The contribution of farm animals to human health

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Farm animals and their products have a longstanding and successful history of providing significant contributions to human nutrition, clothing, facilitation of labour, research, development and medicine and have thus been essential in improving life expectancy and human health. With the advent of transgenic technologies the potential of farm animals for improving human health is growing and many areas remain to be explored. Recent breakthroughs in reproductive technologies, such as somatic cloning and in vitro embryo production, and their merger with molecular genetic tools, will further advance progress in this field. Here, we have summarized the contribution of farm animals to human health, covering the production of antimicrobial peptides, dietary supplements or functional foods, animals used as disease models and the contribution of animals to solving urgent environmental problems and challenges in medicine such as the shortage of human cells, tissues and organs and therapeutic proteins. Some of these areas have already reached the level of preclinical testing or commercial application, others will be further advanced only when the genomes of the animals concerned have been sequenced and annotated. Provided the necessary precautions are being taken, the transmission of pathogens from animals to humans can be avoided to provide adequate security. Overall, the promising perspectives of farm animals and their products warrant further research and development in this field.

Farm animals have made significant contributions to human health and well-being throughout mankind's history. The pioneering work of Edward Jenner with cowpox in the 18th century paved the way for modern vaccination programs against smallpox, as well as other human and animal plagues. To date, more than 250 million people have benefited from drugs and vaccines produced by recombinant technologies in bacteria and various types of mammalian cells and many more will benefit in the future (New Medicines in Development for Biotechnology, 2002; www.phrma.org/newmedicines/biotech/). Further examples of the significant contribution of farm animals to human health are the longstanding use of bovine and porcine insulin for treatment of diabetes as well as horse antisera against snake venoms and antimicrobial peptides. In addition, farm animals are models for novel surgical strategies, testing of biodegradable implants and sources of tissue replacements, such as skin and heart valves.

Progress in transgenic technologies has allowed the generation of genetically modified large animals for applications in agriculture and biomedicine, such as the production of recombinant proteins in the mammary gland and the generation of transgenic pigs with expression of human complement regulators in xenotransplantation research [1]. Further promising application perspectives will be developed when somatic cloning with genetically modified donor cells is further improved and the genomes of farm animals are sequenced and annotated. The first transgenic livestock were born less than 20 years ago with the aid of microinjection technology [2]. Recently the first animals with knockout of one or even two alleles of a targeted gene were reported (Table 1). Somatic nuclear transfer has been successful in 10 species, but the overall

Table 1. Milestones (live offspring) in transgenesis and reproductive technologies in farm animals

<table>
<thead>
<tr>
<th>Year</th>
<th>Milestone</th>
<th>Strategy</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1985</td>
<td>First transgenic sheep and pigs</td>
<td>Microinjection of DNA into one pronucleus of a zygote</td>
<td>[2]</td>
</tr>
<tr>
<td>1986</td>
<td>Embryonic cloning of sheep</td>
<td>Nuclear transfer using embryonic cells as donor cells</td>
<td>[91]</td>
</tr>
<tr>
<td>1997</td>
<td>Cloning of sheep with somatic donor cells</td>
<td>Nuclear transfer using adult somatic donor cells</td>
<td>[92]</td>
</tr>
<tr>
<td>1997</td>
<td>Transgenic sheep produced by nuclear transfer</td>
<td>Random integration of the construct</td>
<td>[93]</td>
</tr>
<tr>
<td>1998</td>
<td>Transgenic cattle produced from fetal fibroblasts and nuclear transfer</td>
<td>Random integration of the construct</td>
<td>[94]</td>
</tr>
<tr>
<td>1998</td>
<td>Generation of transgenic cattle by MMLV injection</td>
<td>Injection of oocytes with helper viruses</td>
<td>[95]</td>
</tr>
<tr>
<td>2000</td>
<td>Gene targeting in sheep</td>
<td>Gene replacement and nuclear transfer</td>
<td>[96]</td>
</tr>
<tr>
<td>2002</td>
<td>Trans-chromosomal cattle</td>
<td>Additional artificial chromosome</td>
<td>[15]</td>
</tr>
<tr>
<td>2002</td>
<td>Heterozygous knockout in pigs</td>
<td>One allele of α-galactosyl-transferase knocked out</td>
<td>[34,35]</td>
</tr>
<tr>
<td>2003</td>
<td>Homozygous gene knockout in pigs</td>
<td>Both alleles of α-galactosyl-transferase knocked out</td>
<td>[36]</td>
</tr>
<tr>
<td>2003</td>
<td>Transgenic pigs via lentiviral injection</td>
<td>Gene transfer into zygotes via lentiviruses</td>
<td>[97]</td>
</tr>
</tbody>
</table>

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efficiency is low and few cloned offspring have been born worldwide (Table 2). Compared with microinjection of DNA constructs into pronuclei of zygotes, somatic nuclear transfer is superior for the generation of transgenic animals (Table 3).

