The perspectives of genetically modified livestock in agriculture and biomedicine

• Agricultural perspectives
• Biomedical perspectives

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Mariensee, Neustadt, Germany
Projected increase in human global population

Quelle: UN, World Population Prospects: The 2010 Revision, 2011
Selected constraints of agricultural production

- ~5% of global land is usable for agriculture
- Increase in affluency if several parts of the world is associated with changes in diet towards more valuable animal proteins
- Environmental constraints of livestock production (~1/3 of climate relevant emission comes from agriculture)
- FAO: 1.3 kg CO$_{2\text{eq}}$/milk (Northern America, Europe); 7.5 CO$_{2\text{eq}}$/milk (Africa)

- **Consequences**: Food production needs to be doubled or tripled

- **Need**: higher productivity without detrimental side effects (sustainable intensification).
A new era in biology: Genome sequencing, somatic cloning and embryonic stem cells

1997

2004: first draft of bovine and chicken genome
Agricultural perspectives of genetically modified farm animals

- Growth and development (myostatin, GH, GHrec, IGF)
- Wool production
- Lactation (amount, composition)
- Hornless cattle (Polled locus)
- Disease resistance (Mx-gene, IgA, BSE, TB, PRRS, etc)
- Reproduction
- Environmental improvements (Phytase)
- Dietetic improvements
- Skewing the gender
## Improvements in economically important parameters of growth hormone transgenic swine

<table>
<thead>
<tr>
<th>Constructs</th>
<th>mMTI-bGH*</th>
<th>hMTI-pGH(cDNA)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain</td>
<td>+ 23</td>
<td>10 - 20%</td>
</tr>
<tr>
<td>Feed efficiency</td>
<td>+ 18</td>
<td>10 - 15%</td>
</tr>
<tr>
<td>Backfat thickness</td>
<td>7.5 mm</td>
<td>significantly reduced</td>
</tr>
<tr>
<td>(from 21 mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>„Side effects“ GH)</td>
<td>+ + +</td>
<td>- (30 - 40ng/ml)</td>
</tr>
</tbody>
</table>

*Pursel et al. 1990; **Seamark and Nottle (BresaGen)
Cattle and sheep with TALEN induced knockout of the myostatin gene

Proudfoot et al., Trans. Res. 24, 2015
Myostatin knockout pigs after employing TALEN

Produced in Korea 2015

Gene-edited minipigs as pets

BGI announced its plan to sell the micropigs as pets at a summit in Shenzhen, China.

Cyranoski, Nature 526, 2015
Transgenic animals with improved fibre production

<table>
<thead>
<tr>
<th>Introduced modification</th>
<th>Application</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovine insulin-like growth factor 1</td>
<td>6.2% more fleece</td>
<td>Sheep</td>
<td>Damak et al. 1996</td>
</tr>
<tr>
<td>Ovine growth hormone</td>
<td>Improved wool production</td>
<td>Sheep</td>
<td>Adams et al. 2002</td>
</tr>
<tr>
<td>Ovine keratine intermediate filament</td>
<td>Improved wool processing and wearing properties</td>
<td>Sheep</td>
<td>Bawden et al. 1998</td>
</tr>
<tr>
<td>Bacterial serine transacytelase and O-acetylserine sulfhydrylase</td>
<td>Improved wool production</td>
<td>Sheep</td>
<td>Ward 2000</td>
</tr>
</tbody>
</table>
### Genetically modified animals with improved milk production

<table>
<thead>
<tr>
<th>Introduced modification</th>
<th>Application</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine α-lactalbumin</td>
<td>Increase milk yield and piglet survival</td>
<td>Pig</td>
<td>Wheeler et al. 2001</td>
</tr>
<tr>
<td>Bovine β- and κ-casein</td>
<td>Improved milk composition</td>
<td>Cattle</td>
<td>Brophy et al. 2003</td>
</tr>
<tr>
<td>siRNA-β-lactoglobulin</td>
<td>reduced allergenicity</td>
<td>Cattle</td>
<td>Jabei et al. 2012</td>
</tr>
<tr>
<td>β-lac-GFP-neo-L ycostaphin</td>
<td>improved udder health</td>
<td>Cattle</td>
<td>Wall et al. 2005</td>
</tr>
<tr>
<td>hlysozyme in β-cas locus</td>
<td>improved udder health</td>
<td>Cattle</td>
<td>Liu et al. 2014</td>
</tr>
</tbody>
</table>
Targeted microRNA expression in dairy cattle directs production of β-lactoglobulin-free, high-casein milk

