implantation embryos. Such genetic diagnosis may aim to detect inherited disorders or important performance traits. Blastomere handling is an inseparable

instrumentation for the production of embryonic stem cells. Embryonic stem cells in animals are a tool desired for the study of cell differentiation and development, gene regulation but more importantly production of transgenic (knock out) animals. The term "knock out" means the generation of null mutations of a gene by inactivation of an endogenous gene by insertion of cloned sequences. ES cells are isolated from the inner cell mass of blastocysts. The problem of establishing ES cells from livestock may be related to the limited availability of embryos with a defined genetic background (Donovan, 1994).

Embryo transfer

The term embryo transfer (ET) includes the collection or production of embryos (*ex vivo* or *in vitro*) from donor animals, the temporary culture and/or manipulation and reintroduction into the physiological system (recipient animal). ET depends on the developmental stage of the embryos either into the oviduct or into the uterine horns of the foster mother. The early stage embryos are transferred to the recipients 60 – 63 h after induction of ovulation. Transfer techniques are:

1) The surgical procedure (Cameron et al, 1989); 2) Transfer *per viam naturalem* through the cervix (Reichenbach and Niemann, 1994); 3) Endoscopic access to the reproductive organs (Besenfelder et al., 1997). The last mentioned method is quick, minimally invasive and causes therefore a minimum of stress at a very high pregnancy rate of the animals.

Final remarks

Concerning "biotechnology" in general and also for "biotechnology in meat production" in particular different questions are arising, which did not find enough satisfying answers until now: a) patenting of transgenic animals, b) biosafety problems of transgenic animals, c) food safety evaluation of transgenic animals d) from a moral point of view: ethical problems of animal transgenesis. The discussions stirred up will last still for a long time in the future.

References

Anderson, G.B., Choi, S.J. & Bondurant, R.H. (1994): Theriogenology **42**, 204 – 212.

Andersson-Eklund, L., Marklund, L., Lundström, K., Haley, C.S., Andersson, K. & Hanssen, I. (1998): Science **263**, 1771 – 1774.

Archer, J.S., Kennan, W.S., Gould, M.N. & Bremel, R.D. (1994): Proceedings of the National Academy of Sciences of the USA **91**, 6840 – 6844.

Armero, E., Flores, M., Barbosa, J.-A., Toldra, F. & Pla, M. (1998): Meat Science **51**, 185 – 189.

Besenfelder, U., Mödl, J., Müller, M. & Brem, G. (1997: Theriogenology **47**, 1051 – 1060.

Besenfelder, U., Müller, M. & Brem, G. (1998) In: The Genetics of the Pig, eds. Rothschild, M.F. & Ruvinsky, A., CAB International **12**, 345 – 369.

Bishop, J.O. & Smith, P. (1989): Molecular Biology and Medicine **6**, 283 – 298.

Brem, G., Brenig, B., Goodman, H.M., Selden, R.C., Graf, F., Kruff, B., Springman, K., Hondele, J., Meyer, J., Winnacker, E.-L. & Kräuszlich, H. (1985): Zuchthygiene **20**, 251 – 252.

Brem, G. (1993) Transgenic animals. In: Rehm, H.-J., Reed, G., Pühler, A. & Stadler, P. (eds.) Biotechnology. VCH, Weinheim, Germany, 745 – 832.

Brem, G. & Müller, M. (1994): In: Animals with Novel Genes, ed. Maclean, N., Cambridge University Press, Cambridge, UK, 179 – 244.

Brem, G., Besenfelder, U., Aigner, B., Müller, M., Liebl, I., Schütz, G. & Montoliu, L. (1996): Molecular Reproduction and Development **44**, 55 – 62.

Brem, G. (1997) In: Proceedings of the International Symposium on "Novel Foods Regulation in the European Union – Integrity of the Process of Safety Evaluation", 101 –105.

Brenig, B. (2000) In: Hülsenberger Gespräche 2000, Schriftenreihe der H. Wilhelm Schaumann Stiftung, 22 – 27.