Here, we have summarized the contribution of farm animals to human health covering (i) the production of pharmaceuticals; (ii) production of xenografts for overcoming the severe shortage of human organs and tissues; (iii) the use of farm animals as disease models; (iv) the production of dietary supplements or functional foods; and (v) the contribution of farm animals to solving environmental problems.

Farm animals for pharmaceutical production

Gene ‘pharming’: production of recombinant human proteins in the mammary gland of transgenic animals

The conventional production of rare human therapeutic proteins from blood or tissue extracts is an inefficient, expensive, labour and time consuming process, which in addition bears the risk of contamination with human pathogens. The production of human therapeutic proteins by recombinant bacteria or cell cultures has alleviated these problems and has made several therapeutic proteins available for patients. However, these recombinant systems have several limitations. They are only suitable for ‘simple’ proteins, the amount of protein produced is limited, and post-translational modifications are often incorrect leading to immune reactions against the protein. In addition, the technical prerequisites are challenging and production costs are high.

Farm animals such as cattle, sheep, goats, pigs and even rabbits [3,4] have several significant advantages for the production of recombinant proteins over other systems, including their potential for large-scale production, correct glycosylation patterns and post-translational modifications, low running costs, rapid propagation of the transgenic founders and high expression stability.

These attractive perspectives led to the development of the ‘gene pharming’ concept, which has been advanced to the level of commercial application [5]. The most promising site for production of recombinant proteins is the mammary gland, but other body fluids including blood, urine and seminal fluid have also been explored [6]. The mammary gland is the preferred production site mainly because of the quantities of protein that can be produced and the ease of extraction or purification of the respective protein.

Based on the assumption of average expression levels, daily milk volumes and purification efficiency, 5400 cows would be needed to produce the 100 000 kg of human serum albumin (HSA) that are required per year worldwide, 4500 sheep would be required for the production of 5000 kg α-antitrypsin (α-AT), 100 goats for 100 kg of monoclonal antibodies, 75 goats for the 75 kg of antithrombin III (ATIII) and two pigs to produce 2 kg human clotting factor IX. All these values are calculated on a yearly basis [3].

Large amounts of numerous heterologous recombinant proteins have been produced by targeting expression to the mammary gland via mammary gland-specific promoter elements. Proteins were purified from the milk of transgenic rabbits, pigs, sheep, goats and cattle. The biological activity of the recombinant proteins was assessed and therapeutic effects have been characterized [3,7]. Products such as ATIII, α-AT or tissue plasminogen activator (tPA) are advanced to clinical trials (Table 4) [5]. Phase III trials for ATIII have been completed and the protein is expected to be on the market within the next 2–3 years. In February 2004 an application was submitted to

### Table 2. Efficiency of somatic cloning of mammals

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of animals</th>
<th>% Viable offspring</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>~3000</td>
<td>15–20</td>
<td>Up to 30% of the cloned calves showed abnormalities, such as increased birth weight</td>
</tr>
<tr>
<td>Sheep</td>
<td>~400</td>
<td>5–8</td>
<td>Same problems as with cattle clones</td>
</tr>
<tr>
<td>Goat</td>
<td>~400</td>
<td>3</td>
<td>Minor health problems reported</td>
</tr>
<tr>
<td>Mouse</td>
<td>~300</td>
<td>&lt;2</td>
<td>Some adult mice clones showed obesity and a reduced life span</td>
</tr>
<tr>
<td>Pig</td>
<td>~200</td>
<td>&lt;1</td>
<td>Some cloned piglets had reduced birth weights</td>
</tr>
<tr>
<td>Cat</td>
<td>1</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>6</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>Mule</td>
<td>1</td>
<td>~1</td>
<td></td>
</tr>
<tr>
<td>Horse</td>
<td>1</td>
<td>~1</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>2</td>
<td>~1</td>
<td></td>
</tr>
</tbody>
</table>

*Estimated total numbers of mammals derived from somatic cloning since 1997.