Anower Jабed, Stefan Wagner, Judi McCracken, David N. Wells, and Goetz Laible

AgResearch, Hamilton 3240, New Zealand; and Department of Biological Sciences, University of Waikato, Hamilton 3240, New Zealand

Edited by R. Michael Roberts, University of Missouri, Columbia, MO, and approved August 28, 2012 (received for review June 22, 2012)

Milk from dairy cows contains the protein β-lactoglobulin (BLG), which is not present in human milk. As it is a major milk allergen, we wished to decrease BLG levels in milk by RNAi. In vitro screening of 10 microRNAs (miRNAs), either individually or in tandem combinations, identified several that achieved as much as a 98% knockdown of BLG. One tandem construct was expressed in the mammary gland of an ovine BLG-expressing mouse model, resulting in 96% knockdown of ovine BLG in milk. Following this in vivo validation, we produced a transgenic calf, engineered to express these tandem miRNAs. Analysis of hormonally induced milk from this calf demonstrated absence of BLG and a concurrent increase of all casein milk proteins. The findings demonstrate miRNA-mediated depletion of an allergenic milk protein in cattle and validate targeted miRNA expression as an effective strategy to alter milk composition and other livestock traits.

nuclear transfer | transgenic cattle

Jабed et al. 2012, PNAS 109, 16811-6
miRNA-mediated depletion of BLG in bovine milk

Jabed A et al. PNAS 2012;109:16811-16816
Targeted microRNA expression in dairy cattle directs production of β-lactoglobulin-free, high-casein milk

<table>
<thead>
<tr>
<th>Cow</th>
<th>Milk</th>
<th>Total</th>
<th>Casein, mg/g</th>
<th>Whey, mg/g</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>α-Lac</td>
<td>BLG-A</td>
</tr>
<tr>
<td>miRNA 6-4</td>
<td>Induced, day 1</td>
<td>98.2</td>
<td>3.9</td>
<td>0.0</td>
</tr>
<tr>
<td>miRNA 6-4</td>
<td>Induced, day 2</td>
<td>96.8</td>
<td>3.5</td>
<td>0.0</td>
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<tr>
<td>miRNA 6-4</td>
<td>Induced, day 3</td>
<td>106.6</td>
<td>4.3</td>
<td>0.0</td>
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<tr>
<td>miRNA 6-4</td>
<td>Induced, day 4</td>
<td>128.6</td>
<td>5.3</td>
<td>0.0</td>
</tr>
<tr>
<td>WT-1</td>
<td>Natural, day 69</td>
<td>39.6</td>
<td>1.5</td>
<td>5.7</td>
</tr>
<tr>
<td>WT-2</td>
<td>Induced, day 5</td>
<td>38.8</td>
<td>1.5</td>
<td>7.6</td>
</tr>
<tr>
<td>WT-3</td>
<td>Induced, day 5</td>
<td>32.5</td>
<td>1.5</td>
<td>7.3</td>
</tr>
<tr>
<td>WT-4</td>
<td>Colostrum, day 1</td>
<td>48.1</td>
<td>1.7</td>
<td>10.1</td>
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<tr>
<td>SEM-*</td>
<td>-</td>
<td>1.27</td>
<td>0.09</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Jabed A et al. PNAS 2012;109:16811-16816
In vitro knockdown of BLG in COS-7 cells.