Brinster, R.L. & Avarbook, M.R. (1994): Proceedings of the National Academy of Sciences of the USA **91**, 11303 – 11307.

Buckingham, M. (1992): Trends in Genetics **8**, 144 – 149.

Cameron, R.D.A., Durack, M., Fogarty, R., Putra, D.K.H. & McVeigh, J. (1989): Australian Veterinary Journal **66**, 314 – 318.

Charlier, C., Denys, B., Belanche, J.I., Coppieters, W., Grobet, L.; Mni, M., Womack, J., Hanset, R., & Georges, M. (1996a) Mammalian Genome **7**, 138 – 142.

Charlier, C., Farnir, F., Berzi, P., Vanmanshoven, P., Brouwers, B., Vromans, H. & Georges, M. (1996b): Genome Research **6**, 580 – 589.

Cockett, N.E., Jackson, S.P., Shay, T.L., Nielsen, D., Moore, S.S., Steele, M.R., Barendse, W., Green, R.D. & Georges, M. (1994): Proceedings of the National Academy of Sciences of the USA **91**, 3019 – 3023.

Cockett, N.E., Jackson, S.P., Shay, T.L., Farnir, F., Berghmans, S., Snowder, G.D., Nielsen, D.M. & Georges, M. (1996): Science **273**, 236 – 238.

Cotinot, C., Kirszenbaum, M., Leonard, M., Gianquinto, L. & Vaiman, M. (1991): Genomics **10**, 646 – 653.

De Koning, D.J., Janss, L.L.J., Van Arendonk, J.A.M., Van Oers, P.A.M. & Groenen, M.A.M. (1998) In: Proceedings 6th World Congress Genetics Applied to Livestock Production **26**, 410 – 413.