<table>
<thead>
<tr>
<th>Integrative efficiency</th>
<th>Random or targeted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Integration site</td>
<td>Random</td>
</tr>
<tr>
<td>Gene deletion</td>
<td>+</td>
</tr>
<tr>
<td>Construction size</td>
<td>~50 kb (Artificial chromosomes)</td>
</tr>
<tr>
<td>Technical feasibility</td>
<td>Technically demanding</td>
</tr>
<tr>
<td>Mosaicism</td>
<td>+ + +</td>
</tr>
<tr>
<td>Expression screen in vitro</td>
<td>+</td>
</tr>
<tr>
<td>Expression pattern</td>
<td>Variable</td>
</tr>
<tr>
<td>Multi-transgenics</td>
<td>+</td>
</tr>
</tbody>
</table>

| Abbreviations: –, not possible; +, weak advantage; ++, moderate advantage; +++, strong advantage |

www.sciencedirect.com
the European Market Authorization to allow Atryn®, the recombinant ATIII from the milk of transgenic dairy goats, to enter the market as a fully registered drug. The enzyme α-glucosidase from the milk of transgenic rabbits has Orphan drug registration and has been successfully used for the treatment of Pompe’s disease [8]. This is a rare glycogen storage disorder, which is fatal in children under 2 years and currently application with recombinant α-glucosidase is the only way to treat this metabolic defect. Biologically active human lactoferrin has been produced in large amounts in the mammary glands of transgenic cows and will probably be developed as a biopharmaceutical for prophylaxis and treatment of infectious diseases [9].

Guidelines developed by the Food and Drug Administration (FDA) of the USA require monitoring of the animals’ health, validation of the gene construct, characterization of the isolated recombinant protein, as well as performance of the transgenic animals over several generations. This has been taken into account when developing ‘gene pharming’, for example by using only animals from prion disease-free countries (New Zealand) and keeping the animals in very hygienic conditions. Successful drug registration of Atryn® will demonstrate the usefulness and solidity of this approach and will accelerate registration of further products from this process, as well as stimulate research and commercial activity in this area.

When considering the ‘gene pharming’ concept, one has to bear in mind that not every protein can be expressed at the desired levels. Erythropoietin (EPO) could not be expressed in the mammary gland of transgenic cattle [10] and was even detrimental to the health of rabbits transgenic for EPO [11]. We have shown that human clotting factor VIII (hFVIII) cDNA constructs can be expressed in the mammary gland of transgenic mice, rabbits and sheep [1,12]. However, the yields of biologically active recombinant hFVIII protein from ovine milk were low because hFVIII was rapidly sequestered into ovine milk. [13]. These results show that the technology needs further improvements to achieve high-level expression with large genes having complex regulation, such as that coding for hFVIII, although higher levels of hFVIII have been reported in transgenic swine [14]. With the advent of transgenic crops that produce pharmacologically active proteins, there is an array of recombinant technologies available that will allow the most appropriate production system for a specific protein to be targeted.

An interesting new development is the generation of transchromosomal animals (Table 1). A human artificial chromosome (HAC) containing the entire sequences of the human immunoglobulin heavy and light chain loci has been introduced into bovine fibroblasts, which were then used in nuclear transfer. Transchromosomal offspring were obtained that expressed human immunoglobulin in their blood. This system could be a significant step forward in the production of human therapeutic polyclonal antibodies [15]. Further studies will show whether the additional chromosome will be maintained over future generations and how stable expression will be.

**Production of a new class of antibiotics: cationic anti-microbial peptides**

With increasing antibiotic resistance in bacterial species, there is a growing need to develop new classes of anti-microbial agents. Cationic anti-microbial peptides (AMP) have many of the desired features [16] because they possess a broad spectrum of activity, kill gram-positive and gram-negative bacteria rapidly, are unaffected by classical resistance genes and are active in animal models [17–19]. AMPs belong to the innate immune defense, which acts as a first barrier ahead of humoral and cellular immune systems, and neutralizes bacteria by interacting specifically with their cell membranes. Their low transmembrane potential of < -100 mV, and the abundant anionic phospholipids are essential for this selective interaction. It is proposed that AMPs physically disintegrate the cell membrane, and in addition can interact with several intracellular target molecules [16]. More than 500 such peptides have been discovered in plants, insects, invertebrates, fish, amphibians, birds and mammals [16–20]. AMPs from livestock species would be superior anti-microbial drugs because they would lack cytotoxic effects that were found for insect peptides; the evolution of resistance would not affect the human specific innate immunity [20].