Jabed A et al. PNAS 2012;109:16811-16816
Production of cattle with elevated concentration of β- and κ-Casein in milk

<table>
<thead>
<tr>
<th>Cow</th>
<th>Cell line</th>
<th>Age at induction, months</th>
<th>Protein&lt;sup&gt;b&lt;/sup&gt;, %</th>
<th>Protein&lt;sup&gt;b&lt;/sup&gt; Casein (CN)&lt;sup&gt;a&lt;/sup&gt;, mg/ml</th>
<th>β-CN&lt;sup&gt;a&lt;/sup&gt;, mg/ml</th>
<th>κ-CN&lt;sup&gt;a&lt;/sup&gt;, mg/ml</th>
<th>β-CN:CN</th>
<th>κ-CN:CN</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td>Cows induced in July 2001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG2</td>
<td>TG2</td>
<td>8</td>
<td>5.6</td>
<td>44.5</td>
<td>15.8</td>
<td>11.2</td>
<td>0.36</td>
<td>0.26</td>
</tr>
<tr>
<td>TG3-1</td>
<td>TG3</td>
<td>7</td>
<td>6.9</td>
<td>54.9</td>
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<td>10.7</td>
<td>0.36</td>
<td>0.20</td>
</tr>
<tr>
<td>TG3-2</td>
<td>TG3</td>
<td>7</td>
<td>5.2</td>
<td>42.0</td>
<td>17.6</td>
<td>11.6</td>
<td>0.42</td>
<td>0.29</td>
</tr>
<tr>
<td>TG3-3</td>
<td>TG3</td>
<td>8</td>
<td>5.9</td>
<td>47.5</td>
<td>20.9</td>
<td>12.0</td>
<td>0.44</td>
<td>0.24</td>
</tr>
<tr>
<td>TG3-4</td>
<td>TG3</td>
<td>7</td>
<td>5.9</td>
<td>47.2</td>
<td>18.4</td>
<td>10.1</td>
<td>0.39</td>
<td>0.22</td>
</tr>
<tr>
<td>TG3-5</td>
<td>TG3</td>
<td>7</td>
<td>5.3</td>
<td>43.0</td>
<td>14.1</td>
<td>8.4</td>
<td>0.33</td>
<td>0.20</td>
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<tr>
<td>Mean</td>
<td>TG3</td>
<td></td>
<td>5.8</td>
<td>46.9</td>
<td>18.2</td>
<td>10.6</td>
<td>0.39</td>
<td>0.24</td>
</tr>
<tr>
<td>CC-1</td>
<td>NA</td>
<td>10</td>
<td>4.8</td>
<td>38.2</td>
<td>14.3</td>
<td>5.1</td>
<td>0.40</td>
<td>0.15</td>
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<tr>
<td>CC-2</td>
<td>NA</td>
<td>10</td>
<td>5.0</td>
<td>39.6</td>
<td>14.8</td>
<td>5.8</td>
<td>0.37</td>
<td>0.15</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>4.8</td>
<td>37.9</td>
<td>14.6</td>
<td>5.5</td>
<td>0.39</td>
<td>0.15</td>
</tr>
<tr>
<td><strong>B</strong></td>
<td>Cows induced in December 2001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG3-6</td>
<td>TG3</td>
<td>9</td>
<td>4.5</td>
<td>33.8</td>
<td>14.0</td>
<td>10.7</td>
<td>0.41</td>
<td>0.32</td>
</tr>
<tr>
<td>TG3-7</td>
<td>TG3</td>
<td>9</td>
<td>6.4</td>
<td>34.8</td>
<td>17.8</td>
<td>13.0</td>
<td>0.51</td>
<td>0.37</td>
</tr>
<tr>
<td>TG3-8</td>
<td>TG3</td>
<td>9</td>
<td>4.5</td>
<td>33.8</td>
<td>17.0</td>
<td>14.1</td>
<td>0.50</td>
<td>0.42</td>
</tr>
<tr>
<td>TG7</td>
<td>TG7</td>
<td>7</td>
<td>3.8</td>
<td>25.6</td>
<td>12.2</td>
<td>5.7</td>
<td>0.48</td>
<td>0.22</td>
</tr>
<tr>
<td>Mean</td>
<td>TG3</td>
<td></td>
<td>5.1</td>
<td>34.1</td>
<td>16.3</td>
<td>12.6</td>
<td>0.48</td>
<td>0.36</td>
</tr>
<tr>
<td>BFF-1</td>
<td>BFF</td>
<td>7</td>
<td>3.8</td>
<td>26.4</td>
<td>14.4</td>
<td>5.0</td>
<td>0.55</td>
<td>0.19</td>
</tr>
<tr>
<td>BFF-2</td>
<td>BFF</td>
<td>7</td>
<td>5.1</td>
<td>24.0</td>
<td>14.9</td>
<td>5.0</td>
<td>0.62</td>
<td>0.21</td>
</tr>
<tr>
<td>BFF-3</td>
<td>BFF</td>
<td>7</td>
<td>4.5</td>
<td>25.5</td>
<td>14.5</td>
<td>5.0</td>
<td>0.57</td>
<td>0.20</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>4.5</td>
<td>25.3</td>
<td>14.6</td>
<td>5.0</td>
<td>0.58</td>
<td>0.20</td>
</tr>
</tbody>
</table>

