GE ANIMALS in New Zealand

2010 - 2025 years

GENETICALLY ENGINEERED ANIMALS: PART 2 - THE SECOND FIFTEEN YEARS

GE Free NZ in Food and Environment has compiled this report from information gathered through AgResearch annual reports and Official Information Act requests (OIA)

Written by Claire Bleakley

PREFACE

Immanuel Kant, the well-known German philosopher once said, "We can judge the heart of a man by his treatment of animals", a testament to his deep ethical insight on this and other matters.

When **GE Free NZ** asked me to look over this report, I remembered an interview I did with TVNZ in 2010 on genetically engineered (GE) animals. I was asked about the progress of these animals in the 2000s, and what conclusions we might draw from this work. Back then, things already looked gruesome for the animals and their offspring. Deaths of adults, young animals and foetuses were common, much more so than with their normal counterparts. Similarly, birth defects, spontaneous abortions and many abnormalities were also common. Sadly in the following years up to 2024, the animals have further suffered from the same range of chronic health problems, and many were killed to relieve the pain and discomfort.

In the first decade of the 2000s, a failed experimental venture on GE sheep resulted in the Scottish biotech company leaving NZ and consequently all its GE animals that were still alive were slaughtered and dumped into a large pit.

Using animals as bioreactors to create pharmaceutical compounds sounds awful, and it is. Animals not only suffer from the complications associated with the genetic engineering and gene editing processes, but their finely tuned metabolism is thrown into chaos, because of foreign proteins or bio-chemicals, often produced in significant amounts – otherwise why use the animal for a bioreactor?

Furthermore, there have been 15 different experimental trials on a range of sentient animals. "Climate Smart" cattle, engineered and edited to have a lighter coat colour. Consideration is not given to the fact that animals living in hot climates come in a range of colours from dark to light and are all well adapted to the heat. Goats have been engineered to produce female-only progeny. Sheep have been engineered and edited for xeno-transplantation of organs to humans. None of these experiments have produced a positive outcome. They have, however, raised serious ethical questions.

I grew up in Southland and every year there was a day when children could bring their calves and lambs to an agricultural event in Gore. The loving bond that children had with their animals was unmistakable. Many people love animals – pets, domestic or wild. It is a vital part of our co-existence with other species.

This experimentation, which has continued to subject animals to pain and death from genetic engineering and gene editing, shows a failure of our duty to care for animals. It has never been something we "needed" to do.

Animals are sentient beings, feeling pain, grief, confusion, happiness and more.

Elvira Dommisse (PhD)
Former GE scientist, Crop & Food,
Lincoln, New Zealand (1985-1993).

CONTENTS

GE Animals App:# ERMA200223

INTRODUCTION

The GE Animals report is a meta-assessment of genetically engineered (GE) and gene edited animals in AgResearch Ruakura New Zealand facility, with a focus on the years 2015-2024. The information is sourced from the AgResearch annual reports to the EPA and OIA requests.

It follows the previous report, GE Animals in New Zealand: The first 15 years (2000-2015). These reports highlight a range of serious concerns about scientific, welfare, and ethical issues arising from GE animal experimentation in cattle, goats, and sheep.

Key Findings:

- **Animal Welfare Issues:** The report documents chronic health problems, deformities, high rates of abortion, premature deaths, and routine euthanasia due to suffering in all GE animal lines. These issues persisted irrespective of technical advances from older transgenic techniques to newer gene editing methods.
- **Experimental Outcomes:** No GE or gene edited animal experiment at the Ruakura facility produced a commercially successful or welfare-positive outcome. Many projects ended with the euthanasia or death of all GE animals by 2024.
- **Widespread Euthanasia:** Animal disposal following failed pregnancies or experiments involved burial in "offal holes," with at least 58 such pits recorded for euthanised animals.
- **Low Live Birth/Survival Rates:** The live birth rate (0-15%) per transgenic pregnancy, remained extremely low, with most offspring dying before or shortly after birth as a result of deformities or chronic health issues.

Cows

- **Transgenic Traits Studied:** Increased casein production, hormones of pharmaceuticals, hypoallergenic milk, and altered coat colour.
- **Outcomes:** High abortion and deformity rates, with all lines discontinued by 2024 except for a small number of climate smart cattle. Embryo transfers rarely resulted in viable, healthy calves, and most animals were euthanised for health reasons or as surplus.

Goats

- **Traits Studied:** Production of pharmaceutical proteins, genes for female-only offspring.
- **Outcomes:** Nearly all GE goats were euthanised or died, with a few live births accompanied by high rates of complications, deformities, and reproductive failures. The last lines were culled in 2024, leaving only conventional goats for future experiments.

Sheep

- **Traits Studied:** gene for male sterility, gene edits for xenotransplantation compatibility, and earlier projects aiming to improve wool properties.
- **Outcomes:** High rates of abnormality, sterility, and death. Xenotransplantation lines suffered chronic illness and reproductive failure. None of the engineered lines achieved their intended biomedical or agricultural outcomes.

Additional Notes

- **Environmental Concerns:** The long-term impacts of disposing GE animal remains are unknown, including risks to soil and microbial ecosystems.
- **Transparency and Oversight:** Adverse effects added to the concerns over animal welfare and scientific merit.
- **GE Ryegrass Trials:** Failures in GE HME ryegrass field and nutritional trials are recorded; yield and methane reduction goals were not met, with performance worse than existing non-GE mixed herbal pastures or seaweed supplement alternatives.

SUMMARY

Genetically engineered animal trials in New Zealand from 2015-2024, at the AgResearch Ruakura facility, consistently failed to deliver viable, healthy animals that would have commercial benefits. The experiments resulted in high mortality, deformities, and chronic suffering among cattle, goats, and sheep, with nearly all GE animal lines ending in mass euthanasia. The ongoing pain, suffering, and lack of positive outcomes from GE animal experiments raise serious ethical questions about the justification and necessity of such research, especially given the consistent animal welfare failures and lack of justifiable outcomes.

GE ANIMALS OVERVIEW

In the years between 2015-2024 there was a high abortion rate in conventional surrogate cows who were recipients of transgenic or gene edited embryos. All euthanised or dead animals that had undergone a transgenic/gene edited pregnancy were buried in offal holes in the facility.

From 2000-2024, the AgResearch Ruakura facility developed cattle engineered with different genetically engineered (GE) proteins intended to be produced in the cow's milk.

Ag Research Facility
Photo: Steffan Browning

Animals in AgResearch Ruakura Facility

Cows

- **Casein + protein** - cows that express an extra casein transgene (TG) gene.
- **Recombinant Human Myelin Basic Protein** (rhMPB) a transgene expression in cow's milk for pharmaceutical purposes.
- **Recombinant human lactoferrin** (rhLF) protein expression in milk. Biotechnology company pharming has licenced the rights to AgResearch for the protein production of recombinant human lactoferrin in transgenic cows. This product will be used for nutraceutical and pharmaceutical products.
- **Recombinant human Follicle Stimulating hormone** (rhFSH). This trial was a horrific failure and two of the surviving cows were found dead after the hormone entered the bloodstream, causing enlarged twisted ovaries and a rupture of the uterine artery. The remaining calf was euthanised.
- **Recombinant Beta LactoGlobulin knockdown** (BLG-). The approved method of transgene insertion failed to produce viable embryos. In 2011, 107 BLG- gene edited embryos were implanted – one calf (Daisy) survived. Daisy had no tail and a fused right back hock making walking difficult.
- **Erbitux** – a recombinant chimeric monoclonal antibody to treat colorectal cancer.
- Coat Colour cows for purported tolerance to heat extremes.
- KDM4B

Goats

- Transgenic non-Mendelian (TRC) inheritance leads to sex-ratio distortion. All were born male.
- Erbitux and Enbrel - a recombinant chimeric monoclonal antibody intended to treat colorectal cancer.

Sheep

- AI on Hooves - breeding of NANOS2 male sterile sheep.
- Germline Deficient sheep gene edited using CRISPR.
- Immune Compatible sheep for xenotransplantation.
- The 2025 ERMA200223 annual report to the EPA show that all GE animals have been euthanised and there are no GE animals alive in the Ruakura facility.

GE COWS

Surrogate cattle are conventional Friesian cows who receive transgenic embryos.

From 2015-2024 the surrogate cows that received transgenic embryos, had an average of 8% live births.