Prominent examples of cationic anti-microbial peptides from farm animals already in advanced clinical trials (Phases II–III) are Iseganan (derived from protegrin-1 peptide of pig leucocytes; Intrabiotics; http://intrabiotics.com/) and MBI-594 (similar to indolicidin peptide from bovine neutrophils; Micrologix, http://mbiotech.com/). To date, there are no documented cases of antimicrobial peptide-resistance for AMPs and combinatorial approaches in peptides (20 possible amino acids in each position) provide great potential for rational drug design [21]. Recombinant production will keep the production costs low.

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**Table 4. Proteins produced in the mammary gland of transgenic farm animals**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Developmental phase</th>
<th>Production species</th>
<th>Therapeutic application</th>
<th>Potential market introduction date</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT III</td>
<td>Phase III</td>
<td>Goat</td>
<td>Genetic heparin resistance</td>
<td>2005</td>
</tr>
<tr>
<td>TPA</td>
<td>Phase I/III</td>
<td>Goat</td>
<td>Dissolving coronary clots</td>
<td>&gt; 2006</td>
</tr>
<tr>
<td>α-AT</td>
<td>Phase I/III</td>
<td>Goat and/or sheep</td>
<td>Lung emphysema</td>
<td>&gt; 2007</td>
</tr>
<tr>
<td>hFVIII</td>
<td>Experimental</td>
<td>Sheep</td>
<td>Hemophilia A</td>
<td>&gt; 2008</td>
</tr>
<tr>
<td>HAS</td>
<td>Phase I</td>
<td>Cattle</td>
<td>Blood substitute</td>
<td>&gt; 2008</td>
</tr>
<tr>
<td>Various antibodies</td>
<td>Phase I/II</td>
<td>Goat</td>
<td></td>
<td>&gt; 2007</td>
</tr>
</tbody>
</table>

*Abbreviations: α-AT, α-A1-antitrypsin; AT III, antithrombin III; hFVIII, human clotting factor VIII; HSA, human serum albumin; TPA, tissue plasminogen activator*
**Xenotransplantation of porcine organs to human patients**

**Solid organs**

Today >250 000 people are alive only because of the successful transplantation of an appropriate human organ (allotransplantation). On average, 75–90% of patients survive the first year after transplantation and the average survival of a patient with a transplanted heart, liver or kidney is 10–15 years. This progress in organ transplantation technology has led to an acute shortage of appropriate organs, and cadaveric or live organ donation cannot cover the demand in western societies. The 2001 figures from the United Network for Organ Sharing (www.unos.org) in the USA show that the ratio of patients with organ transplantation to those on the waiting list is ~1:4 (Table 5). A similar ratio is found in other countries such as the UK, France and Germany. A new person is added to the waiting list every 14 minutes. This has led to the sad and ethically challenging situation in which several thousand patients who could have survived if appropriate organs had been available die every year.

To close the growing gap between demand and availability of appropriate organs, porcine xenografts are considered the solution of choice [22,23]. Today the domesticated pig is considered the optimal donor animal because (i) the organs are similar in size to human organs; (ii) porcine anatomy and physiology are not too different from that of humans; (iii) pigs have short reproduction cycles and large litters; (iv) pigs grow rapidly; (v) maintenance of high hygienic standards is possible at relatively low costs; and (vi) transgenic techniques for modifying the immunogenicity of porcine cells and organs are well established.

The process of generating and evaluating transgenic pigs as potential donors for xenotransplants involves a variety of complex steps and is time-, labour- and resource-intensive.

Essential prerequisites for successful xenotransplantation are:

(i) Overcoming the immunological hurdles.

(ii) Prevention of transmission of pathogens from the donor animal to the human recipient.

(iii) Compatibility of the donor organs with the human organ in terms of anatomy and physiology.

The immunological obstacles in a porcine-to-human xenotransplantation are the hyperacute rejection response (HAR), acute vascular rejection (AVR), cellular rejection and potentially chronic rejection [24]. The HAR occurs within seconds or minutes. In the case of a discordant organ (e.g. in transplanting from pig to human) naturally occurring antibodies react with antigenic structures on the surface of the porcine organ and induce HAR by activating the complement cascade via the antigen–antibody complex. Ultimately, this results in the formation of the membrane attack complex (MAC). However, the complement cascade can be shut down at various points by expression of regulatory genes that prevent the formation of the MAC. Regulators of the complement cascade are CD55 (decay accelerating factor, DAF), CD46 (membrane cofactor protein, MCP) or CD59. MAC disrupts the endothelial cell layer of the blood vessels, which leads to lysis, thrombosis, loss of vascular integrity and ultimately to rejection of the transplanted organ [24].