<sup>a</sup>Based on skim milk.<sup>b</sup>Based on whole milk. Milk samples were collected after hormonal induction in July 2001 (A) and December 2001 (B). Milk from cows induced in July 2001 was analyzed for total protein and casein (CN) using infrared spectroscopy and milk from cows induced in December 2001 was analyzed by total combustion (protein) and HPLC (CN). β- and κ-casein concentrations were determined nephelometrically.

Brophy et al. 2003, Nature Biotechnology 21, 157-162
Milk from hLZ transgenic goats helps children with diarrhea

Maga et al., 2014
TALEN induced mutation of the Polled locus to produce cattle without horns

Tan et al., PNAS 110, 16526-531; 2013
Gene-editing of Polled locus: Spotiguy, born 2015, with two of his clones

Carlson et al., Nature Biotechnology 2016
Approaches towards animals transgenic for enhanced disease resistance

- **General:**
  - Mx 1 protein (pigs)
  - Immunoglobulin A (pigs)
  - Visna virus envelope (sheep)
  - Transmissible Gastroenteritis virus (TGEV; mammary gland specific mouse model)
  - Knockout of specific genes (f.ex. Prion; PRRS, PERVs)
  - siRNA mediated knockdown of pathogenic virus expression

- **Mammary gland:**
  - α-Lactalbumin (pigs)
  - Lysozyme (goats, cattle): antimicrobial effects
  - Lactoferrin (cattle): bacteriostatic, bacteriocidic, iron provider
  - Lycostaphin-transgenesis: St. aureus resistant cows
Transgenic cattle with mit Lysostaphin induced resistance of the mammary gland against infections with *St. aureus*

Resistance against *St. aureus* infusions: Tg: 18/21 (85.3%) (Infection only after very high doses) vs. WT 13/47 (31.7%)
Mastitis resistant cows by transgenic expression of human lysozyme expression from the β-casein locus

Table 4. Infection rate of three types of bacterium infused into mammary glands of five transgenic and five non-transgenic lactating cows. During each challenge experiment, each gland was infused with one of the three types of bacterium and the fourth gland was infused with PBS. TG, transgenic cows; WT, non-transgenic cows.

<table>
<thead>
<tr>
<th>group</th>
<th>mammary glands treated</th>
<th>mammary glands infected&lt;sup&gt;a&lt;/sup&gt;</th>
<th>number of bacteria (× 10&lt;sup&gt;3&lt;/sup&gt; CFU ml&lt;sup&gt;−1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0 h</td>
</tr>
<tr>
<td>TG</td>
<td>5 (Sta. aureus)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TG</td>
<td>5 (Str. agalactiae)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TG</td>
<td>5 (E. coli)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TG</td>
<td>5 (PBS)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>WT</td>
<td>5 (Sta. aureus)</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>WT</td>
<td>5 (Str. agalactiae)</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>WT</td>
<td>5 (E. coli)</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>WT</td>
<td>5 (PBS)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup>Infection was defined as bacterium growth in two consecutive milk samples collected 12–24 h apart.