Between 2015 and 2020 an annual average of 99 conventional cows, and in the four years of 2021-2024 an annual average of 97 surrogate recipient cows who received transgenic genetically engineered embryo transfer (ET) procedures. On average over the five years there was an 85% - 99% spontaneous abortion rate.

In addition, 53 cattle of varying ages were euthanised following veterinary advice. They were seen as; surplus, too old, unsuitable or needing to be put down for humane reasons.

Surrogate Cow Euthanasias.

- **Udder problems.**
- **Black mastitis infection.**
- **Hip dysplasia.**
- **Organ and metabolic problems.**
- **Hydrops.**
- **Pregnancy complications.**
- **85%-99% spontaneous abortions.**
- **2%-8% calves' survival to weaning.**

In 2018, 35 surrogate mothers received embryo transfers using the new method of transgenic gene editing. However, 20 foetuses were aborted. Further to this, 32 cattle of varying ages were euthanised for humane reasons following veterinary advice, due to enlarged udder, hydrops, organ and metabolic complications.

There were no embryo transfers in 2019 and 2020.

In the years 2021-2024, 431 surrogate cows, this includes cows that were repeat recipients. In these years an average of 85% recipients spontaneously aborted, and 63 recipients were euthanised for humane reasons such as hip dysplasia, pregnancy complications, eye cancer and udder problems.

In 2022, 73 surrogate recipient cows were hormonally stimulated to prepare the uterus for receiving embryo transfers. There was a high level of spontaneous abortions. Of the 73 cows 46 had a repeat procedure a few months later. there were 9 transgenic calves born.

In 2024, the Ruakura GE animal facility has 77 recipient cows to be used for embryo transfers.

A study by Turin et al (2007) analysed maternal blood samples for the presence of foetal DNA during gestation and post-partum periods. The Y chromosome-specific DNA was detected in up to 73% of blood samples from naturally mated heifers carrying conventional bull calves and a transgene-specific sequence in up to 50% of recipient cows carrying transgenic foetuses. These results indicate that crossing the species barrier, by using genetic material from humans and other sources to modify the host animal, has serious deleterious outcomes. This conclusion is also supported by experiments on chimeric animals conducted overseas.

Erbitux – Erbitux is a pharmaceutical that was engineered to produce the protein in the milk. After many trials only one female calf, Erbie, was born in 2012.

The recipient cows suffered from locomotor problems, lameness, eye cancer and abnormalities in placenta cotyledons. The report has changed the way it presents data and is missing vital information concerning the animals' health.

Erbie the sole Erbitux calf
Photo: Steffan Browning

In 2019, Erbie, was seven years old when she was killed. She suffered from lameness and back leg stiffness.

There are no surviving Erbitux cows.

This experiment on cattle was a failure. It was subsequently carried out on goats, see page 14.

BLGkd (Beta-Lacto Globulin knockdown). The approval for the original gene gun/Agrobacterium transfection of Beta-Lacto Globulin (BLG-) did not produce any viable embryos until 2011, when the use of gene editing technology was used to engineer the embryo. Out of 4 groups of surrogate recipients, no calves were born until 2013. In 2013, Daisy, a tailless BLGkd calf was born, the first after 12 years of experimentation.

All 17 of the pregnant GE cows and 68 of the recipient conventional cows spontaneously aborted their embryos; 2 conventional recipients calved prematurely with dead calves; the remaining 16 recipients bore live calves – 13 females and 3 male crosses BLG- x rhLF.

This was not approved by EPA, as conditions of the trials specified pure lines. Because no 67A Environmental Protection Agency(EPA) approval had been sought to create hybrid crosses, **this is a case of non-compliance, with respect to the regulations.**

Daisy's eggs were harvested and fertilised. The resulting embryos were transferred to 85 conventional and 17 GM recipient cows.

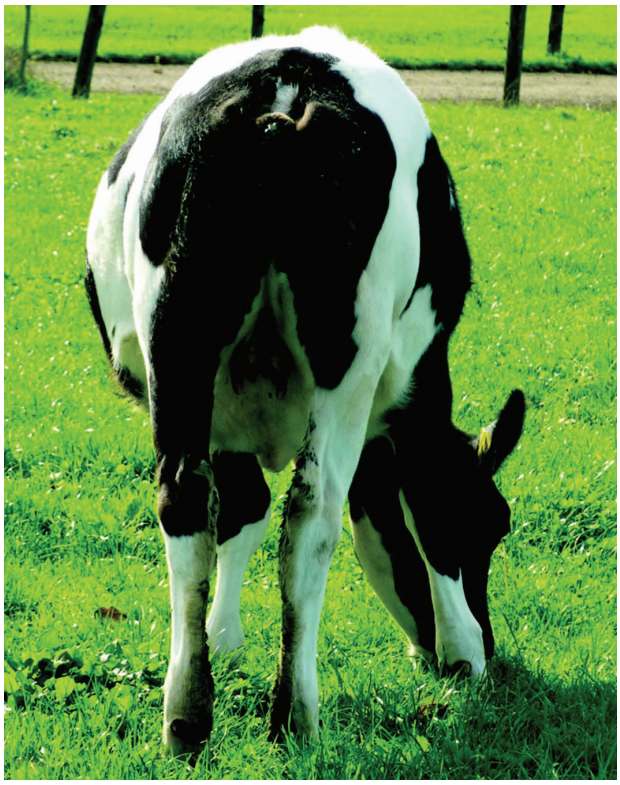
In 2017, Daisy, the sole surviving BLGkd cow was euthanised because of locomotor problems.

She was approximately 4 years old.

From the period 2018-2023, 11 BLGkd calves were born. One calf was born dead and 11 calves were euthanised on humane grounds, because of their age, or because they were simply not needed for further experimentation. This left 13 live heifer calves.

In 2024, all 13 animals were euthanised and there are now no BLGkd cows remaining in the facility.

BLG-/LZ (BLG-/LZ disruption lysosome gene edit) There were BLG-/LZ embryo transfers into 97 recipients/surrogates. Five live calves were born. Two calves (1 male and 1 female) carried the disruption of beta-lactoglobulin and their milk showed absences of the gene. Several calves showed the correct start of the gene, but incorrect integration at the end of BLG-/LZ gene. Data on this line stopped being recorded in early 2017.



Daisy at the age of 7 months.
View of the posterior end showing absence of tail.

Colour images available online at www.liebertpub.com/

hMBP – (human Myelin Basic Protein gene).

In October 2000, 60 recipient cows underwent embryo transfer. By December 2000, only 14 of these were still pregnant. On day 224, there were only 6 recipients still pregnant, of which one was carrying twins that ended up dying and decomposing in utero. Another calf died soon after birth. Ultimately, of the 4 remaining surrogate dams who calved, four calves were born alive and one was born dead.

In 2015, there were two remaining cattle. One 13 year old cow died and in 2017 the remaining 4 year old bull calf was killed, as it was no longer needed. The hMPB milk experiment was a failure, as the cattle suffered from sterility, locomotor problems and ill health.

rhLF – human Lactoferrin. By 2015, 10 surviving transgenic cattle were killed. There were no remaining progeny or cows.

rhFSH calf with enlarged head
Photo: Steffan Browning

rhFSH - Follicle Stimulating Hormone – trial to produce FSH in the milk of the cows. There were 300 embryos produced over the 5 year trial; six animals were born, two were euthanised at birth and one did not express the FSH hormone. The three surviving calves expressed the rhFSH gene. They all showed male-like muscular development, enlarged abdomens, precocious udder development and faster heart and respiratory rates. One had severe deformities in the hind limbs. It was determined that the abnormalities were triggered by the high levels of rhFSH expression, resulting in raised levels of estradiol. This caused a hyper-stimulation of the endocrine system.

The animals were infertile and two were found dead, one from a ruptured umbilical artery and one from a twisted ovary that separated from the uterus. The gene that was supposed to be expressed in the mammary glands leaked into the bloodstream, causing the serious adverse effects. The third was euthanised due to an enlarged head and shortened bow front legs that affected standing.

Histone Demethylase KDM4B

These cattle had an enzyme that removes methyl groups from histones, thereby influencing chromatin structure and gene regulation. The proposed outcome was to stimulate over-expression of the histone demethylase KDM4B enzyme that was fused to a green fluorescent reporter transgene.

The aim of these experimental trials was to undertake fundamental studies for cellular reprogramming and genome editing.

This experiment had no private partners.