Induced xenoreactive antibodies are thought to be responsible for AVR, which occurs within days of a transplantation of a xenograft; disseminated intravascular coagulation (DIC) is a predominant feature of AVR. Despite severe immunosuppressive treatment, a disturbed thrombocyte function and DIC were observed in a pig-to-primate xenotransplant model [25,26]. The endothelial cells of the graft’s microvasculature loose their antithrombic properties, attract leucocytes, monocytes and platelets leading to anemia and organ failure. The underlying mechanism for DIC and thrombotic microangiopathy is thought to be activation of the endothelial cells attributed to incompatibilities between human and porcine coagulation factors [25]. At least three incompatibilities between human and porcine coagulation systems have been identified; the first is the failure of porcine thrombomodulin (TM) to activate human anticoagulant protein C, the second is that the porcine tissue factor pathway inhibitor fails to inhibit human clotting factor Xa and the third is that porcine von Willebrand factor (vWF) binds and activates human platelets [26]. Human thrombomodulin (hTM) and heme-oxygenase 1 (hHO-1) are crucially involved in the etiology of DIC and might be good targets for future transgenic studies to improve long-term survival of porcine xenografts by creating multi-transgenic pigs.

The cellular rejection occurs within weeks after transplantation. In this process the blood vessels of the transplanted organ are damaged by T-cells, which invade the intercellular spaces and destroy the organ. This rejection is observed after allotransplantation and is normally suppressed by life-long administration of immunosuppressive drugs [24].

When using a discordant donor species such as the pig, overcoming the HAR and AVR are the preeminent goals. The most promising strategy for overcoming the HAR is the synthesis of human complement regulatory proteins (RCAs) in transgenic pigs [22,23,27,28]. Following transplantation, the porcine organ would produce the complement regulatory protein and can thus prevent the complement attack of the recipient. Pigs transgenic for DAF or MCP have been generated by microinjection of DNA constructs into pronuclei of zygotes. Hearts and kidneys from these animals have been transplanted either heterotopically, (in addition to the recipient’s own organ) or

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**Table 5. Overview of transplanted organs and demand for organ transplantation (USA)**

<table>
<thead>
<tr>
<th>Type of transplant</th>
<th>Transplantations in 2001</th>
<th>Waiting patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>14 152</td>
<td>52 772</td>
</tr>
<tr>
<td>Liver</td>
<td>5177</td>
<td>17 520</td>
</tr>
<tr>
<td>Pancreas</td>
<td>468</td>
<td>1318</td>
</tr>
<tr>
<td>Kidney/Pancreas</td>
<td>884</td>
<td>2520</td>
</tr>
<tr>
<td>Heart</td>
<td>2202</td>
<td>4163</td>
</tr>
<tr>
<td>Heart/Lung</td>
<td>27</td>
<td>209</td>
</tr>
<tr>
<td>Lung</td>
<td>1054</td>
<td>3799</td>
</tr>
<tr>
<td>Total</td>
<td>23 964</td>
<td>79 845</td>
</tr>
</tbody>
</table>

*Data from United Network for Organ Sharing, 2001 (www.unos.org).*
orthotopically (life supportive) into non-human primates. Survival rates reached 23–135 days with porcine xenografts transgenic for one of the two complement regulators; survival rates were heavily affected by the strength of the immune-suppressive protocol (Table 6) [29–31]. Similarly, transgenic expression of hCD59 was compatible with an extended survival of porcine hearts in a perfusion model or following transfer into primates [32,33]. These data show that HAR can be overcome in a clinically acceptable manner by expressing human complement regulators in transgenic pigs [22].

Another promising strategy towards successful xenotransplantation is the knockout of the antigenic structures on the surface of the porcine organ that cause HAR. These structures are known as 1,3-α-gal-epitopes and are primarily produced by activity of 1,3-α-galactosyltransferase (α-gal). Piglets in which one allele of α-gal locus had been knocked out by homologous recombination in primary donor cells that were employed in nuclear transfer were recently generated [34,35]. The birth of four healthy piglets with disruption of both allelic loci for α-gal has meanwhile also been published. Applying toxin A from Clostridium difficile to cells that already carried one deleted α-gal allele selected a cell clone, which carried an inactivating point mutation on the second allele. This cell clone was then used in nuclear transfer [36]. The usefulness of these animals for xenotransplantation has recently been reported [37].