Mastitis resistant cows by transgenic expression of human lysozyme expression from the β-casein locus

Mastitis resistant cows by transgenic expression of human lysozyme expression from the β-casein locus

Table 3. Raw components of transgenic milk compared with conventional milk. No significant differences were detected between transgenic and non-transgenic groups ($p > 0.05$).

<table>
<thead>
<tr>
<th>Components (g 100 ml$^{-1}$)</th>
<th>Transgenic ($n = 5$)</th>
<th>Non-transgenic ($n = 5$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>4.38 ± 0.39</td>
<td>4.47 ± 0.35</td>
</tr>
<tr>
<td>Protein</td>
<td>3.62 ± 0.28</td>
<td>3.53 ± 0.24</td>
</tr>
<tr>
<td>Lactose</td>
<td>4.69 ± 0.21</td>
<td>4.81 ± 0.38</td>
</tr>
<tr>
<td>Solids</td>
<td>13.89 ± 0.77</td>
<td>13.55 ± 0.69</td>
</tr>
</tbody>
</table>
Increased Resistance against Tuberculosis via gene editing (TALEN) and transgenic technology

- The mouse SP110 gene can control M.bovis growth in macrophages and induce apoptosis in infected cells.
- Transfer of the mouse SP110 gene into the genome of Holstein-Friesian (Macrophage Scavenger Receptor (MSR1)-locus) by TALENs led to an increased resistance against M.bovis infection by macrophage-specific expression of SP110.

Cattle resistant against mycobacterium tuberculosis infection, produced via gene editing and transgenic technologies

Cattle with resistance to BSE after knockout of the prion locus

Figure 1 Generation of \( PRNP^{-/-} \) cattle. (a) \( PRNP^{-/-} \) cattle at 13 months of age. (b) Verification of \( PRNP^{-/-} \) genotype in the ear biopsy fibroblasts by genomic PCR. P, positive control; N, negative.

Richt et al., 2007, Nature Biotechnology
PRRS resistant pigs after CRISPR/Cas mediated knockout of CD 163

CRISPR/Cas-derived CD 163 knockout piglets were kept together with WT-piglets. All animals were infected with PRRS virus. Five days later, the WT-piglets showed typical PRRSV symptoms, while CD 163 KO piglets remained completely healthy.

(PRRS: Porcine reproductive and respiratory syndrome virus, CD163 is a macrophage differentiation antigen belonging to the scavenger receptor cysteine-rich (SRCR) family of membrane proteins)

Whitworth et al., Nature Biotechnology 34, 20-22 (2016)
Foto: University of Missouri
PRRS resistant pigs after CRISPR/Cas mediated knockout of CD 163

PRRS: Porcine reproductive and respiratory syndrome virus

Whitworth et al., Nature Biotechnology 34, 20-22 (2016)
PRRS resistant pigs after CRISPR/Cas mediated knockout of CD 163

Microscopic image of the lung of CD163+/+ and CD163-/- pigs

PRRS: Porcine reproductive and respiratory syndrome virus

Whitworth et al., Nature Biotechnology 34, 20-22 (2016)
PRRS resistant pigs after CRISPR/Cas mediated knockout of CD 163

PRRS specific DNA (a) und antibodies (b)

PRRS: Porcine reproductive and respiratory syndrome virus

Whitworth et al., Nature Biotechnology 34, 20-22 (2016)
Gene-editing record smashed in pigs

Researchers modify more than 60 genes (PERV) in effort to enable organ transplants into humans.

The gene-edited pigs will be raised in isolation from pathogens.

Also ~20 genes altered related to immunology and relevant for Xenotransplantation
Spinach desaturase expression in transgenic pigs alters fatty acid profile in skeletal muscle

Desaturase expression in transgenic pigs leads to meat with more poly-unsaturated fatty acids.