Two groups (82 & 83) of 65 recipient cows underwent embryo transfers. Of these, 62 experienced spontaneous abortion and 2 of the remaining pregnancies were induced to abort. The foetuses of these two pregnancies were recovered for tissue regeneration. In 2022, there was one surviving cow, which had premature abnormal udder development. This cow was euthanised in 2023 for humane reasons. There were no KDM4B animals alive in the Ruakura facility in 2024.

A study by Xiang Y et al (2019), published in the journal *Cancer Research*, identified a distinct mechanism by which ectopic KDM4B induces DNA damage and genome instability. Thus, KDM4B over-expression may play a dual role in tumour formation, either by generating genome instability, or by contributing to the failure of anticancer therapy that relies on induction of DNA damage for enhanced DNA repair efficiency.

The euthanised calves were born with:

- **Enlarged adrenal and thyroid glands.**
- **Poorly developed liver and kidneys.**
- **Severe respiratory distress.**
- **Swollen joints.**
- **Incorrect gene integration.**

Coat Colour calves – The programme was carried out in conjunction with the Climate Smart Cattle. This experiment was to gene edit the transgenic embryos to express the PMEL gene, which lightens the coat colour of cattle from black to brown in the Friesian breed, allegedly to increase the heat tolerance of dairy cattle. AgResearch private partners are the Livestock Improvement Corporation (LIC) and CRV (NZ) whose head office is in The Netherlands, in both the Coat Colour and climate Smart Cattle.

In 2018, the first Coat Colour calves were born. Of the five bull calves born by caesarean section, two were transgenic. However, one of these was euthanised a few hours after birth because it had organ and metabolic problems. The remaining transgenic calf died as a result of septicaemia from bladder and kidney infections, arising from an internal abscess. The experiment was renamed Climate Smart Cattle.

Climate Smart calf
Photo: Dr. Goetz Liable

Climate Smart Cattle (App:# ERMA200223)
Climate Smart Cattle (CS) are gene edited (GE) and developed with the aim of increasing their tolerance to heat. The GE cattle express natural DNA sequence variants from three genes: PRLR (Prolactin Receptor) "SLICK" gene and PMEL (Premelanosome protein) genes.

"SLICK" – Prolactin Receptor (PRLR) gene produces a short sleek coat that improves heat regulation.

Premelanosome (PMEL) protein is a semi-dominant gene that lightens the cattle coat colour.

In 2020, 78 conventional heifer recipients underwent Embryo Transfer with gene edited traits in the three different modification events. All recipients spontaneously aborted their pregnancies by day 83. Some of these animals were reimpregnated following new embryo transfer rounds. No pregnancies survived to full term.

The 10 control pregnancies were aborted at day 45, as **all GE Climate Smart pregnancies had failed.**

In 2021, 14 high breeding cows were hormonally induced every 6 weeks for ovum collection and artificial insemination. There were 35 recipients, 17 were used as non-GE controls and 18 recipient cows who received transgenic embryos in two different rounds. It was predicted that there would be 10 calves born. The conventional control non-GE recipients had 17 calves. All the recipients spontaneously aborted the transgenic embryos resulting in a 0% birth rate.

In 2022, 74 cattle recipients underwent another transgenic embryo transfer. There were 10 bull calves and 7 heifers (10) born. These are the first progeny of this line.

In 2023, 894 embryos were developed and 60 conventional recipients and CS cattle received embryo transfers. Seven SLICK traited calves were born (2 GM female, 5 GM male) and 10 GM PMEL (6 female and 3 male) were born, one bull calf died after birth. Three SLICK bull calves were castrated. Eight calves showed a lightened coat colour phenotype. The 9th calf edited with the PMEL gene for a coat colour mutation was born blind.

Genotyping revealed that the presence of an unintended PMEL allele was an inaccurate gene editing event, where there was a 6 base pair deletion in addition to the intended 3 base pair deletion. This had not been detected when the embryo was biopsied. The unintended allele may possibly be linked to blindness. It was acknowledged that the process of gene editing animals from edited cells by cloning has a low efficacy.

Five calves died or were euthanised due to misadventure, blindness, abnormalities and one was a sudden unexplained death. The recipient cows suffered from uterine infections and loss of condition.

In 2024, 4 bull calves and 4 heifers were born and one bull was euthanised on veterinary advice. There are 31 Climate Smart cattle in the facility, 28 of which are heifers and 3 of which are bulls.

GOATS

Conventional non-GE goat Recipients are used as surrogate carriers of the transgenic embryos. Once they have become pregnant, they are not allowed to leave the facility and are eventually killed and disposed of in the offal pits.

From 2014-2024, documents report that 1702 transgenic/gene edited embryos were created and transferred to surrogate goat recipients.There was a 95% spontaneous abortion rate.

In 2015, to increase the conventional surrogate female goat pool, 107 conventional goats were mated to generate non-GE offspring; these would be used as surrogates for transgenic embryos. Only the female kids were kept with unwanted males being euthanised at birth.

Between 2016-2024, 9 goats suddenly died and 162 were euthanised, as they were either surplus to requirements, unsuitable, or put down after recommendation on veterinary advice.

Conventional Goat difficulties

- **Pregnancy complications.**
- **High spontaneous abortions.**
- **Dystocia.**
- **Black mastitis.**
- **Malformed udder.**
- **Chronic foot problems.**
- **Parasitic worms.**
- **Acute pneumonia.**
- **Respiratory problems.**

Females goats are called does. They are usually pregnant for about 5 months or around 145-155 days. Does can become fertile at 4 months old but they can become stunted and have complications if pregnant too young. It is best to leave impregnation until they are fully developed at 7-8 months.

A male goat is called a Buck or a Billy. He becomes sexually active at 4-6 months. Though it is recommended that he is 1 years old before being used for mating.

Many does died from pregnancy complications as well as acute pneumonia and were euthanised on veterinary advice because of their age, or complications from mastitis, malformed udders or chronic foot problems (lameness).

There were no embryo transfers to recipients or live births in the 2022-2023 period.

There were 2 live births from GM goats in 2021, but of these problems with dystocia (not going into stage 2 of labour) and black mastitis were encountered.

From 2014-2024, the numbers of conventional recipients declined from 88 to 10. The ten remaining conventional mature goats that had undergone repeated pregnancies were euthanised with three of the 29 newly transferred mature goats that were moved into the facility in 2024.

Over the years there have been a total of 152 conventional goat recipients that have been used repeatedly for ET (embryo transfer). This resulted in 126 transgenic kids over a period of 10 years.

A further, 171 conventional goats have been euthanised on humane grounds. In 2024, there are no live transgenic goats in the Ruakura facility. (See **Table 1**, page 12).

ET= Embryo Transfer
Enb/Erb = Enbrel/Erbitux
Non-M I = Non Mendelian Inheritance

Table 1. AgResearch, Ruakura Facility, GM Goats over 15 years.

Year	ET created	ET Recipients	Births *Enb/Erb	Births **Non M I	Euthanised	Died	Live
2010	494	64	0	0	-		0
2011	317	69	4 (1%)	0	16	2	7
2012	368	75	10 (3%)		1	1	15
2013	350	50	25 (6%)		4		36
2014	344	45	8 (2%)		10	1	33
2015	?	56	12	23	3	1	41
2016	?	20	3	0	8	0	43
2017	?	20	33	-	14 NMI	2	46
2018	?	15	9	-	16		39
2019	?	35	4	-	14		33
2020	?	11	1	-	7		27
2021	?	42	1	-	8		20
2022	-	0	3	-	8		15
2023	-	0		-	8		7
2024	-	0	0	-	7		0
2025	-	-	-	-	-	-	-
Total	1702		103 (1-7%)	23	123	7	0

Conventional (non-GE) recipient goats. (App:# ERMA200223)

Non-Mendelian Inheritance; TRC trait Goats (App:# ERMA200223)

The transgenic trait in the bucks (males) had a sex transmission ratio distortion, which meant that only female offspring were sired.

This transgenic edit affected the motility of sperm carrying an X or Y chromosome. AgGenetics, a subsidiary of DairyNZ, were the private partners who in 2023 removed themselves from the Companies Register.

In 2014, twenty two of the NMI/TRC transgenic does and recipients received embryo transfers over a period of two days. Two weeks later there were 13 confirmed pregnancies.

In 2015 there were 23 male kids born, but there were no reports submitted to the Ruakura Animal Ethics Committee (RAEC). As a result there is no available data on the health of the animals. The trait line also changed its approval code from 13089 to 13433.