Further improvements in the success of xenotransplantation will arise from the possibility of inducing a permanent tolerance across xenogenic barriers [38,39]. A particularly promising strategy for long-term graft acceptance is the induction of a permanent chimerism via intraportal injection of embryonic stem cells [40].

Prevention of transmission of zoonoses from the donor animal to the human recipient is crucial for clinical application of porcine xenografts. This aspect gained particular significance when a few years ago it was shown that porcine endogenous retroviruses (PERV) can be produced by porcine cell lines and can even infect human cell lines in vitro [41]. However, until today no infection has been found in patients that had received various forms of living porcine tissues (e.g. islet cells, insulin, skin, extracorporal liver) for up to 12 years [42]. Recent intensive research has shown that porcine endogenous retroviruses probably do not present a risk for recipients of xenotransplants provided all necessary precautions are taken [43–46]. In addition, a strain of miniature pigs has been identified that does not produce infective PERV [47]. Although xenotransplantation poses numerous further challenges to research, it is expected that transgenic pigs will be available as organ donors within the next five to ten years. Guidelines for the clinical application of porcine xenotransplants are already available in the USA and are currently being developed in several other countries.

The ethical challenges of xenotransplantation have been a matter of a worldwide intensive debate. A general consensus has been reached that the technology is ethically acceptable provided the individual well-being does not compromise public health by producing and transmitting new pathogens. Economically xenotransplantation might be viable if the enormous costs caused by patients suffering from severe kidney disease, needing dialysis or those suffering from chronic heart diseases could be avoided by a functional kidney or heart xenograft.

**Table 6. Success rates of RCA-transgenic porcine organs after transplantation to primate recipients**

<table>
<thead>
<tr>
<th>RCA</th>
<th>Organ/kind of transplant</th>
<th>Recipient</th>
<th>Immuno-suppression</th>
<th>Survival (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hDAF</td>
<td>Heart/heterotopic</td>
<td>Cynomologus</td>
<td>++</td>
<td>~135</td>
</tr>
<tr>
<td>hDAF</td>
<td>Heterotopic</td>
<td>Cynomologus</td>
<td>++</td>
<td>~90</td>
</tr>
<tr>
<td>hDAF</td>
<td>Orthotic</td>
<td>Baboon</td>
<td>++</td>
<td>~28</td>
</tr>
<tr>
<td>hDAF</td>
<td>Kidney/orthotopic</td>
<td>Cynomologus</td>
<td>++</td>
<td>~90</td>
</tr>
<tr>
<td>hCD59</td>
<td>Kidney/orthotopic, heterotic</td>
<td>Cynomologus</td>
<td>+++</td>
<td>~20</td>
</tr>
<tr>
<td>hCD46</td>
<td>Heart, heterotic</td>
<td>Baboon</td>
<td>++</td>
<td>~23</td>
</tr>
</tbody>
</table>

*Abbreviations: ++, weak immuno-suppression; +++, moderate immuno-suppression; ++++, heavy immuno-suppression; RCA, regulator of complement activity*
as a novel therapy for liver diseases. On transplantation of porcine hepatocytes to Watanabe heritable hyperlipidemic (WHHL) rabbits (a model for familial hypercholesterolemia) the xenogenic cells migrated out of the vessels and integrated into the hepatic parenchyma. The integrated porcine hepatocytes provided functional low density lipoprotein (LDL) receptors and thus reduced cholesterol levels by 30–60% for at least 100 days [56].

A clone of bovine adrenocortical cells restored adrenal function upon transplantation to adrenalectomized severe combined immunodeficient (SCID) mice indicating that functional endocrine tissue can be derived from a single somatic cell [57]. Bovine neuronal cells were collected from transgenic fetuses, and when transplanted into the brain of rats resulted in significant improvements in symptoms of Parkinson’s disease [58]. Furthermore, xenotransplantation of retinal pigment epithelial cells holds promise for treating retinal diseases such as macular degeneration, which is associated with photoreceptor losses. Porcine or bovine fetal cardiomyocytes or myoblasts might provide a therapeutic approach for the treatment of ischemic heart disease. Similarly, xenogenic porcine cells might be valuable for the repair of skin or cartilage damage [50]. In light of the emergence of significantly improved protocols for genetic modification of donor animals and new powerful immunosuppressive drugs xenogeneic cell therapy will evolve as an important therapeutic option for the treatment of human diseases.