Saeki et al., 2004, PNAS
n-3 and n-6 fatty acids concentration and n-6/n-3 ratios in tail samples from *hfat-1* transgenic and wild-type piglets

<table>
<thead>
<tr>
<th>Fatty acids in tails</th>
<th>Transgenic piglets (n = 8)</th>
<th>Wild-type piglets (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALA (18:3 n-3, %)</td>
<td>0.94 ± 0.10</td>
<td>0.63 ± 0.04</td>
</tr>
<tr>
<td>EPA (20:5 n-3, %)</td>
<td>4.21 ± 0.60</td>
<td>0.26 ± 0.07</td>
</tr>
<tr>
<td>DPA (22:5 n-3, %)</td>
<td>1.69 ± 0.19</td>
<td>0.35 ± 0.05</td>
</tr>
<tr>
<td>DHA (22:6 n-3, %)</td>
<td>1.75 ± 0.23</td>
<td>0.95 ± 0.21</td>
</tr>
<tr>
<td>Total n-3 FA (%)</td>
<td>8.59 ± 0.84</td>
<td>2.18 ± 0.25</td>
</tr>
<tr>
<td>Total n-6 FA (%)</td>
<td>14.28 ± 1.31</td>
<td>18.46 ± 1.41</td>
</tr>
<tr>
<td>n-6/n-3 ratio</td>
<td>1.69 ± 0.30</td>
<td>8.52 ± 0.62</td>
</tr>
</tbody>
</table>

Lai et al. 2006, Nature Biotechnology
Transgenic pigs with expression of Phytase in salivary gland

Golovan et al., 2001, Nature Biotechnology
Transgenic swine expressing Phytase in the salivary gland

Expression of Phytase in the salivary gland of transgenic pigs

Reduction of anorganic phosphorus feeding via improved metabolism

Reduction of phosphorus excretion by up to 75%

Reduction of costs

Environment protection

Golovan et al. (2001), Nature Biotechnol. 19, 741-745
Transgenic animals with agriculturally important traits

- **Lactational performance**

  *Transgenic cattle*: lactoferrin, lysozyme, caseins; but problems in some of the mouse models with milk production, *Mariensee*: Lactase transgenic mice with significant effects on lactose levels
  
  *Transgenic pigs*: bovine α-lactalbumin: elevated lactose levels better piglet performance.

- **Dietetic improvements**

  *Transgenic pigs*: unsaturated fatty acids by introduction of spinach desaturase gene

- **Wool shearing**

- **Reproductive performance**

  (Estrogen receptor gene, inhibin reduction?)
Biomedical perspectives of genetically modified farm animals

• Biomedical perspectives
  
  Gene Pharming (rec. Proteins, mAbs)
  Human blood substitute
  Xenotransplantation
  Inhibitors of chemical weapons

• Basic research
  
  Epigenetic reprogramming
  Models for human diseases
Approved GM vertebrates

- GloFisch, genetically engineered zebrafish, no regulation necessary (FDA statement 2003)

- Atryn (antithrombin III), produced in the mammary gland of transgenic goats, approved by EMA (2006) and FDA (2009)

- Ruconest (C1 esterase inhibitor), produced in the mammary gland of transgenic rabbits, approved by EMA (2010)

- AquAdvantage salmon, added growth hormone from Pacific Chinook salmon, all year long expression, faster growth, approved by FDA (Nov. 2015)
Transgenic chickens are the latest animals engineered to produce ‘farmaceutical’ drugs.

US government approves transgenic chicken
Pigs fed hLZ- milk have improved fecal consistency and activity scores

Towards the ultimate donor pig

**CHOICE CUTS**
Researchers are looking to source an increasing variety of living tissues, including solid organs, from pigs. Many are attempting to genetically engineer the animals to reduce the risk of rejection and infection in humans.

- **LUNG**
  A factory farm is being designed to produce 1,000 pig lungs per year.

- **KIDNEY**
  A kidney with six genetic modifications supported a baboon’s life for 4 months.

- **CORNEA**
  Pig corneas were approved for marketing in China in April.