In 2016, a valuable buck, Hunter, became chronically sick with unnatural weight loss, anaemia and diarrhea and died of an acute roundworm (strongyle) infection. Two other goats were found dead in the paddock. The postmortems found one goat had septicaemia due to clostridial infection, and twisted gut in the second. Seven goats were euthanised on veterinary advice, as they were suffering from lameness and recurrent horn infections. This left 14 one year old bucks.

In 2017, there was only one generation of founder NMI/TRC three year old goats. From these, semen was collected from 9 of the bucks. All 14 remaining buck goats were euthanised on humane grounds, because of foot lesions and lameness. This left no surviving TRC (female only offspring) bucks in the facility.

Because of a 100% spontaneous abortion rate, there was no second generation non-Mendelian Inheritance (TRC) progeny born. Therefore the sex distortion of female-only kids could not be achieved. It is assumed that this TRC trait conferred rejection and sterility.

Transgender goats, females in sterile male bodies.

Photo: Steffan Browning

The goat deaths included:

- **4 died suddenly.**
- **1 goat undergoing embryo transfer surgery.**
- **Five goats were slaughtered to retrieve foetuses for further cloning.**
- **Black mastitis.**
- **Arthritis.**
- **Metritis, uterine inflammation.**
- **Pneumonia.**
- **Post-surgery and uterine infections.**
- **Septicaemia.**
- **Listeria.**
- **Hoof rot.**
- **Spontaneous abortions.**

Somatic Cell Nuclear Transfer (SCNT) Generating Female-Only Offspring

The new method of cloning for the Somatic Cell Nuclear Transfer (SCNT) female-only offspring line started in 2020-2021.

Somatic Cell Nuclear Transfer (SCNT), a cloning technique, produces genetically identical offspring. SCNT transfers the nucleus of a non-reproductive cell (somatic cell) into an egg cell (oocyte) from which the nucleus has been removed. The resulting embryo is then implanted into a surrogate mother, where it develops into a cloned goat.

A total of 138 SCNT clones with the female-only trait were developed. Of these, 48 clones were transferred into goats through surgical embryo transfer, while the rest were introduced via artificial insemination (AI). At 25 weeks, there were 7 foetuses detected in 6 goats, of which four foetuses subsequently died in utero.

The pregnancies resulted in three live males, Whitie and Blackie (AI twins) and a clone named Brownie.

Brownie exhibited health issues such as compromised breathing that later improved, enlarged knees, and bowlegs. Though he gained weight faster than the twins while suckling, he weighed less post-weaning.

Additionally, 88 more clones (53 from CR26, 35 from CR40) were transferred into does; however, by day 35, all pregnancies failed. Pregnancy failures were attributed to toxic cell media, non-viable cell lines, and low doe fertility.

At 6 months, the three bucks were placed with 11 does for natural mating. Despite Brownie's strong mating behaviours, no offspring resulted. Subsequent semen collection with an artificial vagina (AV) initially saw little interest from the bucks. Three months later, Blackie produced good-quality and quantity of sperm in the six collection emissions, which was frozen. Brownie managed only one ejaculation of poor quality, motility, and quantity over six attempts.

It was concluded that Brownie's poor sperm count was due to a dominant-negative transgene affecting sperm quality. Eventually, all three bucks were euthanased as no longer needed.

As of July 2024, there are no surviving SCT goats in the facility.

Genetically engineered goats.
Photo: Jamie Morton NZ Herald.

Erbitux (Cetuximab) sold under the brand name Erbitux, is an epidermal growth factor receptor inhibitor medication used for the treatment of metastatic colorectal cancer and head and neck cancer.

Erbitux Goats (App:# ERMA200223)

The Erbitux Biosimilar programme started with goats in 2010, only 7 kids were born out of the 494 Tg embryos.

At the end of July 2012 there were 75 conventional recipients who received Erbitux embryo transfers (ET) from different trait lines on a cyclical basis. At the end of July 2012 there were 75 conventional recipients who received Erbitux embryo transfers (ET) from different trait lines on a cyclical basis.

As a result ten female kids were born. This increased the number of Erbitux goats in the facility to 15.

The doelings (young females) from each Erbitux biosimilar line were hormonally induced to produce milk at around 4-6 months.

As reported in *FASEB BioAdvances* (2020), transgenic goats produced an improved version of cetuximab in milk. The goats were induced to lactate by being given intramuscular injections of oestrogen and progesterone every second day for a total of seven treatments. For the following three days, the goats received daily intramuscular injections of dexamethasone. From the third estrogen/progesterone injection, the mammary glands were massaged daily to stimulate milk secretion. Milk was taken over 60 days.

Table 2. Erbitux Goat Lines: Number of Embryos, Births and Milk yield.

Transgenic (Tg) Line	Embryos transferred (# of recipients)	Development to term	Development to weaning	Natural lactation	Erbitux Yield in induced kids
GL6.7	56 (8)	2	2	3600ltrs over 60 days	5g/ltr
GL6.7	57 (10)	2	1		5g/ltr
GL8.3	54 (10)	4	4		No-milk
GN97	25 (5)	0	0		
GN97	48 (7)	2	1		
GN97	45 (8)	2	2		
GN99	26 (6)	2	2		15g/ltr
GN100	77 (10)	4	3	494mls over 19 days	15g/ltr
GN118	100 (12)	1	1	8/mls over 1 day	
GN304	24 (5)	1	1		
GN388	25 (4)	0	0		
GN388	35 (7)	2	2		
GN451	37 (7)	3	1		
Total	584	25	20		

- The 3 transgenic does from Erbitux line GL6.7 had four kids (2 singles, 1 twin). One of the twins died at birth leaving 3 kids. The kid was hormonally induced into lactation and the milk contained 5g/ltr of Erbitux biosimilar.
- Line GN118 had 1 kid and she produced a few drops of milk showing the Erbitux biosimilar expression at 5g/ltr.
- Line GL8.3 was unsuccessful with no milk produced.
- Line GN100 gave birth to 3 kids – one died a day later. Only 1 kid was hormonally induced into lactation. She produced milk for 21 days, but the yield decreased at day 10. She produced the Erbitux biosimilar at 15g/ltr.
- Line GN99, with two kids, was induced into lactation at 3 months. They produced milk for 29 days, but the yield decreased after 18 days. They produced Erbitux biosimilar at 15g/ltr.

In 2013, there were 93 transgenic embryos that were transferred to 14 surrogate recipients in two ET runs from Erbitux line GN97. At 30 days gestation, 7 foetuses were detected and 9 recipients had aborted the embryos. Labour was induced for a planned delivery five days before expected full term by administering a combination of prostaglandin and dexamethasone 36 hours beforehand. Four kids were born, one dying a day after birth.

Further, new Erbitux cell clones were developed:

- Clone line GN388, where 25 transgenic Erbitux embryos were transferred to 4 recipients (non-GM) – no pregnancies resulted.
- Clone line GN451, where 37 embryos were transferred to 7 recipients (non-GM). At full-term, one kid died 12 hours prior to delivery, one gave birth to 1 kid and the other had twins; these died after 6 days. Postmortems found that they died of neonatal respiratory distress syndrome. This put the survival rate of Erbitux kids at only 1%.

The transgenic Erbitux does who had given birth naturally underwent normal lactation.

- The one female transgenic doe from line GL6.7 produced 36 litres of milk in total, or 600mls/day over 60 days containing 5g/L of Erbitux. This was only the heavy chain of the antibody.
- The two transgenic does from line GN100 produced a minimal amount of milk, in total 494mls or 26mls/day over 18-19 days,
- The one female transgenic doe from line GN118 failed to lactate and only secreted 8mls of a highly viscous liquid. See **Table 2**, page 14.

The failure to lactate naturally was put down to the transgene copy number or site integration, that affected the milk production.

Over the years 2015-2024 the Erbitux goat embryo transfer experimental programme transferred around 300-400 Erbitux transgenic embryos into 60-120 recipients annually on a rotational basis. There were very high level of spontaneous abortions and only 103 offspring born in the 15 years. This led to significant attempts to raise the levels of live births.

New procedures including:

- Surgical laparoscopic insemination using Saanen semen with hormonally synchronised Erbitux does;
- Super-ovulation, (the hormone-induced release of many mature eggs in one ovulation round) the eggs were flushed out and inseminated the embryos implanted through AI the Erbitux does.