Farm animals as models for human diseases

In mouse genetics the generation of knockout animals is a standard procedure and several thousand strains carrying gene knockouts or transgenes have been developed [Mouse Knockout and Mutation Database (MKMD); http://research.bmn.com/mkmd]. Models have been developed for several human diseases. However, mouse physiology, anatomy and life span differ significantly from those in humans, making the rodent model inappropriate for several human diseases. Farm animals, such as pigs, sheep or even cattle could be more appropriate models to study human diseases in particular non-insulin-dependent diabetes, cancer and neurodegenerative disorders, which require longer observation periods than those possible in mice [59–61]. With the aid of the microinjection technology an important pig model for the rare human eye disease Retinitis pigmentosa (PR) has been developed [62]. Patients with PR develop night blindness early in life attributed to a loss of photoreceptors. The transgenic pigs express a mutated rhodopsin gene and show a great similarity with the human phenotype. Treatment models with value for human patients are being developed [63].

The development of the somatic cloning technology and the merger with targeted genetic modifications and conditional gene expression will enhance the possibilities for creating useful models for human diseases in large animals. A good example is the knockout of the prion gene that would make sheep and cattle non-susceptible to spongiform encephalopathies (scrapie and BSE). Mice models showed that the knockout of the prion protein is the only secure way to prevent infection and transmission of the disease [64]. The first successful targeting of the ovine prion locus has been reported; however the cloned lambs carrying the knock out locus died several days after birth [65]. Prion knockout animals could be an appropriate model for studying the epidemiology of spongiform encephalopathies in humans and are crucial for developing strategies to eliminate prion carriers from a farm animal population.

The pig could be a useful model to study defects of growth hormone releasing hormone (GHRH), which is characterized by a variety of conditions such as Turner syndrome, hypochondroplasia, Crohn’s disease, intrauterine growth retardation or renal insufficiency. Application of recombinant GHRH in an injectable form and its myogenic expression has been shown to alleviate these problems in a porcine model [66].

An important aspect of large animal models for human diseases is the recent finding that somatic cloning per se does not result in shortening of the telomeres. Telomeres are highly repetitive DNA sequences at the end of the chromosomes that are crucial for their structural integrity and function and are thought to be related to lifespan. Telomere shortening is usually correlated with severe limitations of the regenerative capacity of cells, the onset of cancer, ageing and chronic disease with significant impact on human lifespan [67–70]. Expression of the enzyme telomerase, which is primarily responsible for the formation and rebuilding of telomeres, is suppressed in most somatic tissues postnatally. Although telomeres in cloned sheep (Dolly) derived from epithelial cells were shorter than those of naturally bred age matched control animals, telomere lengths in cloned mice and cattle were not different from those determined in age matched controls even when senescent donor cells had been employed [71–75]. Recent studies in our laboratory have revealed that telomere length is established already early in preimplantation development by a specific genetic programme and is dependent on telomerase activity [76].

Dietary modifications of animal products

Application of gene and biotechnology for nutrition and biomedicine is more developed for plants than farm animals [77]. The term nutriceuticals in the farm animal context means that gene and biotechnology are used to enhance farm animal products to improve diet and have concomitant medical applications. Because these products have a proven pharmacological effect on the body, they are regarded as ‘drugs’ and must be tested for efficacy and safety. Functional foods are those designed to provide specific and beneficial physiological effects on human health and welfare and should prevent diet-related diseases. Functional foods from animals could be used to lower cholesterol levels in an effort to battle cardiovascular diseases, to reduce high blood pressure by adding angiotensin converting enzyme (ACE) inhibitors or to increase immunity by adding specific immunostimulatory peptides [78]. However, data showing that nutriceuticals are really beneficial for human health are rare.

Regarding the production of improved quality animal products, interesting observations have been made in several beef cattle breeds, such as Belgian Blue and Piedmontese. These breeds were accidentally bred for
mutations of the myostatin gene, which renders it non-functional or less functional than the wild-type gene [79]. The mutated genes cause muscle hypertrophy and led to improved meat quality. This observation makes targeted modifications of the myostatin gene an interesting option for the meat industry. A diet rich in non-saturated fatty acids is correlated with a reduced risk of stroke and coronary diseases. One transgenic approach to this is the generation of pigs with increased amounts of non-saturated fatty acids. Pigs producing a higher ratio of unsaturated versus saturated fatty acids in their muscles are currently under development in Japan.