- **HEART**
  A genetically modified pig heart implanted in a baboon’s abdomen survived for 2.5 years.

- **LIVER**
  Livers could be engineered to produce their own antibodies against primate immune cells.

- **PANCREAS**
  Phase III clinical trials of insulin-producing islet cells are under way.

Nature 527, Nov. 2015
The domestic pig as a potential donor for human organs

• Domesticated species
• High fertility, great abundance, rapid growth
• Genetics, anatomy, physiology not too different from human
• Strict hygienic conditions possible
• Previous success with porcine insulin, heart valves, skin patches
• Genetic modifications possible

I like pigs; dogs look up to us, cats look down to us, pigs treat us as equal. (Winston Churchill)
Multi-transgenic pigs (GGTA1-KO/hCD46/hCD55/hCD59/hA20/hHO-1) for improved xenotransplantation results

Donor cells | Recipients | Transferred embryos | Pregnant (PR) | Liveborn offspring | Ongoing pregnancies | Delivery dates
---|---|---|---|---|---|---
164/3 (recloning 1706GalKo) | 10 | 983 (Ø = 98) | 7 (70%) | 4 | 3 | 15.11.14, 17.01.15, 18.01.15

Liveborn multi-transgenic piglets “Thomas” and „Müller“.

K. Fischer et al. Scientific Reports 2016
Successful use of TALEN in livestock

Tan et al., PNAS 110, 16526-531; 2013
Application perspectives for genetically modified farm animals

• Agricultural perspectives
  Growth and development (myostatin, GH, GHrec, IGF)
  Wool production
  Lactation (amount, composition)
  Hornless cattle (Polled locus)
  Disease resistance (Mx-gene, IgA, BSE, TB, PRRS, etc)
  Reproduction
  Environmental improvements (Dietetic improvements)

• Biomedical perspectives
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Evolution of farm animal breeding

- Domestication
- Proliferation of „useful“ populations
- Selection according to the Exterieur
- Selection according to specific traits
- Systematic breeding based on population genetics and statistics
- Reproductive technologies (AI, ET, IVP, SCNT, etc.)
- Molecular genetics and genome based breeding concepts (SNPs, GBV, etc.)
- Now entering the era of **Precision breeding**
The future: Targeted and diversified dairy production

- Full fat normal milk
- Defatted milk (knock-out of key lipid enzymes)
- Curd production (enhanced casein expression)
- Cheese production (enhanced casein expression)
- Coffee whitener and Creme liquor (β-casein)
- Hypo-allergenic milk (reduced or omitted β-lactoglobulin)
- Lactose free or -reduced milk (α- lactalbumin knock-out, additional lactase expression)
- Infant milk (enhanced lactoferrin expression)
- Improved udder health (lysozyme, etc.)
- Pharmaceutical proteins
Just the Beginning
Thank you for your attention.
Aquabounty salmon: The first approved genetically engineered animal product

AquAdvantage Atlantic salmon (at back) grow to twice the size of a normal Atlantic salmon (Salmo salar) over the same time.
The practical use of this new genomic information

- Better targeted breeding programmes
  *Genomic breeding values; Direct sequencing*
- Transcriptomics/Proteomics/Phenomics
- Production of genetically modified (transgenic) animals
- New knowledge on genetic diversity
- Descent studies
- Comparative genomics
Summary and Conclusions

• The genomes of farm animals have been sequenced and annotated; informative gene maps are available that can be used for breeding purposes (GBV).
• Novel molecular tools, incl. DNA-nucleases such as ZFNs, TALEN, CRISPR/Cas are compatible with precise genetic modifications (gene editing), that can be induced easily and with high efficiency.
• The use of the new genomic information and gene editing tools allow the development of novel breeding strategies, both for agricultural and biomedical purposes.
• Gene editors are also beneficial in human medicine.
• A complex and complicated legal framework is in place for commercial use of transgenic animals. The application of gene editing is not (yet) legally regulated and could thus be immediately employed in future oriented animal breeding systems.
Novel perspectives for animal breeding in agriculture and biomedicine