The Super-ovulation programme was an ongoing failure, and it was contributing factors were - the age of the recipients led to ovulation failures and ovarian adhesions from repeated hormonal procedures.

An explanation on the extremely high spontaneous abortions was believed to be due to reproductive dysfunction linked to the transgene. Another pre-term risk was the failure to dilate at the second stage of labour (dystocia), resulting in foetal deaths.

There were many deaths of the Erbitux kids before weaning. Issues related to organ and limb deformities and respiratory difficulties. One kid died suddenly, and a postmortem showed an acute abdominal catastrophe where the intestine twists and causes a bowel tear. This torsion of the intestines resulted in tissue necrosis, causing death.

There was regular culling of old animals and males who were deemed surplus to requirements after semen collection or proven sterile. There were continual welfare-related issues from mastitis, lameness, intestinal and skeletal issues leading to euthanasia's on veterinary recommendations.

By 2023, the 7 remaining Erbitux goats were euthanised ending the Erbitux programme in all animals. See **Table 1**, page 12.

Enbrel

Enbrel goats are engineered to produce biosimilar pharmaceutical proteins in their milk. The two transgenic traits are integrated into Chromosome 21 for trait 1 and for trait 2 chromosome 23.

Enbrel is a complex animal derived biosimilar as such is not exactly the same as the reference product. The active ingredient is etanercept and used for Rheumatoid Arthritis and some inflammatory conditions. It has a black box warning due to posing significant risks of serious adverse reactions, which could lead to hospitalisation, death, or permanent damage, especially in vulnerable groups such as pregnant women, children, or older adults.

Between 2017 and 2019, there were 13 Enbrel Biosimilar, 12 females and 1 male, goats bred from founder (F0), and the first generation of offspring (F1). Eight Enbrel goats were euthanised, for chronically bad feet and the buck with horrifically swollen testicles. Multiple Ovulation Embryo Transfer Technology (MOET) was begun with three female goats to increase reproduction, but none of the goats ovulated.

Multiple Ovulation Embryo Transfer Technology (MOET) is the technique of hormonal stimulation using Follicle Stimulating Hormone (FSH) and prostaglandins to induce multiple ovulations. The animal is then artificially inseminated and multiple eggs are fertilised in the reproductive tract. The fertilised embryos are then collected to transfer to recipients.

Of the 5 Enbrel Biosimilar goats, the buck was induced for semen collection with an artificial vagina (AV). However, the quality of the sperm motility and viability was poor resulting in a low yield that was not frozen. In 2020, semen collection was repeated two more times. A small number of straws of higher quality semen were collected for freezing. The single surviving buck was no longer required after semen collection and was euthanised on veterinary advice, two does were euthanised for locomotor problems and one doe was euthanised due to recurring polioencehalomacia. The 1 surviving doe underwent embryo transfer to produce female-only offspring through a new experimental gene edited line, this was not successful.

In Summary on the total Goat trial:

- In the years 2015-2024 there were 246 goats euthanised for health reasons, on veterinary advice or because of old age. Of these,132 were conventional, 21 with the Mendelian inheritance trait, and 93 from the Erbitux/Enbrel lines.
- In 2024, all goats producing pharmaceutical milk proteins (Erbitux and Enbrel) as well as goats producing female-only offspring were killed, leaving no transgenic goats in the facility, but 26 conventional recipient goats for possible new gene editing experimentation.

The quality and quantity of the semen and sperms' motility from the transgenic bucks were compared with the transgenic mice and they both had the same semen alterations, abnormalities and poor quality for oocyte fertilisation. In July 2024, there were no transgenic goat lines existing.

The enormous cost both monetarily and to animal welfare of these disastrous trials should also be considered. The evidence shows that the 10 years of experimentation has produced a high level of suffering, abortions, illness and deformities with no viable transgenic biosimilar production or benefit to pharmaceutical production.

Polioencephalomalacia (PEM) is a non-infectious, nutritional disease characterized by neurological symptoms. PEM is caused by thiamine deficiency due to consuming a diet high in sulphur, fermentable carbohydrates or mouldy feed containing thiaminases. Early clinical signs include anorexia, loss of coordination, grinding teeth, increased salivation, increased sensitivity to pain, touch, noise, and muscle tremors. As the disease progresses it leads to blindness, seizures, and death.

SHEEP

Table 3. AI on Hooves all sheep trait lines.

Year	ET Conventional Recipients	GE Embryos created	Born Tg	Euthanised Tg/Con	Died Tg/ Con	Total Tg sheep in facility
2017	55	186				
2018	93	391	0	0/7	0/7	0/55
2019	162	447	2	0/14	0/7	2/159
2020	156	Not specified	19	4/66	1/7	16/95
2021	96	Not specified	6	2/36	1/0	19/61
2022	0	0	0	2/10	2/0	18/57
2023	66	73	15	5/9	1/1	27/74
2024	0	0		2/23	2/2	24/2

Conventional sheep

In July 2018, there were 55 conventional sheep in the facility and in 2019, a further 119 hoggets were transferred into the facility to be used as recipients. This brought the facility's total of conventional ewes to 159.

In June 2020-2023, 12 conventional lambs had been born (5 female, 7 male). 72 ewes were killed as surplus to requirements and 7 died from polioencephalomalacia. By July 2024, there were only 22 remaining in the Ruakura AgResearch facility.

Sheep with polioencephalomalacia
Photo credit: Dr. Richard Ehrhardt and Michigan State University.

Creation of DAZL transgenic Cell lines

The gene editing of sheep embryos started in late 2017. A slaughterhouse provided the Ovine foetal fibroblasts (OFFs) from a ewe that had been pregnant for 12-14 weeks. The somatic cells were sourced from a multiple breed ram, which was a Perendale, Texel, Coopworth and Romney cross. The 55 blastocytes made up from RNA binding protein in the male ovine foetal fibroblasts in two *DAZL*, cell lines (9 & 25) were developed.

Deleted in Azoospermia Like (DAZL) loss-of-function mutation DAZL produces sheep of both sexes that entirely lack sperm germ cells causing infertility called Sertoli cell-only syndrome.

There were 62 conventional hoggets that were implanted with the two DAZL cell lines using laparoscopic surgery. There were no live births and many pregnancy complications. There were 7 ewes who were euthanised so that the lambs could be recovered on day 141. The two ewes carrying lambs from strain 9 developed symptoms of excessive amniotic fluid (hydroallantois) at full term, the two lambs died within minutes of being born due to respiratory failure.

The lamb from strain 25 was delivered dead on day 146, displaying abnormal fluid in tissues (oedematous) and decaying tissues.

All lambs that died before birth had a variety of anatomical malformations like joint deformities or fusing (ankylosis) of the forelimbs, and abnormal fluid buildup in kidneys (hydronephrosis), something which is common in cloned animals. The main developmental problems in cloned sheep were respiratory, kidney, and musculoskeletal system problems.

NANOS2 genotype sheep (AI-on-Hooves)

In 2019-2020, further gene editing of sheep with the new line NANOS2 to produce a breeding line, was carried out. There were 184 embryos implanted into 51 conventional recipient sheep from two NANOS2+/- sterile male lines, wild type and TAEF chimeras.

Due to the difficulties of the birthing process from the hormonally induced labour and failure to establish 2nd stage labour (dystocia), caesarean sections (C-section) were undertaken, and the ewes euthanised. Some the lambs suffered from foetal distress or inhaled meconium.

Ten lambs were born and of the 8 surviving lambs, six were euthanised on humane grounds as —

- Two lambs suffered from hydrops and died soon after birth.
- Two had cloning problems.
- One was trampled on and had its jaw fractured.
- One lamb had a punctured lung from a broken rib.

Laparotomy surgery was undertaken on six ewes, and 60 egg cells (oocytes) were collected. After the laparotomy surgery, 6-week recovery period was needed and two of the sheep needed oxygen, as they suffered from chronic pneumonia and fluid in the lungs.

In the year ending June 2021, there were 24 births (19 female, 5 male). Three rams and 3 ewes were killed.

Of the existing sheep:

- Hoss was a TAEF chimaeric absolute transmitter.
- Hammer, Bunter, Howie, Rachael and Martha were heterozygous NANOS2 sheep.

The rams, Howie and Hoss, mated with cloned NANOs2-/-ewes Rachael and Martha for two cycles. Howie had enlarged testicles but did not have any mounting problems and his one offspring was born healthy.