De Vries, A.G., Van der Wal, P.G., Long, T., Eikelenboom, G. & Merks, J.W.M. (1994): Livestock Production Science **40**, 277 – 289.
De Vries, A.G., Timm, H.H., Wilson, E.R., Keller, V. & Plastow, G.S. (1997) In: Abstracts 48th Annual Meeting EAAP, Vienna, Wageningen Pers, Wageningen **3**, 340.
De Vries, A.G., Faucitano, L., Sosnicki, A. & Plastow, G.S. (2000): Food Chemistry **69**, 397 – 405.
Donovan, P. (1994): Current Topics in Developmental Biology **29**, 189 – 225.
Dove, A. (2000): Nature Biotechnology **18**, 1045 – 1049.
Dunner, S., Charlier, C., Farnir, F., Brouwers, B., Canon, J. & Georges, M. (1997): Mammalian Genome **8**, 430 – 435.
Eikelenboom, G. & Minkema, D. (1974): Tijdschrift Diergeneeskunde **99**, 421 – 426.
Fujii, J., Otsu, K., Zorzato, F., De Leon, S., Khanna, V. K. & Weiler, J.E. (1991): Science **253**, 448 – 451.
Gerbsen, F., Rettenberger, G., Lenstra, J.A., Veerkamp, J.H. & Te Pas M.F.W. (1997): Mammalian Genome **8**, 328 – 332.
Goldspink, G. (1996): Research Veterinary Science **60**, 193 – 204.
Harms, V.E. & Schmidt, D. (1970): Berliner und Münchner Tierärztliche Wochenschrift **83**, 269 – 288.
Hovenier, R., Kanis, E., Van Asseldonk, T. & Westerink, N.G. (1993): Pig News and Information **14**, 17N – 25N.
Hyttinen, J.M., Peura, T., Tolvanen, M., Alto, J. & Jänne, J. (1996): Molecular Reproduction and Development **43**, 150 – 157.
Jänne, J., Hyttinen, J.M., Peura, T., Tolvanen, M., Alhonen, L., Sinervirta, R. & Halmekyto, M. (1994): International Journal of Biochemistry **26**, 859 – 870.
Johannson Moller, M., Chaudhary, R., Hellmen, E., Höyheim, B., Chowdhary, B. & Andersson, L. (1996): Mammalian Genome **7**, 822 – 830.
Johnson, L.A. (1996): Deutsche Tierärztliche Wochenzeitschrift **103**, 285 – 368.
Kappes, S.M., Keele, J.W., Stone, R.T., McCraw, R.A., Sonstegard, T.S., Smith, T.P., Lopez-Corrales, N.L. & Beattie, C.W. (1997): Genome Research **7**, 235 – 249.
Koohmaraie, M. (1996): Meat Science **43** (193), S193 – S201.
Lavitrano, M., Camaioni, A., Fazio, V.M., Dolci, S., Farace, M.G. & Sparafoda, C. (1989): Cell **57**, 717 – 723.
Le Roy, P., Naveau, J., Elsen, J.M. & Sellier, P. (1990): Genetic Research in Cambridge **55**, 33 – 40.
Lundström, K., Malmfors, B., Stern, S., Rydhmer, L., Eliasson-Selling, L., Mortensen, A.B. & Mortensen, H.P. (1994): Livestock Production Science **38**, 125 – 132.
Matthew, M.E. & Reed, K.C. (1991): Cytogenetics and Cell Genetics **56**, 40 – 44.
Milan, D., Woloszyn, N., Yerle, M., Le Roy, P., Bonnet, M. & Riquet, J. (1996): Mammalian Genome **7**, 47 – 51.
Müller, M., Brenig, B., Winnacker, E.-L. & Brem, G. (1992): Gene **121**, 263 – 270.
Naveau, J. (1986): Journees de la Recherche Porcine en France **18**, 265 – 276.
Niemann, H. & Reichelt, B. (1993): Journal of Reproduction and Ferility (Suppl. **48**), 75 – 94.
Onishi, A., Takeda, K., Komatsu, M., Akita, T. & Kojima, T. (1994): Biology of Reproduction **51**, 1069 – 1075.
Pinkert, C.A., Galbreath, E.J., Yang, C.W. & Striker, L.J. (1994) Transgenic Research **3**, 401 – 405.
Pommier, S.A. & Houde, A. (1993): Journal of Animal Science **71**, 420 – 425.
Rao, K.B. & Totey, S.M. (1992): Indian Journal of Experimental Biology **30**, 775 –777.
Pursel, V.G. & Solomon, M.B. (1993): Food Reviews International **9**, 423 – 439.
Rao, K.B., Pawshe, C.H. & Totey, S.M. (1993): Molecular Reproduction and Development **36**, 291 – 296.
Reichelt, B. & Niemann, H. (1994): Journal of Reproduction and Fertiltiy **100**, 163 – 172.
Sanger, F., Nicklen, S. & Coulsen, A.R. (1977): Science **74**, 5463 – 5467.
Sellier, P. & Monin, G. (1994): Journal of Muscle Foods **5**, 187 – 219.
Simpson, S.P. & Webb, A.J. (1989): Animal Production **49**, 503 – 509.
Soumillon, A., Erkens, J.H., Lenstra, J. A., Rettenberger, G. & Te Pas, M.F.W. (1997): Mammalian Genome **8** (8), 564 – 568.
Te Pas, M.F.W., De Vries, A.G. & Visscher, A.H. (1994) In: Proceedings 40th International Congress on Meat Science and Technology, The Hague, NL, S-VII, 8.
Vaiman, D. (1999) In: The Genetics of Cattle, eds. Fries, R. and Ruvinsky, A., CAB International **6**,123 – 148.
Watson, J.D. & Crick, F.H.C. (1953): Nature **171**, 737.
Weidle, U.H., Lenz, H. & Brem, G. (1991): Gene **98**, 185 – 191.
Willeke, H. (1993): Pig News and Information **14**, 31N – 33N.
Winnacker, E.-L. (2000) in: Hülsenberger Gespräche 2000, Schriftenreihe der H. Wilhelm Schaumann Stiftung, 12 – 20.
Wu, G.M., Qin, P.C., Tan, J.H. & Wang, L.A. (1992): Theriogenology **37**, 323 - 328.