An attractive example for targeted genetic modification could be dairy production [80,81]. Apart from conventional dairy products, it could be possible to produce fat-reduced or even fat-free milk or milk with a modified lipid composition via modulation of enzymes involved in lipid metabolism; to increase curd and cheese production by enhancing expression of the casein gene family in the mammary gland; to create ‘hypoallergenic’ milk by knockout of the β-lactoglobulin gene; to generate lactose-free milk via knockout of the α-lactalbumin locus that is the key molecule in milk sugar synthesis; to produce ‘infant milk’ in which human lactoferrin is abundantly available or to produce milk with a highly improved hygienic standard via an increased level of lysozyme or other anti-microbiological substances in the udder. Lactose-reduced or lactose-free milk could make dairy products suitable for consumption by a large proportion of the world’s population who do not possess an active lactase enzyme in their gut system. However, one has to bear in mind that lactose is the main osmotically active substance in milk and a lack thereof could interfere with milk secretion. A lactase construct has been expressed in the mammary gland of transgenic mice and reduced lactose contents by 50–85% without altering milk secretion [82]. However, mice with a homozygous knockout for α-lactalbumin could not feed their offspring because of the high viscosity of the milk [83]. These diverging findings demonstrate the feasibility of obtaining significant alterations of milk composition by applying the appropriate strategy.

The physicochemical properties of milk are mainly affected by the ratio of casein variants. Therefore, casein is a prime target for the improvement of milk composition. Mouse models have been developed for most of the above modifications indicating the feasibility of obtaining significant alterations in milk composition but at the same time showing that unwanted side effects cannot be ruled out [83,84]. Only one full-scale study in livestock has been reported as yet [85]. The recent report showed that the casein ratio can be altered by overexpression of β- and κ-casein in cattle clearly underpinning the potential for improvements in the functional properties of bovine milk [85].

Towards environmentally friendly farm animals
Phosphorus pollution by animal production is a serious problem in agriculture and excess phosphate from manure promotes eutrophication. Phytase transgenic pigs have been developed to address the problem of manure-based environmental pollution. These pigs carry a bacterial phytase gene under the transcriptional control of a salivary gland specific promoter, which allows the pigs to digest plant phytate. Without the bacterial enzyme, the phytate phosphorus passes undigested into manure and pollutes the environment. With the bacterial enzyme, the fecal phosphorus output was reduced up to 75% [86]. These environmentally friendly pigs are expected to enter the commercial production chains within the next few years.

Precautions and perspectives
Throughout mankind’s history farm animals have made significant contributions to human health and well-being. The convergence of the recent advances in reproductive technologies with the tools of molecular biology opens a new dimension for this area [1]. Major prerequisites will be the continuous refinement of reproductive biotechnologies and a rapid completion of livestock genome sequencing and annotation. The technology developed in the deciphering of the human genome will improve and accelerate sequencing of genomes from livestock [87]. We anticipate genetically modified animals will play a significant role in the biomedical arena, in particular via the production of valuable pharmaceutical proteins and the derivation of xenografts, within the next 5–7 years. Agricultural application might be further away (>10 years) given the complexity of some of the economically important traits and the public skepticism of genetic modification related to food production [1].

A crucial aspect of animal-derived products is the prevention of transmission of pathogens from animals to humans. This requires sensitive and reliable diagnostic and screening methods for the various types of pathogenic organisms. The recent findings (see above) that the risk of PERV transmission is negligible are promising and show that with targeted and intensive research such important questions can be answered within a limited period of time, paving the way for preclinical testing of xenografts. Furthermore, it should be kept in mind that the biomedical applications of farm animals will require strict standards of ‘genetic security’ and reliable and sensitive methods for the molecular characterization of the products. A major contribution towards the goal of well-defined products will come from array technology (cDNA, peptide or protein arrays), which establishes ‘fingerprint’ profiles at the transcriptional and/or protein level [88,89]. Meanwhile improvements of RNA isolation and unbiased amplification of tiny amounts of mRNA (picogram) enable researchers to analyse RNA from single embryos [90]. With the aid of this technology one can gain in-depth insight into the proper functioning of a transgenic organism and thereby ensure the absence of unwanted side effects [88,89]. This would also be required to maintain the highest possible levels of animal welfare in cases of genetic modification.

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