Hoss had difficulty mounting the ewes as he suffered from difficulty breathing, rectal prolapse and front leg deformities.

Bunter was not put with the ewes as he suffered from nasal mucous congestion and difficulty breathing with aggressive bunting behaviours with other sheep. Later, he and another clone tested positive with Johne's disease and were euthanised on veterinary advice.

The rams were also induced to semen collection with an artificial vagina; the semen was collected for artificial insemination (AI). Viable semen was collected for Howie and Hoss, but Bunter did not produce any semen and was euthanised.

- Rachael, who had suffered from lameness and poor condition died suddenly for no reason.
- Martha as she suffered from post-surgery breakdown of abdominal stitches causing a highly painful fist sized hernia.
- Seven of the transgenic offspring of Howie and Hoss died.

Transgenic Sheep problems

- **Enlarged testicles.**
- **Sterility.**
- **Rectal prolapse.**
- **Skeletal deformities.**
- **Respiratory difficulties.**
- **Spontaneous abortions.**
- **Post surgery complications.**
- **Death from birthing problems.**

In 2022-2024, only 25 recipient ewes and the surviving two mature transgenic ewes were artificially inseminated with 45 embryos as there were insufficient viable embryos to transfer to the 95 recipients. There were 16 lambs (10 female, 6 male) born. Only one NANOS2-/- ewe was able to be mated.

There are 24 mixed genotypes in the facility as of June 2024.

The losses were higher than expected and the fourteen sheep (8 ewes, 6 lambs) welfare was adversely impacted.

It is known that cloning is associated with a high percentage of abnormalities causing lamb deaths. This is a serious animal welfare issue and cloning should therefore be prohibited.

Transgenic Lamb postmortems

- **1 twin lamb born dead with an elongated jaw.**
- **3 lambs were euthanised due to short jaw (parrot mouth).**
- **1 lamb euthanised for limb and kidney deformities.**
- **1 lamb euthanised from lung problems, unable to breathe.**
- **1 lamb after birth, struggling to breathe. She was treated with antibiotics and anti-inflammatories and improved. However, on a hot day she suffered from respiratory problems from 50% lung capacity that led to welfare problems, so was euthanised.**

Immune Compatible Sheep for Xenotransplantation (CMAH/GGTA)

The CMAH/GGTA genotypes are edited to produce immune-compatible sheep for human xenotransplantation.

Five females in this group (Sandy, Bertha, Serena, Rhonda, Venus and Minnie) were clinically well, but showed signs of chronic pneumonia.

Sandy died from a twisted gut. Her lamb was bottle fed until well enough to go out to the paddock.

Bertha had an undershot jaw making it difficult for her to eat, therefore leaving her in poor condition. She also suffered from a lame front foot that was treated with antibiotics. As she did not recover health and was considered to be suffering, despite being given longer grass, she was euthanised.

The four remaining transgenic immune-compatible ewes were naturally mated in 2021, but there were no pregnancies. Following observations made, it was concluded that the CMAH/GGTA gene could be connected to immune rejection.

Anephric Sheep for Xenotransplantation.

Anephric (no kidneys) foetuses showed mild to severe renal hypoplasia (small kidneys) or a total loss of kidney development. The loss of the SALL1-/- gene, which was knocked out by gene editing, was thought to be responsible for the failure in kidney development. The possibility of introducing a human kidney/s to replace missing kidneys in the sheep was explored. In July 2024, there were 4 female knockout ewes artificially inseminated. However, there were no offspring, as the sheep appeared to be sterile.

New Zealand's first genetically engineered sheep Lincoln University, Canterbury, 2006.

EVIDENCE FROM EXPERIMENTS NEW ZEALAND AND OVERSEAS

Sheep

In 1995, Lincoln University, Canterbury NZ, created transgenic sheep to improve fleece weight and improve wool properties. The sheep were engineered with a mouse ultra-high sulphur keratin promoter linked to an ovine insulin-like growth factor 1 (IGF1). There were 591 embryos developed and of these, 5 transgenic lambs were born. One male and one female were transgenic.

The transgenic ram was then sired to 43 surrogate non-transgenic ewes. Eighty-five lambs were born, of which 43 were transgenic. When they were first shorn, transgenic rams had improved wool yield compared with the transgenic control group, but the staple strength was 39% lower than the transgenic females staple strength and 29% lower than the non-transgenics controls. However, the improvement was not stably passed down through the generations and eventually there was no difference between transgenic and non-transgenic fleece quality.

No information has been provided on the health of the transgenic animals.

Monkeys

The most recent human-animal trial that has been published is the chimeric monkey (Cao,J. et al 2023). In this study 91 blastocysts were obtained from 206 injected morulae and 74 chimeria blastocytes were implanted into 40 surrogate non-GM monkeys. Of the 40 surrogate monkey recipients, there were 34 spontaneous abortions early in pregnancy. Only 12 embryos continued to the later stages and 6 of these were spontaneously aborted before term. Just six pregnancies survived to full-term.

One chimeric primate male, positive for the transgenic trait was euthanised after 10 days due to respiratory and hypothermia problems.

The very high abortion rate possibly indicated that the injected embryonic stem cells (ESC) had a detrimental effect on normal embryo development.

Recombinetics Gene Edited Hornless cows

Hornless cows, which were created using gene editing technology by the company Recombinetics, were first born in 2016.

The company argued that as they were gene edited using TALENS, they were the product of "*precision breeding*". Recombinetics assumed the gene edits would not be regulated and therefore could be directly released into the market. Recombinetics examined only whether the gene had integrated at the correct site, not any downstream effects that might have occurred in the gene editing process and did not consider any other downstream effects

However, when the USDA did a more thorough examination, a new detection test that looked at the whole genome and not just the insertion site, they detected an unintended alteration. This had come about as a result of the integration of bacterial plasmid DNA into the cow genome. This plasmid DNA contained various sequences designed for use in the editing process, including antibiotic resistance marker genes. Whilst deliberation by the FDA going on, embryos were sent to Australia, where 8 cows were born and three of these euthanised. The remaining five were kept in containment.

A German study, also on hornless cows, (Schuster et al., 2020)

Schuster et al. documented the outcome of experiments into hornless cows in Germany. They produced 70 clones of which 18 viable embryo blastocysts. These were cultivated for 7 days in vitro. Six blastocysts that were ready for implantation were transferred into 6 synchronised surrogate recipients. In addition, two expanded embryos, containing 100 to 125 cells with a thinned Zona pelucida (ZP) were implanted in three recipients. The remaining nine embryos, which were kept as quality controls, were maintained in culture for 8 days. Six of these showed delayed development. On average, cows have a 283 day pregnancy gestation period.

Pregnancies were initially established in six recipients, and were confirmed by ultrasound diagnostic on day 40 of gestation. Four animals did not manage to maintain pregnancy past the first trimester.

One cow was sacrificed on day 90 of gestation in order to analyse the foetus. The calf of the sole remaining pregnancy was successfully delivered by caesarean section. This calf was hornless and had a birth weight of 78 kg. An examination carried out to detect pathologies revealed malformations of some internal organs including the liver, heart, diaphragm, lungs and skull. These abnormalities caused acute cardiovascular failure and the calf died within 24 hours.

This body of research documents poor results, comparable to the deformities and abortions that have been observed in the New Zealand AgResearch GE animals. Regardless of the trait and the genetic technique, genetic engineering continues to raise serious animal welfare issues.

Delivered polled calf.
Photo credit:

Pigs

Revivicor is gene editing pig organs, namely kidneys and hearts, to be used in xeno transplantation to humans. Gene editing has been used to remove the gene that causes rejection following transplantation. Revivicor's pig farm in rural Virginia spliced out the alpha 1,3-galactosyltransferase gene (alpha-gal) that is expressed on the surface of pig cells. This caused rejection in transplant recipient patients.

When the human immune system detects alpha gal on a xeno-transplanted organ, there is a marked antibody reaction, specifically a host T-cell–mediated immune activation, which can lead to a hyperacute rejection. It is assumed that the inactivated alpha-gal gene is not the only cause of rejection. The pigs in this research have had 10 genetic modifications: four inactivated pig genes and six genetically engineered human genes to suppress immune rejection. They also have two additional antigens. One of the inactivated genes knocks out the growth hormone receptor.

The addition of engineered human genes to pigs is done in an attempt to shut down the "complement cascade," in which the immune system proteins in the blood activate each other to cause inflammation and other immune responses. This creates a cytokine storm.

Human-pig kidneys have been used for xeno-transplantation in 6 patients with severe kidney and heart problems. One of these lived for 5 months before the kidney was rejected; all other organ recipients died within 2 months. The deaths were attributed to a number of factors, such as the infection from a cytomegalovirus from a genetically modified pig heart xenotransplant.

This shows that crossing species barriers can pose lethal risks from viral and other infectious diseases.

Table 4. GE Animals alive at Ruakura facility from 2015-2024

Cows GE Trait	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025
Casein+	17	15	13	12	2	0	0	0	0	0	0
MBP	2	1	0	0	0	0	0	0	0	0	0
rhLF*	0	0	0	0	0	0	0	0	0	0	0
BLGkd**	1	17	27	20	19	16	15	14	13	0	0
Erbitux	1	1	1	1	0	0	0	0	0	0	0
KDM4B****				1	1	1	1	1	0	0	0
Climate Smart				3	0	0	0	17	24	31	0
Goats											
Erbitux	33	35	34	33	30	25	20	15	7	0	0
Enbrel	8	8	7	6	6	2	0	0	0	0	0
N-MI	23	14	0	0	0	0	0	0	0	0	0
Sheep											
AI on Hooves					2	16	19	18	27	24	0
Facility Total TG/GE animals	**85**	**91**	**82**	**76**	**60**	**60**	**55**	**65**	**71**	**55**	**0**

TG Transgenic

C+ Casein +(plus) engineered with an extra transgenic casein gene

MBP Myelin Basic Protein engineered with an extra transgenic human MBP gene

LF human Lactoferrin, cows engineered with a transgenic human rhLF gene

FSH human Follicle Stimulating Hormone, cows engineered with a transgenic hFSH gene.

BLG- Beta-Lacto-globulin minus transgenic cows have the BLG gene silenced.

ERBITUX cows engineered with a transgenic ERBITUX gene.

KDM4B Over expression of Histone Demethylase

N-MI Non-Mendelian Inheritance

AI on Hooves Xeno – Human Xenotransplantation, (NANOS Female-only)

Facility Total Total number of transgenic animals in the facility at the end of each year

All data taken from AgResearch ERMA 200223 Annual Reports to EPA. https://www.gefree.org.nz/gm-annual-reports/

Table 5. AgResearch GE/GM cows year created, Genetic technology used and alive in 2024.

Date born	Result	GM animal	Trait	Live 2024
2022	PMEL- and SLICK-edited	Cow	Heat Tolerant	**31**
2018	Gene edited	Cow	Light Coat	**0**
2018	Gene edited	Cow	KDM4B (Male sterility)	**0**
2011	Gene edited	Cow	BLG -	**0**
2011	Transgenic	Cow	Enbrel	**0**
2011	Transgenic	Cow	Erbitux	**0**
2007	Transgenic	Cow	rhFSH	**0**
2005	Transgenic	Cow	rhLF	**0**
2000	Transgenic	Cow	Casein +	**0**
2000	Transgenic	Cow	MBP	**0**
Goats				
2016	Gene edited CRISPR	Goat	Non-Mendelian Inheritance (Female-only)	**0**
2011	Transgenic	Goat	Erbitux	**0**
2011	Transgenic	Goat	Enbrel	**0**
Sheep				0
2020	Gene edited (CRISPR)	Sheep	Xenotransplantation	**?**
2019	Gene edited (CRISPR)	Sheep	Germline deficient NANOS2 Male Sterility	**24**
Total TG and GE animals in facility				**55**

All data taken from AgResearch ERMA 200223 Annual Reports to EPA. https://www.gefree.org.nz/gm-annual-reports/

GE RYEGRASS

Photo: AgResearch: Trials in Missouri, USA, to test GM ryegrass developed in a laboratory in Palmerston North have now ended. *Photo: Jamie Morton NZ Herald.*

In 2005, AgResearch was granted approval to develop a genetically engineered (GE) High Metabolisable Energy (HME) ryegrass. The genetic constructs came from Escherichia coli, parainfluenza virus 5, Sesame indica (cysteine oleosin) and Nasturtium (Tropaeolum majus) and the cells were resistant to the antibiotic hygromycin.

Ryegrass developed using gene gun technology many unwanted integrations in the DNA, therefore, Agrobacterium was used to engineer the plants. These engineered constructs were intended to reduce water use, nitrogen dioxide (NO2) and methane emissions in livestock.
A report by Dr. Terri Dunahay, Genetically Modified Pasture Grasses in New Zealand (2010), stated that AgResearch, along with partner Pastoral Genomics, planned to apply to conduct field trials on the GE HME ryegrass in 2010 in NZ.

These trials were eventually conducted in Missouri, USA, from 2017-2021 at a cost of $25 million, to assess the environmental effects and conduct a short nutritional trial with 16 cows grazing on an established GE ryegrass pasture.

The New Zealand partners who helped to fund the US trials were the Ministry of Business, Innovation and Employment, Dairy NZ, PGG Wrightsons, Grasslands Technology Ltd, who provided governance. In addition Barenbrug Agriseeds provided the elite grass germplasm and a pathway to market.

Though the 5 years results of the trials had some seed production delays, the results were poor. As strong light, high temperatures affected the measurable achievements for nitrogen and water use efficiency, effects on soil Rhizobium bacteria and generation stability, in the field. The performance was so poor that nutritional trials on animals could not be conducted. (MBIE OIA Report C10X1603- CR-6).

The failed field trials in the US led to AgResearch growing the GE HME ryegrass in a glasshouse in Palmerston North. No EPA approval was sought or given.

To conduct nutritional trials, the GE ryegrass was grown in spaced pots in low light and was cut and dried over 18 months, rendering it non-viable. By 2025, there was enough GE ryegrass to start the nutritional trials. These were conducted on 24 lambs, 12 of which were fed GE rye grass and 12 fed on non-GE ryegrass controls.

Lambs were fed for 11 days and placed in methane detectors. A reduction of 10% in methane production was predicted, but the GE ryegrass-fed lambs only achieved a 7% reduction, non-GE controls showed a 4.5% reduction in methane. The 7% reduction in methane production is significantly lower than achieved with non-GE mixed pasture species (13% reduction). Phenomenally, if seaweed is added as a supplement to feed, a reduction of 90% methane can be achieved. As the GE ryegrass feeding trial was too short to gain any meaningful data documenting health effects on the cows, and GE ryegrass hay was grown in laboratory conditions, because of its poor performance in the field, the commercial success of GE ryegrass seems very unlikely.

GLOSSARY

- **Conventional surrogates**: Non-GE animals used as recipients for genetically engineered (GM/GE) embryos.
- **Casein +**: Casein plus.
- **rhMPB**: Recombinant Human Myelin Basic Protein (rhMPB) a transgene expression in cow's milk for pharmaceutical purposes.
- **rhLF**: Recombinant human lactoferrin.
- **rhFSH**: Recombinant human Follicle Stimulating Hormone.
- **BLG**: Recombinant Beta Lactoglobulin knockdown.
- **Erbitux**: trade name Cetuximab, is a recombinant chimeric monoclonal antibody, to treat colorectal cancer.
- **CC**: Coat Colour.
- **KDM4B**: Histone Demethylase KDM4B.
- **CS**: Climate Smart cattle.
- **SLICK**: Prolactin Receptor (PRLR) gene produces a short sleek coat that improves heat regulation.
- **PMEL**: Premelanosome protein is a semi-dominant gene that lightens the cattle coat colour.
- **NMI**: Non-Mendelian Inheritance; TRC trait: skews gender ratio.
- **SCNT**: Somatic Cell Nuclear Transfer Generating Female-Only Offspring.
- **Enbrel**: trade name Etanercept, an animal derived biosimilar used for Rheumatoid Arthritis and some inflammatory conditions.
- **PEM**: Polioencephalomalacia nutritional disease characterised by neurological symptoms.
- **DAZL**: Deleted in Azoospermia Like loss-of-function mutation produces sheep of both sexes that entirely lack sperm germ cells causing infertility called Sertoli cell-only syndrome.
- **NANOS2**: genotype sheep (AI-on-Hooves) produce sterile males.
- **CMAH/GGTA**: Immune Compatible Sheep for Xenotransplantation produce immune-compatible sheep for human xenotransplantation.
- ***SALL1***: gene is a multi-zinc finger transcription factor that regulates kidney formation.
- ***SALL1-/-***: the loss of SALL1 gene produces small or no kidneys.
- **Anephric**: no kidneys or small kidneys.
- **Biosimilar**: a biological medicine similar, but not identical, to versions of an already registered medicine.
- **Bucks**: male goats at breeding age.
- **Does**: female goats of breeding age.
- **Doeling**: young female goat.
- **Kids**: young goats under 1 year of age.
- **Euthanised**: humanely killed.
- **Hypothermia**: dangerously low body temperature.
- **Hygromycin resistant**: antibiotic resistant to Hygromycin.
- **In-vitro**: research conducted in a test tube, culture dish outside of the body.
- **In-vivo**: research conducted on a living organism in body.
- **Locomotor problems**: difficulties with coordination of the bones, joints, muscles, or nervous system.
- **MOET**: Multiple Ovulation Embryo Transfer Technology.
- **Postmortem**: after death.
- **Sesame indica Oleosin**: sesame oil.
- **Superovulation**: the hormone stimulated ovulation inducing the release of many mature eggs at once.
- **Tropaeolum Majus**: Nasturtium plant.
- **Torsion of the intestines**: segment of the intestine twists around itself and its supporting mesentery, causing a bowel obstruction and cutting off blood supply.
- **Tissue necrosis**: cell death of tissues.
- **Xenotransplantation**: the transplantation of organs or tissues from an animal source into a human recipient.

REFERENCES

Official Information Act (OIA) replies
https://www.gefree.org.nz/official-information-act-requests/ regarding GE animals and GE ryegrass

- Ministry of Business Innovation and Employment (MBIE)
- Ministry of Primary Industries (MPI)
- AgResearch
- Environmental Protection Agency (EPA)

Terminology OIA - **https://www.gefree.org.nz/assets/Uploads/GE-Free-NZ-OIA-response-GM-Animals.pdf**

Embryo transfer for all GE animal traits
https://www.gefree.org.nz/assets/pdf/Response-to-Claire-Bleakley-OIA-October-2012.pdf

Annual report 2025 on ERMA200223 - https://tinyurl.com/5xauwk57

Annual Report to Environmental Protection Authority for Activities under ERMA 200223, June 2016
https://tinyurl.com/3wcabpz3

Annual Reports to Environmental Protection Authority for Activities under ERMA 200223 – 2010-2024
https://www.epa.govt.nz/database-search/hsno-application-register/view/erma200223/

Summary and analysis of AgResearch ERMA200223 10-year report
https://tinyurl.com/yfdh8wfp

Sheep deaths Martha **https://www.gefree.org.nz/assets/Uploads/Adverse-event-353-Redacted.pdf**

Transgenic Lamb death from defects
https://www.gefree.org.nz/assets/Uploads/Adverse-event-350-Redacted.pdf

Adverse event Bertha **https://www.gefree.org.nz/assets/Uploads/Adverse-event-352-Redacted.pdf**

Dunahay T (2010) Is the grass always greener? Issues affecting the adoption of genetically modified pasture grasses in New Zealand **https://tinyurl.com/29m4ndsc**

GE Ryegrass report 2019-2020 **https://www.gefree.org.nz/assets/Uploads/C10X1603-CR-5.pdf**
GE Ryegrass report 2022 **https://www.gefree.org.nz/assets/Uploads/C10X1603-CR-6.pdf**
GE Ryegrass nutritional Trials NZ
https://www.gefree.org.nz/assets/Uploads/Letter-GE-Free-New-Zealand-HME-Livestock-Feeding-Trials.pdf

Turin L, Invernizzi P, Woodcock M, Grati FR, Riva F, Tribbioli G, Laible G. Bovine fetal microchimerism in normal and embryo transfer pregnancies and its implications for biotechnology applications in cattle.
Biotechnol J. 2007 Apr;2(4):486-91. doi: 10.1002/biot.200600218. PMID: 17285678.
https://pubmed.ncbi.nlm.nih.gov/17285678/

Pharming licenses lactoferrin production tech to NZ group **https://tinyurl.com/ms8v5z4x**

Mutant cows die in GM trial, Eloise Gibson NZ Herald 1 May, 2010.
https://www.nzherald.co.nz/nz/mutant-cows-die-in-gm-trial/UNAM73ED3VXZJFX7MXU7VYAZ74/

What is Chromatin? **https://www.youtube.com/watch?v=6Z8aQhV_aD4&t=2s**

REFERENCES

XiangY., *et al.* 2019 Histone Demethylase KDM4B Promotes DNA Damage by Activating Long Interspersed Nuclear Element-1 Molecular Cell Biology
https://aacrjournals.org/cancerres/article/79/1/86/634005/Histone-Demethylase-KDM4B-Promotes-DNA-Damage-by

Generation of Climate smart Cattle Report
https://www.gefree.org.nz/assets/Uploads/ReportA-15088-Redacted-1.pdf

Skrzyszowska M, Samiec M. (2021) Generating Cloned Goats by Somatic Cell Nuclear Transfer-Molecular Determinants and Application to Transgenics and Biomedicine. Int J Mol Sci. 22(14):7490.
https://pmc.ncbi.nlm.nih.gov/articles/PMC8306346/

Chug K (2011) Animal death toll ends cloning trials (2011) Stuff
https://www.stuff.co.nz/national/4681283/Animal-death-toll-ends-cloning-trials

Winichayakul, S. et al. (2022) Insight into the regulatory networks underlying the high lipid perennial ryegrass growth under different irradiances', PLOS ONE, 17(10). doi:10.1371/journal.pone.0275503.

Wang Q., Gavin W., Masiello N., Tran K.B., Laible G., Shepherd P.R. (2020) Cetuximab produced from a goat mammary gland expression system is equally efficacious as innovator cetuximab in animal cancer models, Biotechnology Reports, 10.1016/j.btre.2020.e00533, 28, **https://faseb.onlinelibrary.wiley.com/doi/10.1096/fba.2020-00059**

Björn Oback B., Daniel A. Cossey D.A.(2023) Chimaeras, complementation, and controlling the male germline, Trends in Biotechnology, Vol: 41,10; p.1237-1247, ISSN 0167-7799, **https://www.sciencedirect.com/science/article/abs/pii/S0167779923001002**

Damak, S., Su, Hy., Jay, N. et al. Improved Wool Production in Transgenic Sheep Expressing Insulin-like Growth Factor 1. Nat Biotechnol 14, 185–188 (1996). **https://doi.org/10.1038/nbt0296-185**

Su, H., Jay, N. P., Gourley, T. S., Kay, G. W., & Damak, S. (1998). Wool production in transgenic sheep: Results from first-generation adults and second-generation lambs. Animal Biotechnology, 9(2), 135–147. **https://pubmed.ncbi.nlm.nih.gov/9713678/**

Cao, J. et al. (2023) Live birth of chimeric monkey with high contribution from embryonic stem cells. *CellPress*, Vol:186, 23, 4996 - 5014. **https://www.cell.com/action/showPdf?pii=S0092-8674%2823%2901087-5**

Norris, A.L., Lee, S.S., Greenlees, K.J. et al. Template plasmid integration in germline genome-edited cattle. Nat Biotechnol 38, 163–164 (2020). **https://doi.org/10.1038/s41587-019-0394-6**

Schuster F, Aldag P, Frenzel A, Hadeler KG, Lucas-Hahn A, Niemann H, Petersen B. (2020) CRISPR/Cas12a mediated knock-in of the Polled Celtic variant to produce a polled genotype in dairy cattle. Sci Rep.;10(1):13570. PMID: 32782385; PMCID: PMC7419524.
https://www.nature.com/articles/s41598-020-70531-y

THE ORGAN FARM Gene-edited pig kidneys are finally moving the long-stymied field of xenotransplantation forward (2025)
https://www.science.org/content/article/can-gene-edited-pigs-solve-organ-transplant-shortage

ACKNOWLEDGEMENTS

I would like to thank:

Elvira Dommisse for her oversight and editing skills.
Jo Constable for the typesetting and layout.
Steffan Browning for his photos.
Kara Vandeleur for the cover design and table formatting.
Printed by Lamb-Peters Print, Greytown.

GE Free New Zealand
In Food and Environment Inc.
PO Box 13402, Wellington, 6440.
claire@gefree.org.nz
www.gefree.org.nz
027 348 6731